BIOCHEMICAL ALTERATIONS IN THE BLOOD OF IRRADIATED RATS

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ABSTRACT

It is known that biochemical alterations come before the morfological changes, in organisms exposed to radiation. We also know that it is very difficult to study the biochemical changes for, some hours after the irradiation, the effects due to exposure are indistinctive from secondary alterations.

The authors study the changes of Sodium, Chlorine and Iron in the total blood and the modificatiams of the serum proteins observed in rats subjected to acute exposition of 300 r. The determinations done, suggest the existence of a crisis, around 4 hours after the irradiation. It is recognized that the results have only a preliminary character and the irradiation problems of small animals irradiated on the Reactor's holes, is studied.

INTRODUCTION

Biochemical alterations are observed in animals submitted to whole body irradiation. This study is complicated by the neuro-endocrinous reactions which constitute the adaptation syndrome.

To have an idea of the relative importance of the great number of observations that have been published on this subject, it is necessary to remember that we pretend to study the ionizing radiation effects and that they are attached to the alterations observed during the first hours that follow exposure. We know that biochemical alterations come before the appearance of lesions anato-

mically visible and that when the dead cells accumulate on the lymphoid tissue (thymus, spleen, lymphatic ganglion, etc.) - which occurs two hours after irradiation - it is of small use to make any biochemical determination. On the other hand, the biochemical investigation made right after irradiation, still finds the animal in good conditions, i.e. not yet suffering from the shock, infection or consequences of the subsequent bad nutrition.

This work presents results of observations made in Mc Collen rats which were not irradiated (reference) and rats (of the same age, males and pubescent) exposed (whole body irradiation) to gamma radiation at the IEAR-1, twenty four hours after the shut down. The exposure doses were of 300r and the exposition acute (1 minute). The blood was taken from the inferior vein cave.

The serum proteins were determined by paper electrophoresis following the technique described by L.P. Ribeiro et all. and which consists in spreading the material to be analysed over filter paper using as electrolyte a solution of barbital of pH 8,6 and ionic strength 0.06.

The sodium was determined by the method of Albanese and Coll using the Coleman Jr. espectrofotometer. Chlorine was determined by the turbidimeter method described in Fisher's handbook, even though we recognize its deficiency; it was chosen for being a micromethod that may be carried out in our laboratories. The Iron was determined by Wong's method and the results compared with the hemoglobin determinations.

RESULTS

Serum Proteins

We find four proteic fractions in rats serum. This observation agrees with some authors but is contrary to the opinion of others that describe five and which would be the same as the human serum. The presence of four fractions is attributed to the non-separation of globulin alfa 1 and alfa 2. The reading of the dyed strips were made with the Photovolt densitometer and the areas corresponding to different proteic fractions were determined with a

planimeter, its absolute values were calculated from biochemical assays. In Table I we present the absolute quantities in g/100ml and the percentages of the proteic fraction in the serum of non irradiated rats (reference).

TABLE I

	Absolute Values g/100ml	Percentage Values %
Total Proteins	5.85	100
Albumins	2.66	45.47
Globulins (total)	3.19	54.53
alfa	1.54	26.33
beta	0.54	9.23
gamma	1.11	18.97

On the rats exposed to radiation, determinations were made with the blood taken at different times after the irradiation and beginning from 1h. and 30 min. In all cases we observed the increase of total proteins, due basically to the increase of globulin. It could be verified that the increase of globulins is not uniform: alfa increases less than beta and gamma, the increase of beta globulin is the more distinct. On the other hand there is an apparent increase of albumins. In Table II we present the medium results obtained in blood taken four hours after irradiation. We chose this data as they are the ones that present themselves farthest from normal values.

TABLE II

Average results obtained from the serum of rats 4h. after irradiation

	g/100ml	%
Total proteins	7.49	100
Albumins	2.54	33.91
Globulins (Total)	4.95	66.09
alfa	1.54	20.43
beta	2.11	28.46
gamma	1.30	17.20

The variations in relation to the data obtained in the group of non irradiated rats are presented in Table III.

TABLE III

	Tot.Proteins	Albumin	Globulin	alfa	beta	gamma
Absolute increase	1.67	0.44	1.22	0.07	0.73	0.43
Relative increase	100	26.35	73.05	4.19	43.71	25.75

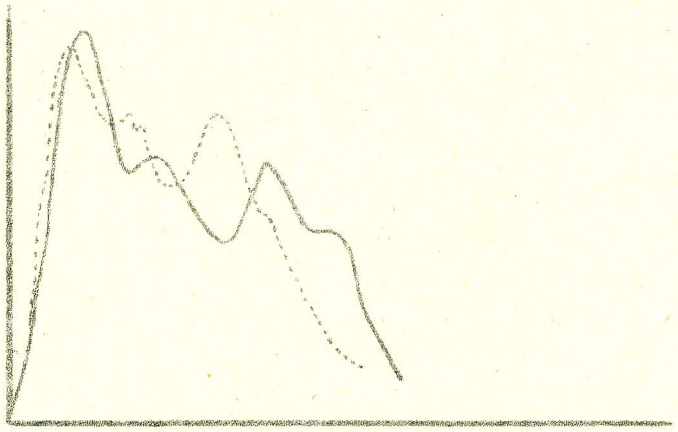
RELATIVE MOBILITY

Beside the proteinic alterations, a variation of the relative mobility was observed. The variation was progressive, reaching a higher significance around 4 hours after the irradiation. It was observed that when the alfa globulin has its mobility increased, the mobility of beta globulin decreases. As a consequence alfa globulin is nearer to albumin and may be confused with the "tail" of the albumin. In Table IV we indicate values found for the mobilities, and the time it took to gather the material, after the irradiation.

TABLE IV

General Table of the Relative Mobilities

	Albumin - 100 % γ globulin - 0%	
Hours or days	α globulin (%)	β globulin (%)
Normal	65 - 75	20 - 30
1h 30min.	63	29
2h	64	21
2h 30min.	70	25
3h	70	25
4h	69	21
4h 30min.	69	20
5h	67	27
6h	69	23
12h	67	22
1 day	63	23
2 days	63	25
4 days	73	25
5 days	67	24
6 days	59	19
7 days	61	22
15 days	65	30
21 days	64	21
22 days	67	27



——— normal
- - - - - crisis after 4 hours

RECOVERY

After the fifth day, the proteinemia tends to return to normal conditions and what is established around the 30th day.

SODIUM

In Table V are the results found for Sodium on the blood of 62 animals exposed to 300r (sharp exposition during 1 minute).

TABLE V

Normal: 121.56 mEq/l

1h.30	158.44 +
2h.30	109.40
3h.0	165.28
3h.30	138.44
4h.0	173.14
4h.30	104.17 +
5h.0	129.79
6h.0	116.13
12h.0	196.20
1 day	151.83
2 days	114.87
4 days	158.85
5 days	150.43
7 days	174.49
12 days	173.91
13 days	138.96
15 days	268.80
21 days	200.15
22 days	251.74
28 days	268.16
30 days	201.81
32 days	167.27

CHLORINE

The obtained results for chlorine are schematized in Table VI

TABLE VI

Normal: 171.21 mEq/l

		mEq/l
1h.30	91.28	183.65
2h.30	92.39	185.90
3h.0	101.79	204.81
3h.30	101.95	205.13
4h.0	102.31	205.84
4h.30	90.93	182.96
5h.0	91.35	183.80
6h.0	103.17	207.59 +
12h.0	119.21	239.85

	m.mol/l	mEq/l
1 day	102.03	205.29
2 days	124.08	249.65
4 days	143.21	288.14
5 days	133.74	269.10
7 days	109.12	219.56
12 days	126.62	254.76
13 days	136.83	275.32
15 days	131.63	264.85
21 days	178.58	359.30 +
22 days	112.01	225.38
28 days	250.99	505.01 +
30 days	104.14	209.53
32 days	322.17	648.23 +

IRON

In table VII we present the results obtained for the total Iron:

TABLE VII

Normal: 42.2 -44.4 mg%

1h.30	41.1 mg%
2h.30	41.2 "
3h.0	42.3 "
3h.30	40.9 "
4h.0	45.3 "
4h.30	42.0 "
5h.0	42.4 "
6h.0	42.7 "
12h.0	42.4 "
1 day	45.2 mg%
2 days	42.7 "
4 days	38.8 "
5 days	43.4 "
6 days	38.6 "
7 days	43.1 "
13 days	40.6 "
15 days	46.9 "
21 days	43.7 "
22 days	34.0 "
28 days	48.8 "
30 days	48.0 "
32 days	41.6 "

CONSIDERATIONS ON THE SODIUM, CHLORINE AND IRON DATA

The examination of the data will permit us to observe that around 4 hours after the irradiation, there is somewhat of a crisis in biochemical composition of the blood. The quantitative alterations are observed from the first hour after irradiation, with a certain chronological rhythm. Around the 5th and 6th day the values of sodium and Iron , and less clearly the ones of Chlorine, are apparently normal. Consequently they vary again to approximate the normal around the 30th day.

CONCLUSIONS

Our observations have still a preliminary character and consequently it is not possible to draw definite conclusions. On the other hand, the measurements were made mostly from materials gathered some time after the exposure and do not represent the results of the radiations themselves, but does express a superposition of the results with the adaptation syndrome. The decrease of the albumin serum occurs from the capillary permeability alterations favouring the passage of the albumin to the intersticial fluid. The tissue retention of the albumin would consist of a mean to reduce the unchained reactions having a direct influence on the changes of ions. The increase of globulin would take care of the transportation of other substances, such as Iron, Lipoproteins, etc. which come from the cellular destruction. Possibly the low mobility of the globulins is associated to the increase of the Lipoproteins. The increase of the gamma globulin is in charge of the destructive lesions by superposition with beta globulin of lower mobility.

The fall of Sodium and Chlorine observed during the first three hours may be interpreted as depending on the establishment of the adaptation syndrome and on capillary permeability alterations that may eventually occur from the action of the radiations. The alterations of the Total Iron are such as to suggest new studies, in which other parameters are determined, as for instance the formation of Metahemoglobin, porfiniric groups, and so on.

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PROTEINS FROM IRRADIATED RATS. CONNECTION ALBUMIN-GLOBULOUS (A/G)

Hours or Days	Total Proteins g/100 ml	Albumin(A) g/100 ml	α-Globulin g/100 ml	β-Globulin g/100 ml	γ Globulin g/100 ml	$\beta+\gamma$ Globulin g/100 ml	Globulin (G) g/100 ml	A/G	Serie
	5.85	2.10	1.47	1.38	0.87		3.73	0.56	Norm.
	4.50-6.50	1.50-2.50	1.00-2.00	0.80-2.00	0.50-1.20		2.50-4.50		
1.30	4.79	1.80	0.88	1.06	0.97		2.82	0.64	B
	8.14	2.33	1.54	2.91	1.45		5.86	0.40	B'
	8.49	2.37	1.28	3.44	1.32		6.03	0.39	C
	6.82	1.85	1.19	2.38	1.23		4.89	0.38	C'
2,00	8.49	3.39	1.50	1.81	1.45		5.24	0.65	D
2.30	5.98	2.11	1.36	1.24	1.10		3.79	0.56	B
	7.35	2.50	1.45	1.98	1.27		4.80	0.52	B'
	8.14	3.03	1.19	2.69	1.10		5.06	0.60	C
	9.06	3.56	1.14	2.47	1.71		5.42	0.66	C'
3.00	7.13	2.59	1.23	1.94	1.23		4.49	0.58	D
	10.07	2.90	2.20			5.06	7.18	0.40	E
	7.79	2.68	1.85			3.17	5.02	0.53	F'
	6.64	1.89	1.85	1.59	1.32		4.84	0.39	F''
3,30	9.10	4.00	1.36			3.74	5.02	0.80	D
	6.63	2.28	1.45	1.63	1.27		4.45	0.51	E
	10.91	3.34	1.54			6.03	7.66	0.44	E'
4.00	10.07	3.03	1.89	3.44	1.58		7.00	0.43	B
	8.18	3.87	1.54	1.59	1.19		4.40	0.88	C
	10.64	3.12	2.55	3.22	1.80		7.62	0.41	C'
	7.13	2.20	1.76	1.68	1.32		4.84	0.46	C_1
	7.70	2.46	1.67	1.98	1.41		5.15	0.48	C'_1
	7.57	3.43	0.92	2.16	0.97		4.14	0.83	B
	7.92	2.15	2.25	1.20	1.23		5.72	0.38	E

	7.61	2.55	1.23			3.74	5.06	0.50	F
	6.38	2.15	1.19			2.86	4.14	0.52	G'
	7.43	2.24	1.10			3.92	5.11	0.44	G''
	5.54	1.80	1.10	1.63	0.79		3.66	0.49	K
	7.14	2.33	1.58	1.72	1.41		4.80	0.49	K'
	7.04	2.06	1.67	1.72	1.45		4.89	0.42	K''
	7.35	2.46	1.67	1.90	1.14		4.80	0.51	312
	6.99	2.42	1.63			2.91	4.62	0.52	311
	5.63	2.37	0.88			2.47	3.30	0.72	313
4.30	7.79	2.37	1.94	2.65	0.70		5.37	0.44	D
5.00	11.66	3.65	1.23	5.03	1.67		8.01	0.46	B
	11.00	4.04	1.98			4.93	7.00	0.58	B'
	6.95	2.02	1.63	1.76	1.44		4.98	0.41	B''
	9.85	3.73	1.76	3.17	1.27		6.17	0.61	C
	6.25	1.80	1.58	1.63	1.06		4.36	0.41	C'
6.00	10.38	3.43	1.98	3.17	1.89		7.05	0.49	B
	7.61	2.02	1.10	2.82	1.49		5.50	0.37	C
	7.87	2.64	2.16	1.54	1.45		5.24	0.50	D
12.00	5.89	1.76	1.41			2.69	4.10	0.43	J
	6.07	2.33	1.54	1.50	0.66		3.74	0.62	J'
1 day	8.88	2.81	2.11	2.03	1.67		6.08	0.46	F
	5.59	1.67	1.45			2.29	3.83	0.44	F'
	6.11	1.58	1.50	2.07	1.06		4.58	0,35	G
	5.59	1.76	1.36	1.45	0.97		3.92	0,45	G''
2 days	5.76	1.63	1.10	1.41	1.63		4.18	0.39	F
	9.45	4.04	1.32	2.47	1.63		5.46	0.74	F'
	6.16	2.24	1.36	2.03	0.70		4.01	0.56	F''
	6.03	2.24	1.32	1.01	1.27		3.70	0.61	J

	7.48	2.50	1.85	2.03	0.97		4.93	0.51	J_1
4 days	8.67	2.94	1.72	1.81	2.11		5.68	0.52	E
5 "	8.84	2.90	2.51	2.69	0.88		5.99	0.48	D
6 "	7.79	3.60	0.97	1.81	1.41		4.27	0.85	B
7 "	7.13	2.15	1.63	1.46	1.80		4.93	0.43	F
	5.89	1.67	1.45	1.59	1.10		4.14	0.40	J
15 "	6.60	2.55	1.54	1.46	0.92		3.96	0.64	D
21 "	6.60	1.98	1.28	1.59	1.80		4.67	0.42	C
	6.77	1.89	1.89	1.54	1.27		4.80	0.39	E
	6.82	2.15	1.41			2.95	4.76	0.45	G
22 "	5.98	1.14	1.72	1.98	0.97		4.76	0.25	D
30 "	10.29	4.04	1.36			4.84	6.25	0.84	C
	5.54	1.71	1.80			2.20	3.92	0.44	C'
	6.42	2.72	1.01			2.64	3.74	0.73	D