or biological value. In fact, there appeared to be no influence of rate of infusion on nitrogen retention whether the amino acid mixture was given as a sole source of nitrogen or as a supplement to small or large protein intakes by mouth.

Summary. 1. Seven subjects (including normal individuals and patients) were given daily intravenous infusions of a 10% solution of amino acids at widely varying rates of infusion. 2. There was no correlation between the daily non-protein nitrogen excretion and the rate of infusion. Very rapid rates of infusion did not increase the nitrogen excretion and thus did not reduce the biological value of the amino acid solution.

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Protective Effect of Pitressin and of Epinephrine Against Total Body X-Irradiation. (19388)

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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 by the authors 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.

Methods. "Labile phosphate" (ATP*) content and cytochrome oxidase activity of muscle of rats as influenced by pitressin and epinephrine administration or by limb occlusion. Phosphate content and cytochrome oxidase activity determinations were done on gastrocnemius muscle samples from normal male Sprague-Dawley rats weighing 220-300 g. The rats were starved for 24 hours with water *ad libitum*, and were sacrificed 15 or 30 minutes following intraperitoneal injection of pitressin (Parke, Davis) or epinephrine (Abbott). Control animals received equivalent volumes of 0.9% saline or no treatment prior to sacrifice. Treated and untreated controls showed no significant difference and were grouped in the calculations. Phosphate determinations on lyophilized tissue were carried out according to 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 were anesthetized with sodium pentobarbital (45 mg/kg) and a tourniquet consisting of 5 turns of a rubber band (Eberhard Faber No. 30) was applied to the right hind leg as high as possible. 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 ½ hour or 4½ hours after tourniquet application. Gastrocnemius muscle samples were taken from the occluded limb as well as from the opposite hind limb of the tourniquet animal and from a 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 g were irradiated in pairs,

* Adenosinetriphosphate.

TABLE I. Effect of Pitressin and Epinephrine on Muscle Phosphorus.

Treatment	Sacrificed, min	No. of rats	Phosphorus, avg ± S.D. Inorganic, mg % dry wt	Phosphorus, avg ± S.D. Labile, mg % dry wt
Control		40	42.2 ± 8.2	211.2 ± 24.9
Pitressin 5 units IP	15	12	49 ± 8.9 p=.1	248.7 ± 29.2 p<.05
	30	12	51.7 ± 9.3 p<.05	229.4 ± 27.9 p<.2
Epinephrine .2 mg IP	15	12	32.7 ± 8.1 p<.05	165.9 ± 27.5 p<.05
	30	12	34.5 ± 6 p<.05	188.3 ± 47 p=.2

p<.1 considered statistically significant.

TABLE II. Effect of Tourniquet Occlusion on Muscle Phosphorus. Average values ± S.D.

Exp. conditions	No. of rats	Inorganic P, mg % dry wt	Labile P, mg % P dry wt	Inorganic P / Labile P
Tourniquet on ½ hr—sacrificed without release				
Control—no tourniquet	6	39.2 ± 4.3	187.5 ± 18.2	.21 ± .03
" leg-tourniquet animal	10	49.5 ± 7.7	187.9 ± 18.5	.27 ± .04
Occluded leg- " "	10	77.5 ± 11.9	259.1 ± 24.7	.31 ± .07
Tourniquet on 4½ hr—sacrificed without release				
Control—no tourniquet	5	34.9 ± 1.3	183.4 ± 21.1	.19 ± .03
" leg-tourniquet animal	10	44 ± 9	188.9 ± 24.7	.24 ± .05
Occluded leg- " "	10	115.9 ± 20.1	361.3 ± 37.8	.32 ± .07

one serving as a control, the other as a treated animal. Each pair was exposed to total body x-irradiation for 22 minutes in a single exposure. Radiation factors were: 200 kv, 6 ma, ½ mm Cu 1 mm Al filter, target distance approximately 29 cm, and 40 r/min. dosage rate measured in air.† Five units (0.25 ml) of pitressin or 0.2 mg (0.2 ml) of epinephrine 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.

Results. As can be seen from Table I, following pitressin injection the inorganic and "labile phosphate" (ATP) values for muscle tissue were elevated at 15 minutes. At 30 minutes the "labile phosphate" was slightly elevated but the difference from the control was no longer significant. On the other hand, epinephrine administration produced at 15 minutes a lowering of the inorganic and "labile phosphate" (ATP) content of muscle tissue. Again the "labile phosphate" difference at 30 minutes was no longer significant. Preliminary investigations have indicated that

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

TABLE III. Effect of Tourniquet Occlusion on Muscle Cytochrome Oxidase. Average values±S.D. Sacrificed without release of tourniquet.

	No. of determinations	Qo_2
Tourniquet on ½ hr		
Control—no tourniquet	8	300 ± 58
" leg-tourniquet animal	8	359 ± 87
Occluded leg- " "	8	289 ± 66
Tourniquet on 4½ hr		
Control—no tourniquet	6	276 ± 49
" leg-tourniquet animal	6	301 ± 74
Occluded leg- " "	6	173 ± 61

cytochrome oxidase activity of muscle tissue was slightly but definitely decreased following pitressin administration and increased following epinephrine injection.

To demonstrate the possible 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 II illustrates that occlusion of the limb for ½ hour and for 4½ hours produced a pronounced increase in the inorganic and

TABLE IV. Effect of Pitressin and Epinephrine on Survival of Rats Exposed to X-Radiation.

Treatment	Time of treatment relative to x-radiation, min	Total roentgens	No. of rats	Survival							
				1st wk		2nd wk		3rd wk		4th wk	
				No.	%	No.	%	No.	%	No.	%
P,* 5 units IP	5 before	880	42	39	93	36	86	36	86	36	86
C*			43	25	58	6	14	5	12	5	12
P, 5 units IP	"	1000	18	14	78	9	50	8	44	7	39
C			18	2	11	0	0	0	0	0	0
P, 5 units IP	"	1120	19	10	53	6	32	5	26	5	26
C			14	0	0	0	0	0	0	0	0
P, 5 units IP	20	880	31	29	94	22	71	20	65	19	61
C			31	11	35	4	13	3	10	3	10
P, 5 units IP	40	880	26	23	88	15	58	12	46	12	46
C			27	15	56	7	26	6	23	6	23
P, 5 units IP	5 after	880	29	24	83	12	41	11	38	11	38
C			31	18	58	8	26	7	23	7	23
E,* .2 mg IP	5 before	880	34	29	85	20	59	20	59	20	59
C			34	19	56	8	24	8	24	8	24

* P = Pitressin, E = Epinephrine, C = Control.

"labile phosphate" (ATP) content of the muscle tissue of the tourniquet-occluded limb. Cytochrome oxidase activity was found to decrease gradually, depending on the length of time the limb was occluded (Table III). After ½ hour occlusion, no significant change was observed. However, with 4½ hours occlusion, the occluded limb showed a significant decrease in cytochrome oxidase activity.

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 IV. 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 was 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 only very slight, if any, protection. *Threshold studies,* employing greater x-ray dosages, indicate that with pitressin injection 5 minutes before irradiation the threshold could be raised from about 800 r to about 1100 r.

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.

The phosphorus and cytochrome oxidase activity results 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 arterial 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 glycolytic processes followed by increased formation of ATP. The results obtained with tourniquet-occluded

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

It is known that an immediate but short-lived increase in the oxygen consumption 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 and the vasoconstrictive action of epinephrine, decreasing the tissue supply of oxygen, may lead to a temporary tissue anoxia.

It is generally assumed that many of the biological effects of x-irradiation can be attributed to the action of the decomposition products of water which result from the irradiation. It is also 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).

Summary. 1. The survival rate of rats, exposed to lethal x-ray dosage, was found to be significantly increased after pretreatment with pitressin or epinephrine. 2. The protective effect of these principles may be due to their property of producing a temporary tissue anoxia.

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Comparative Antigenic Potency of Killed Irradiated Tubercle Bacilli and Living BCG Vaccine. (19389)

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Criticism is raised against artificial immunization of man with living BCG vaccine on the assumption that the organisms may survive for years and gradually regain virulence(1). The U. S. Public Health Service(2) and the American Trudeau Society(3) recommend further research to develop a tuberculosis vaccine containing only dead organisms. It is well known from the experimental and clinical studies(4) that such vaccines hitherto have yielded less effective protection against a challenge virulent infection than living BCG vaccine. The question has arisen whether or not the prolonged heating required to kill tubercle bacilli vitiates against their antigenic potency. In order to eliminate this factor, vaccine has recently been prepared by killing virulent human tubercle bacilli by brief ultraviolet irradiation(5-7). Such vaccines are reported to be equal or superior to living BCG vaccine on the basis of guinea pig and mouse protection tests. The advantages of a killed tuberculosis vaccine are twofold since it would

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