tal causes the fall in the concentration of free, bound and total glycogen content in rat liver. The question is raised concerning the relationship between the fall in glycogen concentration and the above-mentioned complex of biochemical[4,7-9] and structural events[4] which follow phenobarbital treatment. First of all, this question is related to the increased activity of drug metabolizing enzymes[4]. It was found that the activity of drug metabolizing enzymes in parenchymal liver celles, under certain experimental conditions is parallel to the quantity of liver glycogen[10-12]. That is why FOUTS et al.[13] have postulated that there is correlated dependence between the glycogen quantity and the activity of drug metabolizing enzymes. On the contrary, data of WOOLES[14] does not support the concept of FOUTS et al.[13]. Analyzing the influence of prolonged phenobarbital treatment on the activity of drug metabolizing enzymes, ORRENIUS[7] has found that its activity is increased progressively until the fifth day of treatment. If our results are compared with the results of ORRENIUS[7], the conclusion is reached that there is no direct dependence between the level of glycogen and the degree of activity of drug metabolizing enzymes in the parenchymal liver cells of phenobarbital treated animals. However, we cannot exclude the possibility that the observed decrease in glycogen concentration is at least partly the consequence of increased synthesis of liver microsomal enzymes.

On the other hand, the growth-promoting effect of phenobarbital can also be related to the above-mentioned decrease of glycogen concentration. It is already known that intensification of the proliferative power of tissues in the case of cancerization[15] as well as in the case of normal regeneration of the liver[16,17] is always followed by a decrease in glycogen concentration. The same antiparallel relationship between the quantity of glycogen and mitotic activity of the liver is evident from the present and also our earlier published observations[8,18].

*Résumé.* On a constaté qu'une injection du phénobarbital provoque la diminution de la concentration du glycogène libre de 34% et la diminution du glycogène total de 22,6% seulement 24 h après leur injection. La répétition des injections du phénobarbital diminue le glycogène libre respectivement de 36% et de 28%, le glycogène lié de 46% et de 27%, et le glycogène total de 41% et 24%.

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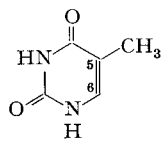
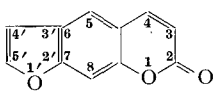
## A Comparison Between the Photoreactivity of Some Furocoumarins with Native DNA and Their Skin-Photosensitizing Activity

Some years ago we found that photosensitizing furocoumarins give a photoreaction with nucleic acids by irradiation at 365 nm[1,2]. A $C_4$-cyclo-addition reaction of furocoumarins to the pyrimidine bases of the macromolecules takes place. Pyrimidine bases always react with their 5,6-double bond and furocoumarins may react either with their 3,4 or with their 4',5' double bond[3-6].



Thymine



Psoralen

This photoreaction appears to explain the mechanism of the photosensitizing action that furocoumarins exert on bacteria[7-9], on mammalian cells in vitro grown[10], on DNA-viruses[11] on mouse Ehrlich ascites tumour cells[12], on sea-urchin sperm[13], and also their skin-photosensitizing activity[14-17] (outcome of erythema on human and guinea-pig skin after a latent period), even if at present the connection between the damage to DNA and the outcome of the erythema is not clear.

In order to test this connection, we have evaluated the photoreactivities of a number of furocoumarins with native DNA by irradiation at 365 nm and we have com-

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pared the results so obtained with those previously obtained in determining the skin-photosensitizing activities of the same substances by irradiation also at 365 nm.

In our early research in this field[14,15], we used a test to evaluate the skin-photosensitizing activity of these substances. The test consisted in placing 5 μg of substance for $cm^2$ of skin (the backs of human volunteers were used), in irradiating at 365 nm in standard conditions and in determining the minimum irradiation time necessary for the outcome of erythema. Considering the activity of psoralen (the parent compound in this group) as equal to 100, the relative activities of the other substances were calculated.

Another test was used later with strictly analogous results, by PATHAK et al.[16,17], operating on guinea-pig skin and determining the minimum amount of substance necessary to produce erythema after a constant period of irradiation.

Recently we have modified our previous test by making it more suitable for assaying the very active methyl-derivatives of psoralen[18]; guinea-pig skin was used, reducing the quantity of substance applied (2.5 $\mu g/cm^2$), but always determining the minimum irradiation time (in standard conditions) necessary to obtain erythema. The relative skin-photosensitizing activities of the various furocoumarins reported in the Table are referred to this test.

To evaluate the photoreactivity of some furocoumarins with native DNA, we have studied the rates of the photoreactions between the same furocoumarins and DNA by irradiating at 365 nm aqueous solutions of the substances for different periods, operating always in the same conditions.

We have used 8 furocoumarin derivatives (see Table), chosen from among those which have a very high activity on human and guinea-pig skin, or only a moderate activity, or from among those which are inactive. All of them were labelled with tritium[19] and had a specific radioactivity in the range $4.5 \times 10^8$–$8.82 \times 10^{10}$ dpm/m$M$.

To an aqueous 0.1% solution of calf-thymus native DNA (Mann Research Laboratories, New York; hypochromicity was higher than 38%) were added small quantities of alcoholic solutions of the furocoumarins to give final concentrations of 10 μg/ml (the alcohol concentration in the various solutions was always less than 1%). 2 ml of the solutions so obtained were irradiated into glass calibrated tubes, 1.2 cm in diameter, immersed in a small cell with glass walls, in which thermostatically controlled water circulated. The irradiations were performed at 22 °C by means of 2 Philips HPW 125 lamps, which emit almost exclusively at 365 nm, placed on both sides of the cell, at a distance of 3.5 cm. In these conditions, the total amount of the incident irradiation on the 2 ml of solution was equivalent to $2.9 \times 10^{16}$ quanta/sec [determined by means of 0.15 $M$ potassium ferrioxalate chemical actinometer[20]].

Applying a procedure which had already been used in our studies on the photoreaction between furocoumarins

Relative photoreactivity with DNA and relative skin-photosensitizing activity of furocoumarins

| Furocoumarins | Irradiation time necessary for a 20% linkage to DNA (sec) | Relative photo-reactivity with DNA (psoralen = 100) | Relative skin-photo-sensitizing activity[18] (psoralen = 100) |
|---|---|---|---|
| 8-Methyl-psoralen | 90 | 373 | 540 |
| 5-Methyl-psoralen | 144 | 233 | 450 |
| Psoralen | 336 | 100 | 100 |
| Xanthotoxin (8-methoxy-psoralen) | 558 | 60 | 71 |
| Bergapten (5-methoxy-psoralen) | 1026 | 33 | 61 |
| Angelicin (iso-psoralen) | 2160 | 15 | 12[a] |
| 4',5'-Dihydro-psoralen | – | – | inactive |
| Xanthotoxol (8-hydroxy-psoralen) | – | – | inactive |

[a] The low skin-photosensitizing activity of angelicin did not allow a correct determination on guinea-pig skin, which has a lesser sensitivity than human skin[18]. Therefore this datum is referred to the test on human skin[14,15].

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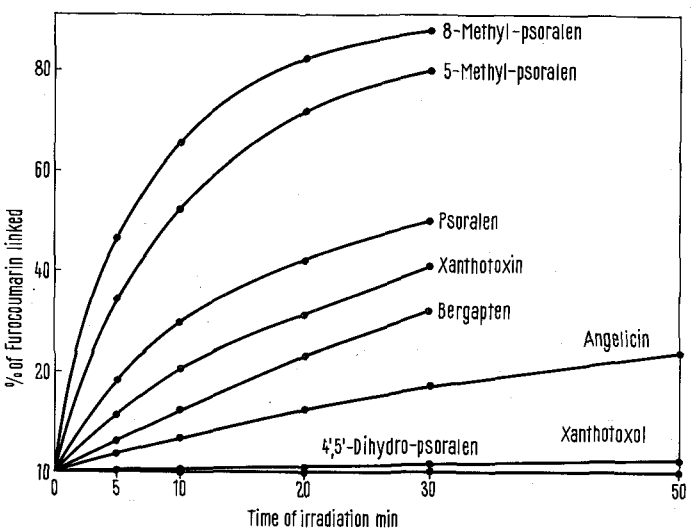


Photoreactions between some furocoumarins and native DNA by irradiation at 365 nm. Percentages of furocoumarins (referred to the amount initially present) linked to DNA as a function of the period of irradiation are reported.

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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[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

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