The effect of Ultraviolet Irradiation and 8-Methoxypsoralen on copper and glutathione levels in tissues of Albino Rats

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It is well known that the exposure of the skin to ultra-violet light will produce a photobiological response. This response is followed by erythema of the skin which occurs within few hours after exposure. The erythema may persist for a few days depending on the severity of the exposure, and pigmentation gradually occurs. With repeated exposures the pigmentation may become intense and will persist for many weeks or months without further ultra violet exposure. The intensity of effected pigmentation is more marked in dark individuals than fair kinned subjects. The pigmentation is associated with the formation and migration of melanin particles.

Various suggestions have been given to explain the new formation of melanin which occurs after ultra-violet irradiation and the possible factors playing a role in the process[1]:—

I. Ultra-violet irradiation enhances the oxidation of tyrosine to dopa and the small amount of dopa thus formed then catalyzes the tyrosine tyrosinase reaction.

II. Changes in the redox potential of the skin occurs on iraadiation, so as to favour dark melanin formation.

III. Increased skin temperature accompanying the ultra-violet erythema accelerates the tyrosine tyrosinase reaction.

IV. A drop in the tyrosinase inhibitory epidermal sulfhydryl groups occuring as a result to such irradiation.

V. Ultra-violet irradiation oxidises the sulfhydryl content of the skin and so lowers it by 20%.[2]. It also helps the transformation of the pigment dihydroxyphenyle alanin to melanin. Reduced melanin can be oxidised to the dark form by exposure to ultra-violet rays.

FLESH, P. and ROTHMAN 1948[3] noted that exposure of the shaved skin of rabbit to ultra-violet rays in vivo causes an immediate reduction of the sulphydryl contents in the skin. This suggests that pigmentogenic produced stimuli act by elemination of the sulfhydryl group inhibitors allowing the enzymic oxidation of pigment precursers to occur.

The thickening of the horny layer of the skin brought about by increased keratin formation in response to ultra-violet irradiation is accompanied by excessive formation of disulfide (S.S.). The disulfides bridges from -SH groups

of reactive proteins. This finding is considered as a further proof that increased pigmentation is brought about by reduction of the -SH groups.[3]

Under normal conditions, an equilibrium seems to exist within the melanocyts and basal cell layer of the epidermis; between the sulfhydryl compounds and the copper containing enzyme. Under the action of pigment producing stimuli such as ultra violet irradiation, this equilibrium is disturbed by the oxidation of—SH groups, the enzyme being enabled to act freely on the sugstrate to form the pigment. ROTHMAN 1923[4] noted that with development of sun tanning the tyrosine level of blood serum dropped considerably. It was concluded that after ultra-violet irradiation, circulating tyrosine is taken up by the activated epidermal melanocytes to form melanin. Ultra-violet irradiation will indirectly increase the activity of tyrosine through the process of —SH oxidation to —S—S which results in activating the copper containing enzyme —tyrosinase—to enhance melanin formation.

8—Methoxypsorallen Increases Skin Tolerance to Ultra-violet Irradiation

FITZPATRICK, T.P. CE HOPKINS, DELPERT D.,[5] proved that 8-Methoxypsoralen taken orally two to three hours before exposure, increased skin tolerance to ultra-violet irradiation and that the normal areas of skin in leukodermic patients pigmented more intensely than usual and a few stated that the vitiligo patches showed an increased tolerance to solar radiation. In a previous study oral 8-Methoxypsoralen was reported to decrease the erythma response to artificial ultra-violet light.[6] Bearing in mind these observations by several investigators we thought that it is worthy to study the levels of blood, liver glutathione and copper in rats exposed for different periods of time to ultra-violet irradiations either alone or with the administration of Ammoidin.

Experimentation

In this work albino rats weighing from 150-200 gm. were used. The rats were divided into two groups. Both the 1st and the 2nd groups of rats were exposed to ultra-violet light from a mercury lamp 500 V and at a distance of 70 cm. and only the members of the second group were given 8-methoxypsoralen in addition. Group I was divided into three sub-groups: a, b, and c.

Three experiments were done:—

1. In a short experiment animals included in sub-group A were exposed to ultra-violet light for two minutes daily for 15 days.

2. In a medium experiment the rats in sub-group C were exposed to ultra-violet for two minutes/day for 30 days.

3. In a long experiment the albino rats in sub-group C were exposed to ultra-violet light for 5 minutes daily for 47 days.

Group II was exposed to ultra-violet light for 5 minutes daily over a period of 30 days. The rats were also given Ammoidin in doses of 300 mgm/K.G.B.W. over the same period. The drug was given orally with food as an emulsion forced fed with a polythene tube attached to an all glass syringe.

At the end of the experimental period the animals were sacrified by decapitation. The blood was collected from the cut neck and the animal was

immediately dissected and the liver quickly removed washed in physiological saline, blotted against filter paper to remove extra fluid then weighed and kept in the deep freeze until it was analysed.

Any change that occured during the experimental period in the colour of albino rats was noted. The blood copper was estimated by the method of GOBLER et al.[7] The blood glutathione was estimated by the method of THOMPSON and WATSON 1952.[8]. The liver copper content was estimated by the same procedure of GOBLER et al using tissue homogenate preparations. Liver glutathione was estimated by the method of EL HAWARY.[9]

The results obtained for the rats receiving ultra-violet alone are shown in Table I, II, III.

TABLE I

Subgroup A : U.V.R, for 2 min, daily for 15 days.

	No. of rats.							
Tissues	1	2	3	4	5	6	Mean	NOTES
Blood Cu.	128	131	135	126	140	130	132	
Blood GSH	20.4	21.4	18,4	22.6	24.2	20,2	21,2	
Liver Cu.	98	104	112	96	94	112	103	
Liver GSH	91	79.5	108	73	73	83	86.7	

TABLE II

Subgroup B : U.V.R, for 2 min, for 30 days,

	No. of rats.							
Tissues	1	2	3	4	5	6	Mean	NOTES
Blood Cu.	138	136	128	142	144	132	137	The hair
Blood GSH	18.8	20.4	21,6	19.2	23.2	24,1	19,9	attained
Liver Cu.	92	108	96	82	102	104	97	as yellowish
Liver GSH	78	84	94	110	102	96	94.5	tint.

TABLE III

Subgroup C : U.V.R, for 5 min, daily for 47 days.

	No. of rats.							
Tissues	1	2	3	4	5	6	Mean	NOTES
Blood Cu.	138	159	128	146	160	151	147	A deeper yellow
Blood GSH	18.8	19.4	20,6	17,2	18.2	19.1	18,9	tint of hair was
Liver Cu.	92	108	96	82	102	104	97	observed especi-
Liver GSH	78	84	92	110	102	96	93	ally on the back.

These figures in Tables I, II, III show that exposure of albino rats to ultra-violet rays although showing a slight increase in the blood copper from a normal mean value of 131 to a mean value of 147 microgram %, yet in some of the rats particularly in the long span experiments showed high values of blood copper upto 160 microgram %. This was associated with a decrease of liver copper from a normal mean value of 115 to 97 mgm/100gm. fresh tissue weight.

As regards the blood glutathione there is a slight decrease from a normal mean value of 23.8 to 18.9 microgram %. Liver glutathione showed no significant variations. The changes obtained for the copper and glutathione contents in blood and liver were proportional to the period of exposure to ultra-violet rays. The blood copper was raised to 132 microgram when the experimental period was 2 minutes for 15 days, and it was 137 micragram % when the exposure was 2 minutes for 30 days, and it was 147 microgram % when the period of exposure was 5 minutes for 47 days. The results obtained for rats exposed to ultra-violet rays and taking the drug Ammoidin at the same time are shown in Table IV.

TABLE IV

U.V.R, for 5 min, daily for 30 days+300 mgm, Ammoidin/K/G/B/W.

Tissues	No. of rats. 1	2	3	4	5	6	Mean	NOTES
Blood Cu.	290	378	362	350	360	422	360	Deeper yellow tint
Blood GSH	20.2	27.2	29,3	21,2	28	29.2	19.2	colouration deve-
Liver Cu.	92	86	72	66	78	82	79	loped especially
Liver GSH	108	110	82	96	88	104	98	on the back.

From the data given in Table IV, it is clear that prolonged exposure to ultra-violet in combination with Ammoidin (8-Methoxypsoralen) administration have the same effect as giving Ammoidin alone but the changes are more marked. There is a dramatic rise in blood copper from a normal mean value of 131 to a mean value of 360 microgram % and this was associated with a decrease in the level of liver copper from the normal 115 microgram down to 79/100 gm. fresh tissue weight.

Regarding the effect of the combination of ultra-violet rays and Ammoidin administration on blood glutathione there was also a slight decrease in the level of blood glutathione from a normal mean value of 23.8 to 19.2 microgram %. Liver glutathione showed no marked changes.

The colour of albino rats attained a darker yellowish tint than in the previous experiments.

Discussion

As it has been reported exposure of skin to ultra-violet irradiation will result in pigmentation.

Various explanations have been suggested. The most reasonable is that the UVR decreases the sulfydryl groups of the skin by their oxidation and disulfide formation. As a result of this reaction the equilibrium between the sulfydryl compounds and the copper containing enzyme in the melanocytes and basal cell layer of the epidermis is disturbed and the enzyme being enabled to act freely on the substrate to form melanin pigmentation.

The findings in our experimentations showed that exposure of albino rats to UVR alone resulted in slight decrease in blood glutathione levels. This

(The results are graphically represented in Fig. 1 and Fig. 2).

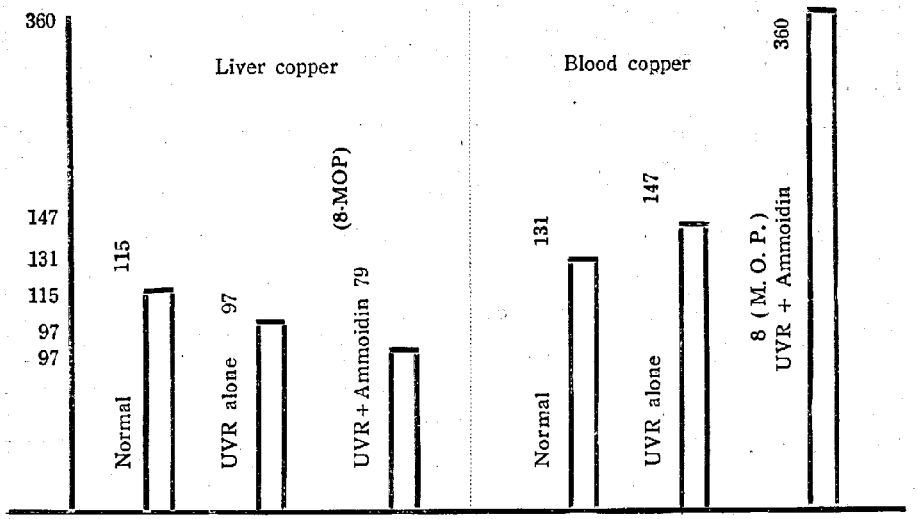

Fig. 1: Changes in blood and liver copper after exposure to ultraviolet alone and with 8-methoxypsoralen.

1 - Exposure to ultraviolet increases blood copper and associated with a decrease In liver copper content.

2 - The combined exposure to ultraviolet and administration of 8-methoxypsoralen made the changes more marked.

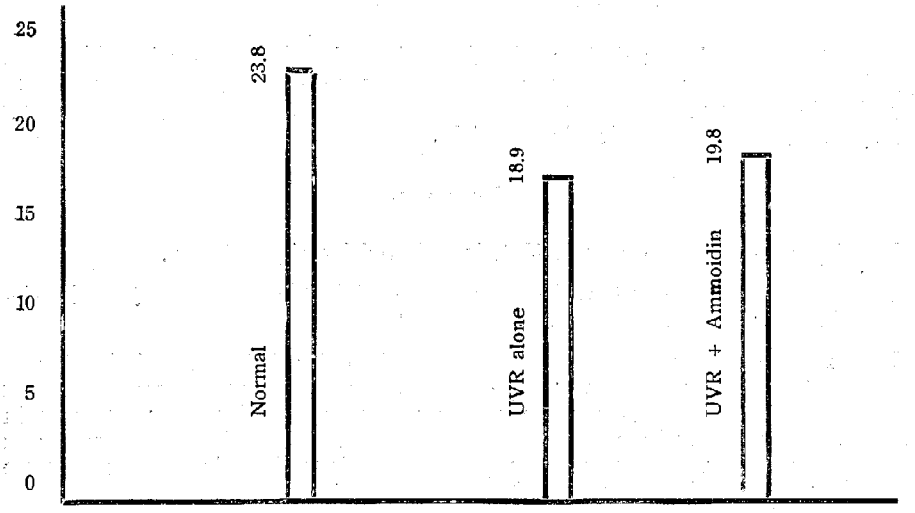

Fig. 2: Changes in blood glutathione after exposure to ultraviolet alone and together with administration of 8-methoxypsoralen.

- Exposure to ultraviolet caused lowering of blood glutathione.
- The combined exposure to ultraviolet and administration of 8-methoxypsoralen showed no significant variation.

was demonstrated in both the short and prolonged experiments. This decrease of blood glutathione was associated with increase in blood copper contents although slight, but high values of blood copper was obtained in the long span experiments. As regards liver copper there was also a slight decrease in its level.

The combination of UVR and Ammoidin was found to cause marked mobilization of copper from liver to blood up to 360 microgram % in blood and down to 79 microgram % in the liver.

As regards their effect on glutathione it is the same as UVR alone.

From these findings UVR could be considered as a hypercuprimic agent although it is a weak one. The combined effect of UVR and Ammodin on tissue glutathione and copper contents might throw light on its mode of action and therapeutic value in speeding up the process of repigmentation in vitiligo. Succinc dehydrogenase enzymatic activity of guinea-pig skin disappears in presence of psoralen or 8-methoxypsoralen. Succinic dehydrogenase activity in the skin depends on the presence of sulphhydryl groups[10]. This supports that psoralen and 8-methoxypsoralen decreases the —SH group of the skin.

From this brief summary we may conclude that UVR has a very definite synergestic action on psoralens. The phenomenon of the yellow colouration of the hairs of albino rats after exposure to ultraviolet light alone for more than 30 days, and together with 8-methoxypsoralen administration remains to be explained. It is known, that albino skin and white spotted skin, presumably without melanocytes catalyse soluble yellow pigment formation from amino acid tryptophane[11]. (This yellow colour is supposed to be not melanin but pheomelanin[12]. It is plausible to assume that ultra-violet and 8-methoxypsoralen help the formation of yellow colour from tryptophane.

This experiment dealing with formation of yellow colour in albino rats adds evidence that in absence of melanocytes, yellow colour can be formed under the effect of ultraviolet and 8-methoxypsoralen. Both stimulating agents may have synergestic action in this field.

Summary

1 Exposure of the skin to U.V.R. is followed by pigmentation.

2 Various suggestions have been given to explain the new formation of melanin after U.V.R. exposure.

3 Exposure of the albino rats to U.V.R. alone for different periods of time caused a slight increase in the blood copper from a normal mean value of 131 to 147 microgram percent. This was associated with a decrease in the liver copper from a normal mean value of 115 to 97 mgm/100 gm. fresh tissue weight. As regards the blood glutathione there is a slight decrease from a normal mean value of 23.8 to 18.9 microgram %.

Liver glutathione showed no significant variation.

4 Exposure of albino rats to U.V.R. in combination with 8-methoxypsoralen (Ammodin) administration caused a dramatic rise in blood copper from a normal mean value of 131 to a mean value of 360 microgram % and this

was associated with a decrease in the level of liver copper from a normal mean value of 115 down to 79 mgm/100 gm fresh tissue weight.

Regarding the combined effect of U.V.R. and 8-methoxypsoralen administration on blood glutathione there was a slight decrease in the level of blood glutathione from a normal mean value of 23.8 to 19.2 microgram %. Liver glutathione showed no marked change.

5 The colour of albino rats attained a darker yellowish tint in both experiments.

Acknowledgement

This work comprises steps in the series of investigations of the problem of the treatment of vitiligo by psoralen compounds. The crystalline extracts of the Egyptian herb Ammi Majus Linn originally started and supervised by Dr. Abdel Monem El-Mofty. The crystalline 8-methoxypsoralen is manufactured and kindly supplied by the Memphis Chemical Co., Cairo to whom we are grateful.

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