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EFFECTS OF SOME RADIOPROTECTIVE SUBSTANCES UPON PRE-NATAL SURVIVAL OF OFFSPRING TO ROENTGEN IRRADIATED MALE MICE

by

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Since KAPLAN & LYON (1953) published their report on experiments in which mercaptoethylamine was used in an attempt to protect the hereditary material against ionizing radiation, an increasing number of experiments with a similar goal have been reported. The greater number of these later works, have, however, not primarily been intended as studies of the purely genetic consequences of irradiation but have rather been concerned with clarifying the ability of different substances to prevent radiation-induced changes in the individuals' reproductive mechanism. Thus, RUGH & WOLFF (1957) showed that the sterility effects following irradiation of ovaries could be diminished with the help of chemical agents, and two years later WANG, KUSKIN & RUGH (1959) reported that cysteamine had similar effects on males. MANDL, in two experiments from 1959, found that degeneration of gametes following irradiation was partly prevented by prophylactic treatment with mercaptoethylamine. Histologic studies showed that radiation damage to female gametes appeared in lower frequencies following a similar pretreatment.

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Table 1

Irradiated spermatozoa protected with cysteamine (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Cysteamine					
A	0 R	60	146	867	113	6	87.93
B	300 R	60	139	679	201	4	77.27
C	600 R	60	127	487	259	2	65.11
		NaCl					
A	0 R	60	140	848	108	4	88.33
B	300 R	60	140	650	236	3	73.12
C	600 R	60	121	387	273	0	58.64

Table 2

Irradiated spermatids protected with cysteamine (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Cysteamine					
A	0 R	60	148	974	80	4	90.17
B	150 R	60	128	661	157	2	80.61
C	300 R	60	121	434	213	5	60.56
D	600 R	60	44	79	76	0	50.97
		NaCl					
A	0 R	60	137	880	83	2	91.19
B	150 R	60	108	510	154	6	76.12
C	300 R	60	87	271	190	1	58.66
D	600 R	60	28	41	57	0	41.83

The positive effects of radioprotective substances in a genetical test were first shown in 1961 by LÜNING, FRÖLÉN & NELSON. There cysteamine reduced the mutation rate in spermatozoa by an estimated 25 %. Since then, similar experiments have been reported by LEONARD & MAISIN (1963, 1964) and

Table 3

Irradiated spermatozoa protected by cysteamine (albino)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Cysteamine					
1	0 R	75	157	974	57	2	94.29
2	200 R	75	140	755	151	5	82.88
3	400 R	75	145	650	231	9	73.03
		NaCl					
1	0 R	75	145	854	58	0	93.64
2	200 R	75	160	817	187	5	80.97
3	400 R	75	118	475	211	0	69.24

EHLING (1964). The experiments now to be reported have been in progress since 1962 and may be considered as complementary tests of genetic effects of radioprotective substances known to be somatically functional.

Material and Methods. In all but one experiment, highly inbred CBA males have been used. The exceptional experiment was made with males from a non-inbred Albino strain to test the protective effect of cysteamine on the genetical material of males more sensitive to irradiation than CBA males (FRÖLÉN 1965).

The males were irradiated when about 70 days old. There was always a control group of the same size as the test groups given various agents. In the control, the males were given saline solution. All injections were given intraperitoneally 15 min before irradiation, 4 mg of the substance (cysteamine, cystamine, AET, glutathion or serotonine) in 0.4 ml saline. Each injected group was subdivided into three parts which were given different amounts of irradiation. When not otherwise stated the dose levels were: 0 R, 300 R and 600 R, given as whole body irradiation. The roentgen equipments was a Müller MG 300 apparatus operated at 160 kV and 10 mA with filters 0.5 mm Cu + 4 mm Al. The dose rate was 84 R/min. The distance between tube and object was 40 cm.

The genetic effects, determined as the rate of intrauterine death of offspring to the irradiated males, was studied in matings within the first week (spermatozoa) and the third week (spermatids), respectively.

Table 4

Irradiated spermatozoa protected by AET (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		AET					
A	0 R	60	124	781	67	7	91.35
C	300 R	60	107	555	158	4	77.41
D	600 R	60	92	316	199	3	61.00
		NaCl					
A	0 R	60	127	786	83	10	89.42
C	300 R	60	107	516	175	4	74.24
D	600 R	60	100	344	214	1	61.54

Each male was mated to 3 females from the CBA strain. In tests of effects on spermatozoa the males were mated immediately after irradiation while in spermatid tests, they were withheld from matings for 14 days after the irradiation.

The females' uterine contents were analysed on the 17th or 18th day after start of mating. The number of living fetuses, dead embryos and placental resorptions (moles) was recorded for each female. From these data the rate of intra-uterine death was calculated. The corpora lutea were not counted.

Results

The results are presented in Tables 1—11. Table 1 refers to an earlier report of LÜNING et coll. (1961).

The mutation-reducing effects of the substances are reflected by the frequencies of living embryos on the 18th day of pregnancy. This parameter, the survival frequency, has been computed as the percent living of total implantations, and is presented in the right hand columns of the tables. These figures represent those individuals whose paternal genome had escaped lethal damages of irradiation and control conditions. The material does not give any information as to what extent the chances of survival of very young non-implanted zygotes have been influenced by the substances, since an analysis of the number of eggs released by the female has not been made. There was, however, a dose-dependent decrease in implantation rate.

Table 5

Irradiated spermatozoa protected with cystamine (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Cysteamine					
A	0 R	60	150	839	116	10	86.94
C	300 R	60	134	517	193	0	72.82
D	600 R	60	137	401	269	1	59.76
		NaCl					
A	0 R	60	151	851	104	6	88.55
C	300 R	60	150	599	239	6	70.97
D	600 R	60	103	314	182	3	62.92

Table 6

Irradiated spermatozoa, comparison between cysteamine and AET (CBA)

Ser.	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Surv. freq. %
		NaCl					
A	0 R	42	102	603	53	3	91.23
C	300 R	42	79	353	115	3	74.95
D	600 R	42	92	296	218	3	55.12
		AET					
A	0 R	42	95	550	66	7	88.29
C	300 R	42	105	474	138	4	76.94
D	600 R	42	90	265	194	4	57.24
		Cysteamine					
A	0 R	42	99	620	44	4	86.83
C	300 R	42	91	415	146	1	73.84
D	600 R	42	92	318	172	4	64.37

Table 7

Irradiated spermatozoa, comparison between cysteamine and AET — same males as in table 6

		NaCl						Cysteamine					
Ser.	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Surv. freq. %	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Surv. freq. %
A	0 R	42	107	664	58	2	96.71	42	100	621	52	2	90.80
C	300 R	42	72	175	163	1	51.62	42	81	223	169	3	56.46
D	600 R	42	15	21	26	0	44.68	42	33	59	53	0	52.68
		AET											
A	0 R	42	103	667	48	5	92.64						
C	300 R	42	84	221	169	5	55.95						
D	600 R	42	38	53	71	0	42.74						

Table 8

Irradiated spermatozoa protected by glutathion (CBA)

		Glutathion					
Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
A	0 R	60	105	680	51	2	92.77
B	300 R	60	119	558	174	5	75.72
C	600 R	60	118	369	285	4	65.08
		NaCl					
A	0 R	60	120	741	64	4	91.60
B	300 R	60	125	620	142	5	80.84
C	600 R	60	131	438	268	2	61.87

Table 9

Irradiated spermatozoa protected by serotonine (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Serotonine					
A	0 R	40	88	600	42	13	91.60
B	300 R	40	93	450	140	5	75.63
C	600 R	40	85	233	216	4	51.43
		NaCl					
A	0 R	40	83	543	66	4	88.58
B	300 R	40	90	465	130	5	77.50
C	600 R	40	78	270	193	3	57.94

Table 10

Irradiated spermatids protected by serotonine (CBA)

Series	Dose	Irrad. ♂♂	Pregnant ♀♀	Foetuses	Moles	Late deaths	Survival frequency %
		Serotonine					
A	0 R	40	94	653	54	8	91.33
B	300 R	40	66	224	122	0	64.74
C	600 R	40	36	48	56	0	46.15
		NaCl					
A	0 R	40	94	652	63	8	90.18
B	300 R	40	81	196	191	4	50.13
C	600 R	40	30	46	46	3	48.42

Table 11

M-value for the different substances $1-\frac{m_1-m}{m_3-m_4}=M$

Strain	Data from table	Dose	Substance	Gamete stage	m_1	m_2	m_3	m_4	M
CBA	1	300	Cysteamine	Spermatozoa	0.258	0.129	0.313	0.123	0.321
	1	600			0.429	0.129	0.534	0.123	0.270
	2	150		Spermatids	0.216	0.104	0.271	0.092	0.374
		300			0.407	0.104	0.533	0.092	0.213
		600			0.674	0.104	0.871	0.092	0.268
ALB	3	200		Spermatozoa	0.118	0.060	0.211	0.066	0.117
		400			0.314	0.060	0.368	0.066	0.159
CBA	4	300	AET		0.256	0.090	0.298	0.112	0.108
		600			0.494	0.090	0.485	0.112	−0.083
	5	300	Cystamine		0.317	0.139	0.343	0.120	0.202
		600			0.515	0.139	0.463	0.120	−0.096
	6	300	Cysteamine		0.303	0.141	0.287	0.091	0.173
		600			0.440	0.141	0.595	0.091	0.407
		300	AET		0.262	0.124	0.287	0.091	0.296
		600			0.558	0.124	0.595	0.091	0.106
	7	300	Cysteamine	Spermatids	0.572	0.096	0.661	0.086	0.172
		600			0.641	0.096	0.806	0.086	0.243
		300	AET		0.571	0.076	0.661	0.086	0.139
		600			0.850	0.076	0.806	0.086	−0.075
	8	300	Glutathion	Spermatozoa	0.278	0.075	0.213	0.088	−0.624
		600			0.430	0.075	0.480	0.088	0.094
	9	300	Serotonine		0.279	0.088	0.254	0.121	−0.436
		600			0.664	0.088	0.545	0.121	−0.358
	10	300		Spermatids	0.434	0.090	0.693	0.103	0.417
		600			0.773	0.090	0.725	0.103	−0.098

$m_1 = m_{x\text{subst}}$ $m_3 = m_{x\text{NaCl}}$

$m_2 = m_{0\text{subst}}$ $m_4 = m_{0\text{NaCl}}$

Earlier studies (FRÖLÉN 1965) showed the spontaneous intrauterine death rate for our CBA-strain to be about 10 %. This has been reconfirmed by the present investigation. As is evident from a comparison of the survival frequencies in the control groups, and the corresponding frequencies for treated animals in the non-irradiated series, neither cysteamine, cystamine, AET, glutathion nor serotonine per se have had detrimental effects on embryonic survival. For all the non-irradiated groups it is approximately 90 %. Parallel

comparisons of survival frequencies within each experiment show, as expected, a sharply reduced embryo survival at higher radiation dosages.

The reduction of the survival frequency is of the same magnitude in the treated groups as in the control groups within each series in all cases except that comprising tests of cysteamine (Tables 1, 2, 3, 5 and 7).

This implies that AET, cysteamine, glutathion and serotonine, which all have a relatively good protective effect, are genetically without effect. Cysteamine on the other hand has a rather good protective effect on both spermatozoa and spermatids. Table 3 shows the effect of cysteamine on spermatozoa from the heterozygous albino population. With regard to the great somatic radiosensitivity of these animals a different dose scale has been used than that applied to the CBA animals. The effect of the protective substance, as is seen from the survival frequencies, is only suggested.

Discussion

The frequency of occurrences which can lead to embryonic death, i.e. spontaneous and radiation-induced lethal damage, is generally supposed to have a Poisson-distribution. The survival frequency in each series, i.e. the frequency of zygotes whose irradiated genome has escaped lethal mutation can thus be expressed with a factor e^{-m} in the frequency function. In this expression, e = the natural logarithm base, and m = the mean for the number of lethal factors per genome at a given dose. As an example, the treatment of data from Table 2 shows the following:

Dose	*Cysteamine*	*NaCl*
0 R	$e^{-m_1} = 0.9017$	$e^{-m_2} = 0.9119$
	$m_1 = 0.104$	$m_2 = 0.092$
150 R	$e^{-m_3} = 0.8061$	$e^{-m_4} = 0.7612$
	$m_3 = 0.216$	$m_4 = 0.271$
300 R	$e^{-m_5} = 0.6656$	$e^{-m_6} = 0.5866$
	$m_5 = 0.407$	$m_6 = 0.533$
600 R	$e^{-m_7} = 0.5097$	$e^{-m_8} = 0.4183$
	$m_7 = 0.674$	$m_8 = 0.871$

The ratio $m_1 : m_2$ expresses the relationship between the mean number of spontaneous lethals per genome for the non-irradiated cysteamine-treated males compared with the same mean for the series NaCl-treated animals. Theoretically, this ratio should be equal to 1, if the cysteamine per se did not

have any effect on the mutation frequency. The value obtained is 1.13, which in this case does not comprise a significant deviation from the expected ratio.

The other *m*-values contain two different components, one of which corresponds to the control values m_1 and m_2 and the other which is caused by irradiation. Only this radiation-induced portion should be used as a basis for judging the protective effect of a substance. An estimate of this radiation-induced portion may be obtained by subtracting the *m*-values for the non-irradiated groups from the corresponding series. A practical measurement of the protective effect can thus be computed by the formula:

$$M = 1 - \frac{m_{x_{\text{subst}}} - m_{0_{\text{subst}}}}{m_{x_{\text{NaCl}}} - m_{0_{\text{NaCl}}}}$$

where *x* indicates an irradiated series and 0 the corresponding non-irradiated series. If the tested substance is without effect then the value of *M* will be around 0. The greater the positive value of *M* obtained, the greater the genetically protective effect of the substance. On the other hand a high negative *M*-value would indicate that the substance had a mutation-enhancing effect.

If one accepts the *M*-value as the best expression of a certain substance's mutation-reducing effect at a certain dose, then we can see from Table 11 that cysteamine alone among these five somatically effective radio-protectors has a clear protective effect on the hereditary material at all dose levels and gamete stages tested.

The data obtained in the experimental series with cysteamine give, as mentioned above, a convincing picture of the genetically radio-protective effects of the substance. Within comparable groups, however, there are great differences. In the series comprising irradiated spermatozoa (Tables 1 and 6) one finds that in one case the *M*-value at 300 R is 0.321 and at 600 R 0.270, while the corresponding data from a later repetition gives $M = 0.173$ and 0.407 respectively. In the first case the mutation-reducing effect is greater at the lower dose, but in the later experiment the protective effect at the higher dose is more than double that at the lower dose.

In the series comprising cysteamine-protected spermatids the same pattern was found in the repetition as in the first experiment at doses 300 R to 600 R. At a dose of 150 R, however, the protector was of considerably greater effectivity. The varying results in the spermatozoa tests are difficult to explain logically. Random influences in combination with a certain heterogeneity in the animal material may have contributed, and so may the fact that the irradiations were performed with a 6-month interlude.

The three AET series show partially contradictory results. According to LEONARD & MAISIN (1963), this substance has a certain protective effect on

different stages of spermatogenesis, as shown with cytological techniques. In addition, EHLING (1964), in dissection results following AET injection of the fathers, found a higher survival frequency among foetuses conceived in the 1st to 3rd weeks after irradiation. The material is comparatively small, and a breakdown into results for the different weeks is not given. It is mentioned, however, that the effect was most marked for the 3rd week. These later embryos are derived from cells which at the time of irradiation were for the most part in the spermatid stage. A substance with a relatively small protective effect could because of variations in a small material give partially contradictory results, such as we have found for AET.

The possibility that this substance has a certain protective effect is therefore not excluded; it is however considerably less than that of cysteamine.

From the comparisons now presented between the effectiveness of different somatic radio-protectors, as genetic radio-protectors, it is apparent that there does not necessarily exist a correlation between the genetic and the somatic protective effect.

In the event that substances with a somatic protective effect are put into practical use, it is of great importance to examine the consequences of such usage from a genetic point of view, since it has now been further documented that such protective effects are possible.

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SUMMARY

The genetic radio-protective effects of cysteamine, AET, cystamine, glutathion and serotonine have been studied. Only cysteamine showed a clear mutation-reducing effect on spermatids and spermatozoa.

ZUSAMMENFASSUNG

Die genetisch strahlenschützenden Wirkungen von Cysteamin, AET, Cystamin, Glutathion und Serotonin wurden studiert. Nur Cysteamin zeigte eine eindeutige mutationsherabsetzende Wirkung auf Spermatiden und Spermatozoen.

RÉSUMÉ

L'auteur a étudié l'effet radioprotecteur génétique de la cystéamine, de l'AET, de la cystamine, du glutathion et de la sérotonine. Seule la cystéamine réduit nettement le nombre des mutations sur les spermatides et les spermatozoïdes.

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