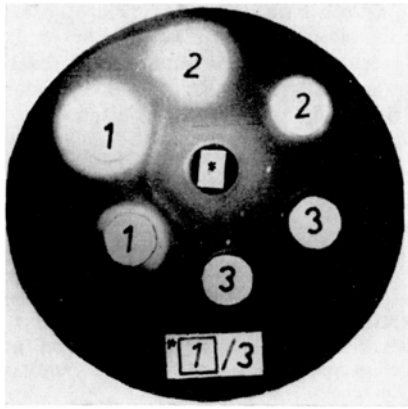

Fig. 1

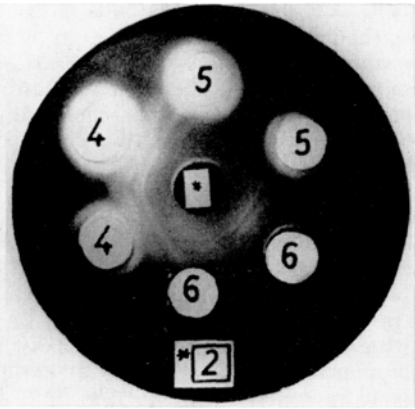

Fig. 2

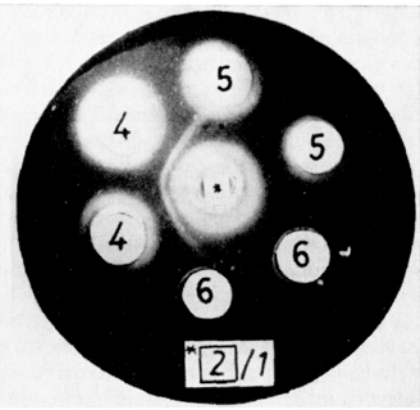

Fig. 3

Antigens: 1, smooth-muscle myogen extract; 2, striated-muscle myogen extract; 3, plasma; 4, smooth-muscle structure-protein extract; 5, striated-muscle structure-protein extract; 6, mixed-tissue myogen extract. Antibodies: [1], immune serum against antigen No. 1; [2], immune serum against antigen No. 4; [1]/3, supernate of immune serum No. [1] after absorption by antigen No. 3; [2]/1, supernate of immune serum No. [4] after absorption by antigen No. 1.

It has been noted in the foregoing that immune sera produced against SM StrProt-s do not precipitate antigens prepared from striated muscle StrProt-s (Figure 2), so that the latter are not identical with the specific antigenic component of the SM StrProt extract, nor is this SM structural component identical with that contained in the SM myogen extract (Figure 3). Considering that, according to our other investigations, the structure extracts contain not more than 10 to 15% actomyosin complex, it is possible that the specific factor remaining in the SM structure extract belongs to IVANOV's T-fraction, or else it may be a different structure component.

Zusammenfassung. Es gelang, zwei charakteristische Antigene im 0,154 *M* KCl-Extrakt glatter Hundemuskeln durch Geldiffusion nachzuweisen, die möglicherweise eine Folge des spezifischen Stoffwechsels der glatten Muskulatur sind. Die weitere Möglichkeit besteht, dass ein Teil der Struktureiweisse der glatten Muskeln bereits bei niedriger Ionenkonzentration gelöst wird. Beim spezifischen Antigen des Weber-Edsall-Extrakts glatter Muskeln handelt es sich wahrscheinlich nicht um Aktomyosin bzw. Myosin, sondern um ein anderes Eiweiss oder Kollagen.

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July 29, 1964.

Photosensitizing Furocoumarins: Interaction with DNA and Photo-Inactivation of DNA Containing Viruses

Some furocoumarins show a well-known photosensitizing activity on the human and guinea-pig skin[1-3]; the lethal photosensitization of bacteria[4-6] and of mammalian cells, adapted to in vitro growth[7], was also studied.

The relationships between the photosensitizing activity of furocoumarins and their chemical structure are now well clarified[2,3].

The photosensitizing effects displayed on the skin are characteristic and different from those of many other photodynamic substances, such as hematoporphyrin, hypericin, methylene blue, fluoresceinic dyes, etc.[8]. The last compounds act by a photooxydative process. The furocoumarins, on the contrary, are lacking in photooxydative properties; the mechanism of their photosensitizing effect is at present not completely understood, in spite of much research done in this field[2,8,9-11].

Studies of the photoreactions between photosensitizing furocoumarins and flavin-mononucleotide (FMN) seemed to suggest a first approach to this problem; in fact only the active substances photoreact, the inactive ones do not form new compounds. The strict parallelism observed suggested the possibility of an explanation, through the photochemical in vitro reactivity with FMN, of the in vivo

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properties of the furocoumarins[8,12]. This hypothesis, however, does not agree with all the facts observed.

Recently, on the basis of the photosensitization of mammalian cells in vitro by psoralen, one of us[6] suggested a damage to nucleic acids. From the results of development of mutants in *Sarcina lutea* after photosensitization by a skin-photosensitizing furocoumarin (8-methoxypsoralen or xanthotoxin) an action on DNA was also suggested by MATHEWS[7].

The present experiments were performed in order to verify the possibility of a direct interaction between nucleic acids and furocoumarins.

From the chemical side, the binding of furocoumarins to DNA and RNA was investigated. From the biological side, the inactivation of animal viruses was tested by irradiation, with long-wave ultraviolet light (3655 Å), of furocoumarin treated virus suspensions.

On the binding of furocoumarins to DNA, some data were obtained a few years ago by the equilibrium-dialysis method[13]. Now the problem has been reinvestigated determining the solubilization of furocoumarins in aqueous solutions of DNA and RNA[14,15]. Control was made (by means of high-speed centrifugation) to make sure that not one colloidal suspension was formed, as in the case of benzpyrene[16].

The results obtained, summarized in the Figure and in Table I, clearly confirm the preceding data[13], indicating that the furocoumarins have a specific capacity of binding to DNA; however, a binding to RNA also occurs to a much lesser extent.

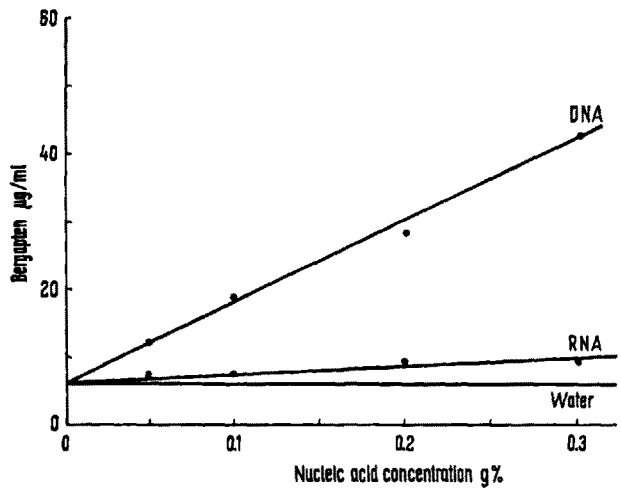
The ability of binding to DNA does not seem conclusive for explaining the mechanism of the skin-photosensitiza tion, since such a property has been shown either by skin-active or inactive furocoumarins, as it appears from Table I.

In the experiments of virus photo-inactivation, three DNA viruses [*Pseudorabies virus* (PrV), *Infectious Canine Hepatitis virus* (ICHV), *Fowl Pox virus* (FPV), *Pigeon strain*] and three RNA viruses [*New Castle Disease virus* (NDV), *Foot and Mouth Disease virus* (FMDV) *C type*, *Teschen Disease virus* (TDV), *Mödling strain*] were tested by determining the infectivity titers after furocoumarin treatment of viral suspensions, followed by irradiation with long wavelength UV-light (3655 Å).

The results are summarized in Table II. Treatment in darkness with psoralen, the most active natural furocoumarin, had no effect on the infectivity titers of all the viruses so far tested, while irradiation (without psoralen) at the above wavelength appeared not to affect virus infectivity.

On the contrary, after irradiation at 3655 Å of psoralen-treated viral suspensions, the first three (DNA viruses) were inactivated; for the other three (RNA viruses) the experimental treatment appeared to be quite harmless, in spite of a longer period of irradiation.

However, preliminary experiments show that different conditions of treatment, such as those used by MELNICK et al. with other photodynamic substances[17,18], seem to



The solubility of bergapten in water and in aqueous solutions of DNA and RNA. The DNA was extracted from calf thymus (P = 7.32%; N/P = 1.68); RNA (from yeast) was furnished by Sigma Chemical Company. The intimate suspensions of bergapten in water and in aqueous solutions of DNA and RNA were shaken for 4 h in a thermostatic bath at 20°; after centrifugation at 12,000 *g* for 40 min, the concentration of furocoumarin in the supernatant was determined spectrophotometrically. The concentrations remained unchanged also after centrifugation of the solutions at 100,000 *g* for 2 h (see [16]).

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Table I. The solubility of some furocoumarins in water and in 0.2% DNA and RNA solutions; experimental conditions as in the Figure

Furocoumarins	Solubility µg/ml			Solubility ratio	
	Water	DNA 0.2%	RNA 0.2%	Soluble in DNA / Soluble in H_2O	Soluble in RNA / Soluble in H_2O
Psoralen	35.4	77.2	42.4	2.18	1.19
Bergapten	5.0	28.4	8.2	5.68	1.64
Xanthotoxin	36.05	151.5	36.3	4.21	1.02
4'-Methyl-psoralen	5.4	16.9	5.4	3.12	1.00
5',4,8-tri-methyl-psoralen	3.5	27.7	2.8	7.87	0.80
Angelicin	41.5	120.9	37.8	2.91	0.91
Bergaptol*	22.4	76.1	36.8	3.39	1.66
Imperatorin*	8.08	12.12	7.4	1.50	0.89

* Inactive on the skin.

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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