Table II. Infectivity titer of various viruses after treatment with psoralen

Virus	Virus titration system[d]	Infectivity titer, neg. log 10[a]			Time of irradiation in min
		Irradiated[b] without Psoralen	Psoralen[c] treated without irradiation	Psoralen treated[c] and irradiation[b]	
1 Pseudorabies	PK cells	5.4	5.4	1.0	80
2 Infectious canine hepatitis	DK cells	4.5	3.7	1.7	80
3 Fowl pox (pigeon strain)	Chick embryo	2.6	2.2	1.0	90
4 Teschen disease	PK cells	6.2	6.2	6.2	100
5_1 New Castle disease[e]	Chick embryo	7.0	7.5	7.7	120
5_2 New Castle disease[e]	PK cells	5.7	6.2	5.7	120
5_3 New Castle disease[f]	Chick embryo	6.2	6.0	5.7	120
5_4 New Castle disease[f]	PK cells	4.2	4.5	3.7	120
6 Foot and mouth disease (type C)	CK cells	5.75	5.75	4.24	90

[a] REED and MUENCH's method (Am. J. Hyg. *27*, 439 (1938)). [b] 3 ml of viral suspension in 60 mm Petri dishes, opened, were irradiated by an analysis quartz lamp original Hanau, mod. Q-500, with 366 mμ filter, at 25 cm of distance, at room temperature. [c] Psoralen was added to the viral suspension at the final concentration of 18.7 μg/ml (10^{-6} *M*). [d] PK = established line of pig kidney cells (W. A. MALMQUIST, Am. J. vet. Res. *23*, 241 (1962)); DK cells = primary culture of dog kidney cells; CK cells = primary culture of calf kidney cells. [e] Chick embryo stock. [f] Cell culture stock.

make also RNA-viruses, treated by furocoumarins, photosensitive.

Further research is in progress, both in the field of cell photosensitization and virus photoinactivation, and in the field of interaction between furocoumarins and DNA after UV-irradiation, with the aim of clarifying the mechanism of the biological photosensitization by furocoumarins.

Riassunto. Le furocumarine possono legarsi in vitro al DNA ed, in misura molto minore, all'RNA. Per irradiazione UV (3655 Å) in presenza di psoralene, tre virus a DNA sono stati inattivati, mentre nessun effetto è stato notato su altri tre virus ad RNA.

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Evidences of a Photoreaction of the Photosensitizing Furocoumarins with DNA and with Pyrimidine Nucleosides and Nucleotides[1]

In connection with our researches on the mechanism of action of the skin-photosensitizing furocoumarins[2], we have published a note[3] on the interaction of furocoumarins with nucleic acids.

We have found that the furocoumarins have a specific capacity of binding to DNA, while their binding to RNA occurs to a much less extent. No relationship exists, however, between such a property (which operates in absence of any irradiation) and the skin-photosensitizing activity of furocoumarins; both active and inactive furocoumarins are able to bind to DNA.

We have also noticed that inactivation of some DNA-containing viruses occurs after irradiation with long-wave UV-light (3655 Å) in the presence of psoralen, the most skin-active furocoumarin.

Now we have investigated the modifications occurring in the DNA and furocoumarin solutions, when they are irradiated with long-wave UV-light.

No significant results have been obtained by examining the variations of viscosity, UV-spectra and rotatory power of the DNA solutions, when irradiated both in the presence and absence of furocoumarins.

On the contrary, we found a strong modification of the fluorescence spectrum after irradiating solutions of DNA and of some furocoumarins by long-wave UV-light.

Figure 1 reports the fluorescence spectra of a solution of DNA and psoralen, before and after irradiation, as determined by an Aminco-Bowman spectrophotofluorimeter. There is an evident shift of the maximum from 450 mμ to 400 mμ, and an increase of the fluorescent intensity.

The fluorescence spectrum of psoralen, irradiated alone, does not show a similar change, as appears from Figure 2.

[1] This study was presented at the IV International Photobiology Congress, Oxford, 26–30 July 1964.

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Analogous modifications in the fluorescence spectra were also observed for solutions of DNA added with other skin-photosensitizing furocoumarins such as xanthotoxin, bergapten, 4'-methyl-psoralen and 4, 4', 8-trimethyl-psoralen. The results are reported in Table I.

On the contrary, no modification was observed after irradiating a solution of DNA in the presence of skin-inactive furocoumarins (bergaptol, imperatorin, isopimpinellin).

With the nucleotides, the nucleosides and the purine or pyrimidine bases, which occur in DNA and RNA, we have obtained these results: (a) On irradiating aqueous solutions containing one of these compounds and psoralen, modifications in the fluorescence spectra were obtained only with the nucleotides and nucleosides derived from a pyrimidine base (Table II). The modifications are identical in all cases, and they are similar to those observed for the DNA solutions. (b) With the nucleotides and nucleosides derived from a purine, no modification was observed. (c) Neither do modifications occur with the simple purine and pyrimidine bases [4].

Table I. Modifications of the fluorescence spectra observed in the aqueous solutions of DNA[b] 0.2% and furocoumarins after irradiation at 3655 Å

Furocoumarins	Concentration μg/ml	Activating wavelength[a] λ_{max}	Fluorescence λ_{max} Before irradiation	After irradiation	Shifts
		mμ	mμ	mμ	mμ
Skin-photosensitizing					
Psoralen	18.6	330	450	400	−50
Xanthotoxin	16.0	325	495	470	−25
Bergapten	6.0	335	450	410	−30
4'-Methyl-psoralen	3.7	330	450	395	−55
4,4',8-Trimethyl-psoralen	2.1	335	460	385	−75
Skin-inactive					
Bergaptol	15.0	340	450	450	0
Imperatorin	8.5	310	440	440	0
Isopimpinellin	8.6	350	440	440	0

[a] The activating wavelengths were the same before and after irradiation. [b] DNA was extracted from calf thymus (P = 7.32%; N/P = 1.68).

Table II. Shifts (mμ) of the λ_{max} of the fluorescence spectra of aqueous solutions of bases, nucleosides and nucleotides (0.1%) added by psoralen (16 μg/ml) and irradiated (90 min at 3 655 Å)

Bases	Nucleosides		Nucleotides	
Thymine 0	Thymidine	−50	Thymidylic acid	−50
Cytosine 0	Cytidine	−50	Cytidylic acid	−50
	Deoxy-cytidine	−50	Deoxy-cytidylic acid	−50
Uracil 0	Uridine	−50	Uridylic acid	−50
Adenine 0	Adenosine	0	Adenylic acid	0
	Deoxy-adenosine	0	Deoxy-adenylic acid	0
Guanine 0	Guanosine	0	Guanylic acid	0
	Deoxy-guanosine	0	Deoxy-guanylic acid	0

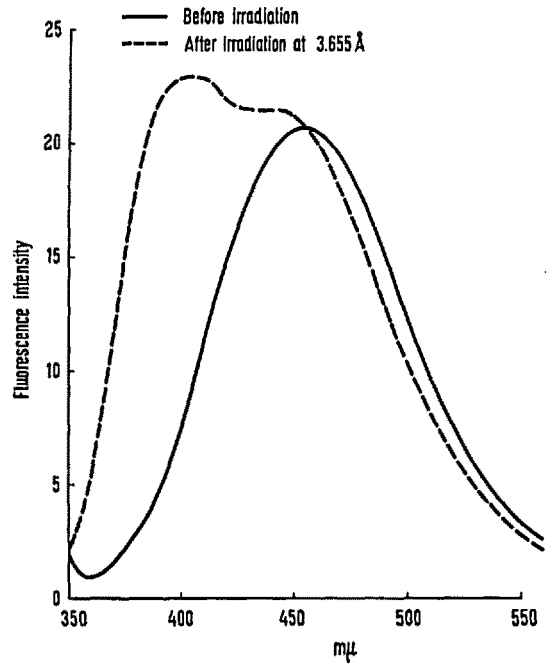

Fig. 1. Fluorescence spectra (before and after irradiation) of an aqueous 0.2% DNA solution containing 16 μg/ml of psoralen. 90 min of irradiation at 3 655 Å (Philips HPW 125 lamp at a distance of 25 cm). Activating wavelength: 330 mμ.

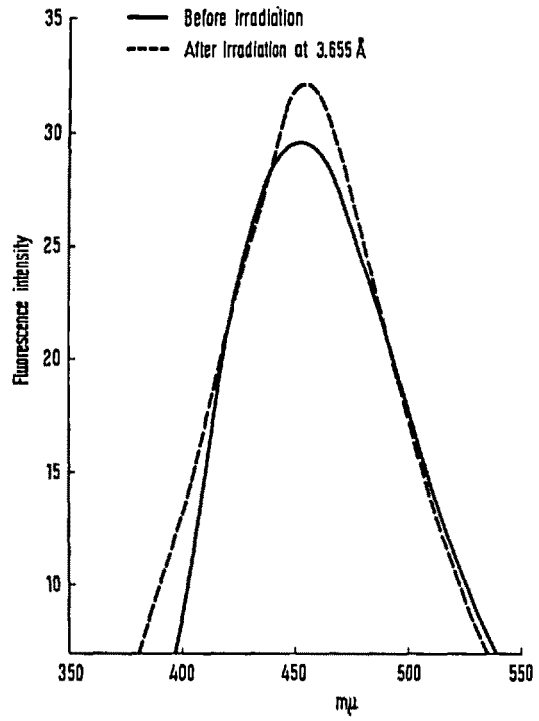

Fig. 2. Fluorescence spectra of an aqueous solution of psoralen (16 μg/ml) before and after 90 min of irradiation at 3 655 Å. Activating wavelength: 330 mμ.

[4] Added in proof: When irradiated in frozen aqueous solutions, the pyrimidine bases (thymine, cytosine, uracil) also react with the photosensitizing furocoumarins, showing modifications in the fluorescence spectra and new chromatographic spots.

The formation of a new compound on irradiating (3655 Å) an aqueous solution of psoralen and a pyrimidine nucleoside was also confirmed by paper chromatography. After irradiation, in the chromatogram (examined at 3655 Å UV-light), within the spot of psoralen and other very slight spots due to the photolysis products of furocoumarin[5], there is an evident new spot with violet fluorescence.

The new spots obtained with various nucleosides have all the same violet fluorescence but different Rf values, as appears in Table III.

The spots of the nucleosides can be observed at 2537 Å UV-light and their Rf values are slightly smaller than those of the new compounds.

Preliminary experiments show that the formation of the new compounds takes place better by irradiating the mixture of psoralen and nucleosides in the solid state.

Irradiating an intimate solid mixture of thymidine and psoralen (2:1 mol) for 1 h with a Philips HPW 125 lamp (3655 Å) at a distance of 25 cm, preparing the chromatogram of the product obtained and eluting with methyl-alcohol the new spot with violet fluorescence and Rf 0.64 (see Table III), we have obtained a solution, which demonstrates a fluorescence spectrum (Figure 3) with a λ_{max} at 395 mμ, very similar to those (λ_{max} 400 mμ) shown by the irradiated solutions of psoralen and DNA or other pyrimidine nucleosides and nucleotides.

These are the first results of research work now under progress. We think we have obtained sufficient indications that a photoreaction occurs when a solution of DNA is irradiated in the presence of a *skin-active* furocoumarin.

At present, our goal is the isolation of the new-formed substances, the study of their biological significance, and the extension of these experiments to RNA.

Table III. Fluorescence and Rf values of new compounds formed in the photoreactions between psoralen and pyrimidine nucleosides (experimental conditions as in Table II)

Pyrimidine nucleosides	New chromatographic spots	
	Fluorescence (at 3 655 Å)	Rf butanol-acetic acid-water 4:1:5
Thymidine	violet	0.64
Cytidine	violet	0.32
Deoxy-cytidine	violet	0.52
Uridine	violet	0.43

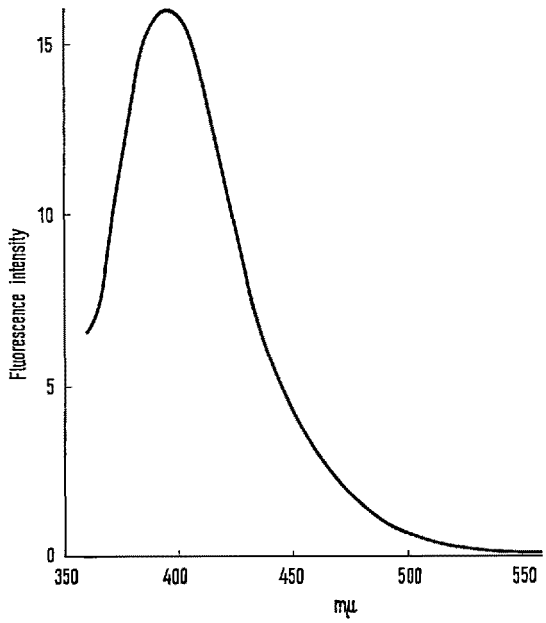

Fig. 3. Fluorescence spectrum of the new chromatographic spot obtained in the photoreaction between psoralen and thymidine. Activating wavelength: 330 mμ.

Riassunto. Modificazioni negli spettri di fluorescenza si hanno irradiando (3655 Å) soluzioni di DNA o di nucleosidi e nucleotidi pirimidinici in presenza di furocumarine fotosensibilizzatrici. La formazione di nuovi composti nelle fotoreazioni tra psoralene e nucleosidi pirimidinici è stata confermata per cromatografia su carta.

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August 10, 1964.

[5] L. MUSAJO, G. RODIGHIERO, F. DALL'ACQUA, and G. CAPORALE, Gazz. chim. ital., in press.

The Steroid Hormone Synthesis in the Brown Adipose Tissue of Mice

The histological and biochemical differences between brown and white adipose tissue are so great that there are reasons for regarding them as different tissues. Although brown adipose tissue (BAT) has recently become the object of renewed interest, its physiological role has not yet been clarified. In extracts of BAT of hibernating and non-hibernating animals, the presence of steroid hormones has been stated[1-6]. The question whether the steroids revealed in BAT are not only stored up but also

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