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EFFECT OF THE SEROTONIN-CREATININE SULFATE COMPLEX ON THE ACTION OF ULTRAVIOLET RADIATION ON PLANARIANS *(Dugesia dorotocephala)*

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ABSTRACT

Populations of planarians were exposed to a wide range of UV, doses in order to analyze the possible protective effect of 5TH. In contrast to the results obtained with low and high LET ionizing radiations, 5TH did not protect planarians against UV radiations either at the level of $LD_{50/60}$ or in the general behavior of the dose-mortality curve. The cause of this is attributed to the different ionizing and excitation mechanisms of the radiation.

RESUMEN

Poblaciones de planarias fueron expuestas a una amplia gama de dosis de radiación UV para analizar el posible efecto protector de la 5HT. A diferencia de los resultados obtenidos con radiaciones ionizantes de bajo y alto LET, la 5HT no protegió a las planarias de la acción de las UV, tanto a nivel de la $LD_{50/60}$ como en el comportamiento general de la curva dosis-mortalidad. La causa se adjudica al diverso mecanismo de acción ionizante y excitante de esas radiaciones.

INTRODUCTION

Ultraviolet radiations induce alterations in biological systems such as formation of thymine dimers and inhibition of DNA synthesis (Setlow, 1963), mutations in microorganisms (Lemontt, 1971, retardation of cell division (Han, 1971), chromosomal aberrations in chinese hamster tissue culture (Bender *et al.*, 1973), skin cancers (Setlow and Hart, 1973), etc.

ABBREVIATIONS:		
	5HT	5-Hydroxytryptamine
	UV	Ultraviolet radiation
	$LD_{50/60}$	Mean lethal dose in 60 days
	LET	Linear energy transfer

Some authors have worked with different protectors against UV. Blum *et al.* (1952), on irradiating eggs and sperms of sea urchin with UV, found a recuperation process by illuminating with visible radiation. Bawden and Kleczkowski (1959) described the protection provided by the tobacco mosaic virus protein against the action of UV on its nucleic acids. Matsuyama *et al.* (1967) have related that some halogenic alkalines protect bacteria and yeasts against UV and Bhuttacharya and Mishra (1969) found protection against UV in *Aspergillus terreus,* especially when the thiourea was present at the moment of irradiation. Ortu *et al.* (1969) observed an increase in damage of erythrocyte membranes irradiated with UV caused by the presence of substances derived from iodine or by the presence of N-ethyl-maleimide. Nevertheless, these same substances protected *Escherichia coli* from the action of UV, increasing their survival.

The radioprotective effect of 5HT against ionizing radiations has benen demonstrated in various organisms: in mammals (Maggiora and Brun, 1963, Pantev and Bokova, 1974), in invertebrates (Laguarda-Figueras and Villalobos-Pietrini, 1967; Villalobos-Pietrini 1969), in plants (Lozeron *et al.,* 1964, Villalobos-Pietrini and Laguarda-Figueras, 1967) and also in rat liver mitocondrial systems (Alexander *et al.,* 1975).

In these investigations low LET radiations were used. It was also found that in mice, 5HT is effective in reducing the effect of neutrons (Vogel *et al.,* 1960). Although x radiation and UV are part of a continuous spectrum of radiations (Casarett, 1968), the information available on protection against x radiation cannot be generalized to UV because the mechanisms of action of the two types are different. However, the fact that substance which are photosensitizers for UV are also radiosensitizers, suggests that those agents which protect against x radition might also protect against the damage caused by UV (Kahn and Curry, 1974).

The present work was designed in order to determine the effects of 5HT on the mortality of planarians irradiated with non-ionizing radiations such as UV.

Material and Methods

Planarians *(Dugesia dorotocephala)* were collected, handled and maintained as described previously (Laguarda-Figueras and Villalobos-Pietrini, 1967). The sample size was calculated according to a modification of the iterative test (Sokal, 1969) and was N = 90 per dose per experiment. The planarians were exposed to an ultraviolet lamp (Westinghouse Sterilamp 782H-10, 10 watts) that emits wavelengths in the range 7800-2700 Å. The planarians were placed at a distance of 50 cm from the source and the exposure times ranged from 15 to 85 min (Table I). Dosimetry was accomplished by using a Latarjet dosimeter (Latarjet *et al.,* 1953). The control groups were maintained in purified water during irradiation and the treated groups were placed in a 3.14×10^{-5}M solution of 5HT (Hycel, Houston), selected on the basis of previous work (Laguarda-Figueras and Villalobos-Pietrini, 1967). The temperature of the solutions was kept invariant at 21°C during the time of exposure and their volumes also remained constant.

Surviving planarians were counted 60 days after treatment. The serotonin solutions were analyzed in a spectrophotometer (Carl Zeiss, PMQ II30392) before and after irradiation for all doses used, in order to detect possible alterations in the chemical composition of the serotonin caused by UV. No differences were found in the cases studied.

Results and Discussion

The mortality rate of planarians with and without serotonin was analyzed by means of the Probit transformation (Finney, 1964) and the regression lines were obtained for both groups. On applying the X^2 test, no significant differences were found among the repetitions of the experiments and thus the average values of each lot of treated and control planarians were used.

Fig. 1 shows the probit regression lines and their equations; when the X^2 test was applied to the two lines, no difference was found ($P > 0.05$). The values of $LD_{50/60}$ (Fig. 1) for the controls and the treated group were compared by means of "Student's t" test and no significant difference between the values was found ($P > 0.05$).

In view of the previous results, Table I shows the average of all the experiments on the mortality of planarians caused by UV with and without serotonin, analyzed by the Probit method in which the weighting coefficient was calculated. From these data, an $LD_{50/60}$ ($\pm$ S. E.) of 17,536 $\pm$ 1119.00 ergs was obtained.

From these results it can be concluded that even though 5HT was effective in protecting against low LET radiations (Laguarda-Figueras and Villalobos-Pietrini, 1967; Villalobos-Pietrini and Laguarda-Figueras, 1971) and high LET radiations, (Vogel *et al.*, 1960, it was ineffective against UV.

A possible explanation of these discrepancies could be the fact that ionizing radiations, besides acting directly on organic molecules, also produce secondary effects by forming free radicals in the intra and extra cellular watery medium (Casarett, 1968). This does not happen with UV whose secondary effects are small and whose main action is the excitation of electron energy levels. Furhternnore, since 5HT is a strong electron donor (Szent-Gyorgyi, 1960; Radda, 1966), its effect would manifest itself more in deionization or in the scavenging of free radicals (Alexander *et al.*, 1955) than on the electrons excited by UV.

On the other hand, Kahn and Curry (1974) observed a strong protection against UV when human erythrocytes were irradiated in the presence of anthracene (photosensitizer) in addition to serotonin and a moderate protection when irradiated in the presence of serotoni and protophorphyrin or chlorpromazine. No protection was found with 5HT and the photosensitizer tribromsalam. Nevertheless, these authors did not investigate the effect of 5HT alone against UV, but always used it in combination with photosensitizers, which could foster the formation of some protecting complex that might have covered the radiosensitive sites (Dukor, 1962). Kahn and Curry (1974) attribute the protection to the anoxia produced by 5HT thatreduce the formation of free radicals in the radiolysis of tissue water.

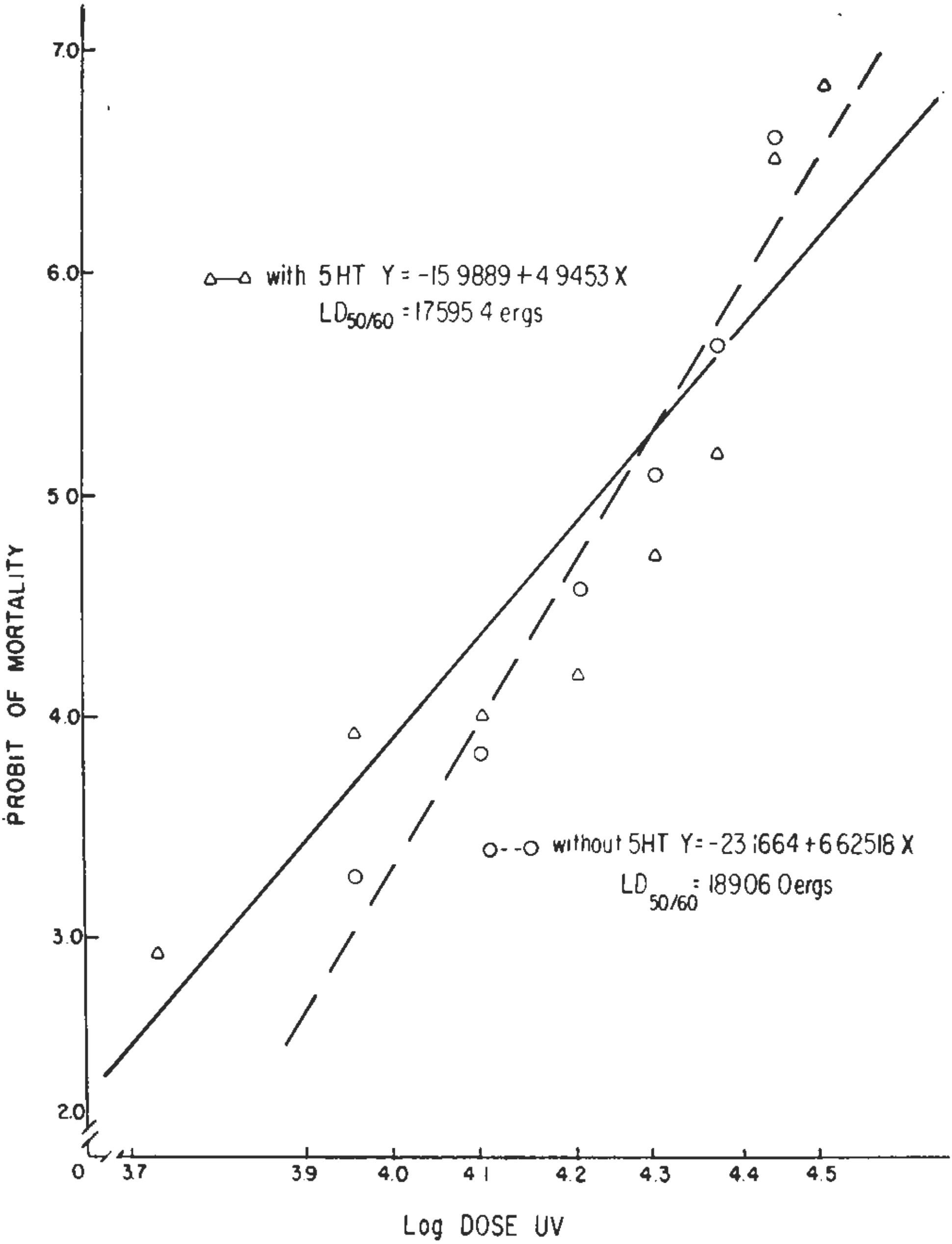

Fig. 1. Probit regression lines for estimating the mortality induced in *Dugesia dorotocephala* by UV. (Mortality is the result of the average experiments with and without 5HT. See the tex.).

TABLA I

MORTALITY IN PLANARIANS *(Dugesia dorotocephala)* PRODUCED BY DIFFERENT DOSES OF UV

Exposition time (min)	*Doses (ergs)*	*Log Doses (x)*	*Mortality*	*Probit Empirical*	*Probit Theoretical*	*Working (y)*	*Weighting coefficient (w)*	*nw*	*nwx*	*nwx²*	*nwy*	*nwy²*
15	5,400	3.7324	1.1	2.7096	2.5	2.716	0.04979	4.48	16.721	62.409	12.168	33.047
25	9,000	3.9542	8.3	3.6148	3.6	3.595	0.30199	27.18	107.475	424.978	97.712	351.275
35	12,600	4.1004	14.4	3.9375	4.3	3.973	0.53159	47.84	196.163	804.347	190.068	755,141
45	16,200	4.2095	26.6	4.3750	4.9	4.421	0.63431	57.09	240.320	1011.627	252.395	1115.838
55	19,800	4.2967	45.8	4.8945	5.3	4.886	0.61609	55.45	238.252	1023.697	270.929	1323.758
65	23,400	4.3692	66.1	5.4152	5.7	5.386	0.53159	47.84	209.023	913.263	257.666	1387.790
75	27,000	4.4314	93.3	6.4985	6.0	6.366	0.43863	39.48	174.952	775.282	251.330	1599.965
85	30,600	4.4857	96.6	6.8250	6.3	6.690	0.33589	30.23	135.603	608.274	202.239	1352.977

$Snw\ (x-\bar{x})^2 = 5615.382$
$Snw\ (y-\bar{y})^2 = 7605.904$
$Snw\ (x-\bar{x})\ (y-\bar{y}) = 6535.293$
$x = 4.2589 = 4.9566$

Equation:

$y = -18.3557 + 5.5033x$
$LD_{50/60} \pm S.E. = 17536.6 \pm 1119.00$ ergs
$LD_{50/60} \pm S.E. = 4.2439 \pm 0.4884$

* Mortality is the result of the average of the experiments with and without 5HT.

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