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Subproject under Effect of Total Body Irradiation on the Various Enzyme and Endocrine Systems. AMRL Project No. 6-59-08-10-(1)

MEDICAL RESEARCH AND DEVELOPMENT BOARD
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REPORT NO. 64

THE PROTECTIVE EFFECT OF PITRESSIN AND OF EPINEPHRINE AGAINST TOTAL BODY X-IRRADIATION*

by

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Army Medical Research Laboratory
Fort Knox, Kentucky
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RECOMMENDATIONS

The influence of starvation and of antibiotics on mortality from total body x-irradiation should be studied. It also should be determined whether tissue anoxia produced by various other agents will exert a general protective action against total body x-irradiation injury.

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THE PROTECTIVE EFFECT OF PITRESSIN AND OF EPINEPHRINE AGAINST TOTAL BODY X-IRRADIATION

I. INTRODUCTION

Pendergrass and his associates (1) have demonstrated a protective effect of pitressin and epinephrine against local x-irradiation injury of the leg of the rat. It was assumed that local tissue anoxia, produced by these principles, was involved in the protective mechanism. It became of interest, therefore, to investigate the possible effect of these agents on the aerobic and anaerobic metabolic cycles, as well as to study the effect of these agents on the survival rate after total body x-irradiation.

II. EXPERIMENTAL

"Labile phosphate" (ATP*) content and cytochrome oxidase activity of muscle of rats as influenced by pitressin and epinephrine administration or by limb occlusion. ATP has been implicated as the key component in the respiratory (aerobic) and the glycolytic (anaerobic) processes. Cytochrome oxidase requires gaseous oxygen for its action and is thought to be the last oxidizing enzyme to act in the chain of enzymes that bring about the oxidation of metabolites.

Phophate content and cytochrome oxidase activity determinations were done on gastrocnemius muscle samples from normal male, Sprague-Dawley rats weighing 220-300 grams. They were starved for 24 hours with water ad libitum, and were sacrificed 15 to 30 minutes following intraperitoneal injection of pitressin (Parke, Davis) or epinephrine (Abbott). Control animals received equivalent volumes of 0.9% saline.

Phosphate determinations on lyophilized tissue were carried out according to a modification of the method described by Umbreit (2). Cytochrome oxidase activity was determined according to the method of Schneider and Potter (3).

In the occluded limb experiments, the rats first were anesthetized with nembutal (45 mg/kg); a tourniquet consisting of 5 turns of gum rubber band (Eberhard Faber #30) was applied as high as possible around the right hind leg; and the teeth were clipped to prevent tourniquet removal or self-laceration. Control animals were anesthetized and the teeth were clipped. All animals were sacrificed without tourniquet release at 1/2 hour or 4-1/2 hours after tourniquet application. Gastrocnemius muscle samples were taken from the occluded limb and from the opposite hind limb of the tourniquet animal as well as from the hind limb of the control animal.

The effect of pitressin or epinephrine administration against total body x-irradiation.

Male, Sprague-Dawley rats weighing 220-300 grams were irradiated in pairs, one serving as a control, the other as a treated animal. Each pair

*Adenosinetriphosphate

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was exposed to total body x-irradiation for 22 minutes in a single exposure. Radiation factors were: 200 kv, 6 ma, 1/2 mm. Cu 1 mm Al filter, target distance approximately 29 cm., and 40 r/min. dosage rate measured in air.* Five units (0.25 cc.) of pitressin (Parke, Davis) or 0.2 mg (0.2 cc.) of epinephrine (Abbott) were injected intraperitoneally either 5, 20, or 40 minutes before or 5 minutes after exposure. Animals were housed in individual cages and weighed daily until death or termination after 28 days.

III. RESULTS

As can be seen from Table 1, following pitressin injection, the inorganic and "labile phosphate" (ATP) values for muscle tissue were elevated for the period of time observed, 15 and 30 minutes. On the other hand, epinephrine administration produced a lowering of the inorganic and "labile phosphate" (ATP) content of muscle tissue. Cytochrome oxidase activity of muscle tissue was found to be slightly but definitely decreased on pitressin administration and increased on epinephrine injection. The results on cytochrome oxidase activity will be published in detail in another report.

These findings are interpreted as indicating that administration of either pitressin or epinephrine produces a temporary local tissue anoxia. The mechanism producing the anoxia differs for the two substances.

Geiling and DeLawder (4) found that after the injection of pitressin the muscles pass for a short time into a state suggesting that their activity was being carried out under anaerobic conditions. The blood coming from the muscles was arteriol in color; it had a low CO_2 and a high O_2 content. The pitressin-induced tissue anoxia with the consequent disruption of the aerobic cycle leads to an increase in the rate of the glycolytic processes followed by increased formation of ATP.

It is known that an immediate but short-lived increase in the oxygen comsumption follows the administration of epinephrine. The mechanism of this action is not known. Epinephrine, by causing increased cell activity (oxygen consumption), brings about an increased utilization of ATP leading to a lowering of the "labile phosphate" content. The increase in the oxygen consumption, following epinephrine administration, may lead to a temporary tissue anoxia. In addition, the vasoconstrictive action of epinephrine decreases the tissue supply of oxygen.

To demonstrate further the effect of anoxia on muscle phosphate content and cytochrome oxidase activity, these constituents were determined in rat muscle tissue of tourniquet occluded limbs. In local x-irradiation studies, Pendergrass and his associates (1) found that the greatest increase of radiation threshold was obtained with tourniquet-occlusion of the exposed limb.

Table 2 illustrates that occlusion of the limb for 1/2 hour and for 4-1/2 hours produced a pronounced increase in the inorganic and "labile

*The authors wish to express their appreciation to the Radiobiology Branch of this laboratory for advice and assistance in the irradiation procedure.

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phophate" (ATP) content of the muscle tissue of the tourniquet occluded limb. Cytochrome oxidase activity was found to decrease, depending on the length of time the limb was occluded (see Table 3).

These results are similar to those obtained after administration of pitressin and substantiate the explanation given for the effect of pitressin.

As an extension of these findings, a study was made of the possible protective effect of pitressin and epinephrine against total body x-irradiation.

The effect of pitressin or epinephrine on mortality after total body x-irradiation is illustrated in Table 4. As would be expected with an 880 r dose in total body exposure, the majority of deaths occurred between 6 and 14 days. Pitressin, given 5 minutes before irradiation, afforded considerable protection with a survival rate of 86% as compared with 12% survival for the untreated irradiated group. Similarly, pitressin injected 20 minutes before exposure decreased the mortality significantly. With injection 40 minutes prior to irradiation the protection was diminished but still definite. The beneficial effect of pitressin pretreatment is further indicated by the observation that the surviving pitressin-treated irradiated animals lost less weight and recovered weight very rapidly compared with irradiated control animals.

Epinephrine, administered 5 minutes before irradiation, also seemed to give protection, but apparently not to the same degree as pitressin.

Pitressin, given 5 minutes after irradiation, would appear to give slight but definite protection.

Threshold studies, employing greater x-ray dosages, indicate that with pitressin injection 5 minutes before irradiation the threshold was raised from about 800 r to about 1100 r.

IV. DISCUSSION

The protective effect of pitressin and epinephrine against local (leg) and total body x-irradiation injury may be due to the tissue anoxia produced by these principles, leading to a reduction in the oxygen content of the tissue fluid.

It is generally assumed that many of the biological effects of x-radiation can be attributed to the action of the decomposition products of water which result from the irradiation. It is becoming increasingly evident that the degree of x-radiation effects is, to an important extent, dependent on the oxygen content in the cellular fluids.

Dowdy, Bennett and Chastain (5) exposed rats in very low oxygen tension (5%) and found that the acute effects of radiation are only half as severe. Remarkable decreases in many x-ray induced cellular phenomena by reduction of oxygen concentration during irradiation have been reported (6, 7).

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V. SUMMARY

The survival rate of rats, exposed to lethal x-ray dosage, was found to be significantly increased after pretreatment with pitressin or epinephrine.

The protective effect of these principles may be due to their property of producing a temporary tissue anoxia.

VI. RECOMMENDATIONS

The influence of starvation and of antibiotics on mortality from total body x-irradiation should be studied. It also should be determined whether tissue anoxia produced by various other agents will also exert a general protective action against total body x-irradiation injury.

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TABLE 1

EFFECT OF PITRESSIN AND EPINEPHRINE ON MUSCLE PHOSPHORUS

Treatment	Sacrificed Min.	No. of Rats	Phosphorus Average ± S. D.	
			Inorganic mg % Dry Wt.	Labile mg % Dry Wt.
Control - No treatment		17	41.3 ± 8.5	204.9 ± 25.5
Pitressin 5 Units I.P.	15	12	49.0 ± 8.9	248.7 ± 29.2
Control*	15	5	40.7 ± 11.3	218.9 ± 26.5
Pitressin 5 Units I.P.	30	12	51.7 ± 9.3	229.4 ± 27.9
Control*	30	6	45.8 ± 7.3	216.6 ± 34.0
Epinephrine 0.2 mg I.P.	15	12	32.7 ± 8.1	165.9 ± 27.5
Control*	15	6	43.4 ± 5.6	222.0 ± 20.5
Epinephrine 0.2 mg I.P.	30	12	34.5 ± 6.0	188.3 ± 47.0
Control*	30	6	41.4 ± 10.1	206.4 ± 21.1

*Controls received equivalent volume of 0.9% NaCl I.P.

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TABLE 2

EFFECT OF TOURNIQUET OCCLUSION ON MUSCLE PHOSPHORUS
AVERAGE VALUES ± S.D.

Experimental Conditions	No. of Rats	Inorganic P mg% Dry Wt.	Labile P mg% P Dry Wt.	Inorganic P / Labile P
Tourniquet on 1/2 hour Sacrificed without release				
Control - no tourniquet	6	39.2 ± 4.3	187.5 ± 18.2	0.21 ± 0.03
Control leg - tourniquet animal	10	49.5 ± 7.7	187.9 ± 18.5	0.27 ± 0.04
Occluded leg - tourniquet animal	10	77.5 ±11.9	259.1 ± 24.7	0.31 ± 0.07
Tourniquet on 4-1/2 hours. Sacrificed without release				
Control - no tourniquet	5	34.9 ± 1.3	183.4 ± 21.1	0.19 ± 0.03
Control leg - tourniquet animal	10	44.0 ± 9.0	188.9 ± 24.7	0.24 ± 0.05
Occluded leg - tourniquet animal	10	115.9 ±20.1	361.3 ± 37.8	0.32 ± 0.07

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TABLE 3

EFFECT OF TOURNIQUET OCCLUSION ON
MUSCLE CYTOCHROME OXIDASE
AVERAGE VALUES ± S.D.

Sacrificed Without Release of Tourniquet	No. of Determs.	Q_{O_2}
Tourniquet on 1/2 Hour		
Control - No Tourniquet	8	300 ± 58
Control Leg - Tourniquet Animal	8	359 ± 87
Occluded Leg - Tourniquet Animal	8	289 ± 66
Tourniquet on 4-1/2 Hours		
Control - No Tourniquet	6	276 ± 49
Control Leg - Tourniquet Animal	6	301 ± 74
Occluded Leg - Tourniquet Animal	6	173 ± 61

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TABLE 4

EFFECT OF PITRESSIN AND EPINEPHRINE ON SURVIVAL OF RATS EXPOSED TO X-RADIATION

Treatment	Time of Treatment Relative to X-radiation	Total Roentgens	No. of Rats	Survival							
				1st Week		2nd Week		3rd Week		4th Week	
				No.	%	No.	%	No.	%	No.	%
Pitressin 5 Units IP	5 min. before	880	42	39	93	36	86	36	86	36	86
Control			43	25	58	6	14	5	12	5	12
Pitressin 5 Units IP	5 min. before	1000	18	14	78	9	50	8	44	7	39
Control			18	2	11	0	0	0	0	0	0
Pitressin 5 Units IP	5 min. before	1120	19	10	53	6	32	5	26	5	26
Control			14	0	0	0	0	0	0	0	0
Pitressin 5 Units IP	20 min. before	880	31	29	94	22	71	20	65	19	61
Control			31	11	35	4	13	3	10	3	10
Pitressin 5 Units IP	40 min. before	880	26	23	88	15	58	12	46	12	46
Control			27	15	56	7	26	6	23	6	23
Pitressin 5 Units IP	5 min. before	880	29	24	83	12	41	11	38	11	38
Control			31	18	58	8	26	7	23	7	23
Epinephrine 0.2 mg IP	5 min. before	880	34	29	85	20	59	20	59	20	59
Control			34	19	56	8	24	8	24	8	24

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