AN ANALYSIS OF THE INFLUENCE OF IRRADIATION BY MEANS OF A MERCURY VAPOUR LAMP UPON THE HEALTH AND FERTILITY OF A BREEDING STOCK OF GUINEA-PIGS AND UPON THE HEALTH OF THEIR OFFSPRING DURING THE FIRST SIX WEEKS OF LIFE.

By G. F. PETRIE,

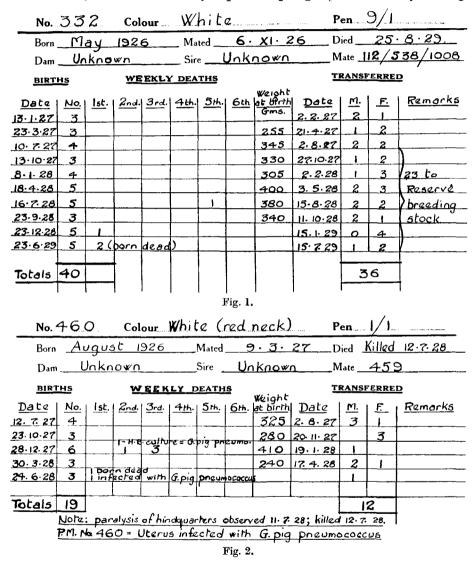
(Serum Department, Lister Institute, Elstree.)

(With 3 Text-figures.)

THE maintenance of a constant supply of healthy guinea-pigs is an important part of the work of laboratories which are engaged in the production of diphtheria and tetanus antitoxin. The subacute infective processes to which malnutrition predisposes enhance the effect of the test dose of toxin, with the result that irregular deaths among the animals under test render difficult the titration of the antitoxin. For this reason it is desirable that breeding stocks should be kept in suitable animal houses under the best hygienic conditions possible. This policy has been followed in the Serum Department of the Institute for many years; there have been comparatively few introductions of stock from outside sources, and within recent years special attention has been devoted to diet. •

Since October 1924 a pneumococcal infection, which is mainly incident upon the breeding sows, has existed among the stock of guinea-pigs at Elstree; the outbreak was apparently attributable to some deficiency in the diet. The disease is well known in France and in the United States, but there are only a few references to its prevalence in Germany and in this country. It is hoped to give, in a later communication, a detailed account of the disease, as it has been observed here. The chief lesions are those of a pleuro-pneumonia or of a metritis which brings about still-births, and may cause the death of the mother soon after parturition. The incidence upon the breeding boars is much less; they occasionally die of the pneumonic type of infection. Fortunately the young stock, from the age of 1 month to the time of mating, that are reserved for experimental and testing purposes, rarely show signs of the disease. The infection has proved to be a persistent one in spite of providing the stock with an abundant and varied diet, which includes a regular and ample supply of green food. Vaccination with the guinea-pig strain of pneumococcus has been tried, but without success. The mortality, especially among the newly born and very young animals, shows an increase in the first quarter of the year, a seasonal relation which suggests the exhaustion, during the first half of the winter, of a vitamin reserve in the breeding sows that had been stored up in

summer, with a consequent lowering of the resistance of both parent and young to the attack of the pneumococcus. A deficiency in vitamin D was considered as a possible factor, and since cod-liver oil cannot be administered to guineapigs because they do not readily digest oily substances it was thought that irradiation by means of a mercury vapour lamp might prove useful by making



good the presumed deficiency or by acting as a therapeutic agent which would strengthen the defences against the microbic invasion. A system of recording the history of each breeding sow had been in operation during 18 months before the experiment began, so that the machinery already existed for collecting data which might afford an estimate of the influence of irradiation upon

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the incidence of the pneumococcal infection, the fertility of the breeding animals, and the health and nutrition of their progeny. The actual record cards of two breeding sows are reproduced (Figs. 1 and 2): one of them the best breeding animal of which we have had experience, and the other an example of an animal which, for part at least of its breeding life, was evidently a carrier of the guinea-pig strain of pneumococcus.

METHOD OF IRRADIATION EMPLOYED IN THE EXPERIMENT.

During the period of the experiment the breeding stock of guinea-pigs was kept in two wooden buildings, which were in every way similar. Each had a floor area of about 45 ft. by 14 ft., and on either side of a central passage there was a row of breeding pens, each of which measured about 5 ft. by 2 ft. 6 in. and housed six sows and a boar. The stock was equally divided between the two buildings, care being taken to avoid conscious selection, and the guineapigs in one of them were irradiated while those in the other house served as a control. In an adjacent building there is a double row of kindling pens, each of them about 18 in. square. When a sow shows obvious signs of pregnancy she is transferred from the breeding house to a vacant kindling pen and is kept there until the young are ready to be removed to the experimental or reserve breeding stock. When the experiment began, the kindling pens were divided by a partition into two sections, one for irradiated sows and their litters, which continued to receive routine irradiation, and the other for sows from the control house, which were given no light treatment. Young guinea-pigs, born of irradiated mothers, that were destined for the breeding stock, continued to receive the treatment until the experiment ended. The stock as a whole included a large proportion of white and light-coloured animals (95 per cent.); 59 per cent. were pure white.

The lamp used was the Ulviarc Medical Lamp supplied by the Hewittic Electrical Co., Ltd., and was fitted with a 3000 c.p. mercury vapour quartz burner. From 14. ii. 28 to 19. v. 28 the original burner was used for a total period of 118 hours. A fresh burner was then fitted and was in use until 23. vi. 28-a total of 105 hours' illumination. Thereafter a new lamp was in operation for 229 hours, until the end of the experiment. There was thus little time for any serious deterioration in the emission power of the burners to have taken place. For the first 2 months of the experiment, dating from 14. ii. 28, the lamp was suspended at a distance of 3 ft. 7 in. from the floor of the pens on a wire support placed midway along the central passage of the breeding house. The area irradiated at each exposure was 5 ft. by 5 ft., that is, the area of two pens. The area irradiated in the kindling pens was 3 ft. by 3 ft., that is, the area of four pens. On 16. iv. 1928 the conditions for irradiation were improved by suspending the lamp directly over the centre of the breeding pens at a height of 2 ft. 6 in. from the floor of the pens, and on the same date the lamp was lowered over the kindling pens so that the animals in six pens received treatment at each exposure. The time of exposure was increased from 1 minute

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per day for the first 3 weeks to 5 minutes per day four times a week and 2 minutes per day twice a week in the period 4. iv. 28 to 19. x. 28. Between the latter date and the end of the experiment on 14. ii. 29 the time was 3 minutes per day for 6 days in the week. The total time of exposure for each individual of the initial stock which survived till the end of the experimental year was 15 hours 9 minutes.

Since any effect due to irradiation was unlikely to have disappeared abruptly when the treatment was stopped it was thought best to include the subsequent 3 months, that is, until 15. v. 29, in the analysis of results. During the experimental year a total of 613 adult breeding animals (519 sows and 94 boars) were observed, and to these, up to 15. v. 29, 3620 young were born. The analysis which follows is thus based on the observation of 613 adults and of 3620 young during the first 6 weeks of life—a total of 4233 animals.

ANALYSIS OF DATA RELATING TO THE BREEDING SOWS.

In each of the two groups of breeding sows a number of the animals which began the experiment did not survive till its close. These were replaced by substitutes, of which a certain number likewise died before the end of the experimental year. The data in the tables have accordingly been arranged in four groups of irradiated animals and four corresponding groups of control animals, as follows:

- 1. Survivors of initial stock: irradiated.
- 2. Survivors of initial stock: control.
- 3. Survivors of replacements: irradiated.
- 4. Survivors of replacements: control.
- 5. Initial stock dead during experiment: irradiated.
- 6. Initial stock dead during experiment: control.
- 7. Replacements dead during experiment: irradiated.
- 8. Replacements dead during experiment: control.

General death-rate. The monthly figures are set forth in Table I and are shown in graphic form in the chart (Fig. 3), which represents the combined figures for boars and sows. The graph prepared from the figures for breeding sows alone is very similar. Dr P. L. McKinlay, of the London School of Hygiene and Tropical Medicine, has very kindly examined the data in this and the other tables. He has calculated the probable errors for the combined groups (boars and sows), both irradiated and control, and has found that the observed differences in the mortality data can be regarded as substantial in 4 only of the 16 months. These are shown in Table II. He notes that in each

Table II. Showing the probable errors for death-rate differences in the combined groups (boars and sows), irradiated and control, as set forth in Table I.

Difference between irradiated and control (boars and sows).

Aug. 1928		 7.7 ± 2.4
Feb. 1929	•••	 5.6 ± 1.2
Mar. 1929	•••	 $4 \cdot 1 \pm 1 \cdot 2$
Apr. 1929	•••	 3.6 ± 1.2

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instance the difference is in favour of the irradiated guinea-pigs, and that in the remaining months of the experiment the differences are statistically negligible, and, moreover, are evenly distributed among the two groups, since in

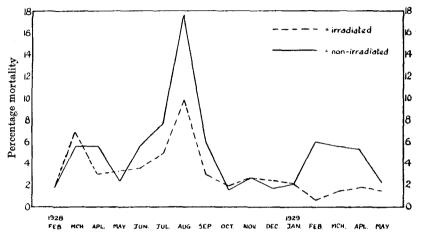


Fig. 3. Comparing the deaths from all causes among the irradiated and the non-irradiated breeding guinea-pigs (boars and sows) in each of the 16 months from the date of beginning the experiment.

five the irradiated animals and in six the control animals show the more favourable experience. In the remaining month the mortality in the two groups is equal.

Deaths from pneumococcal infection. Table III indicates that there is no material difference in this respect between the two groups.

Table III. Comparing the mortality from pneumococcal infection among the irradiated and control adult female guinea-pigs: the figures represent monthly averages.

veruges.	Average monthly population	Month	nly average	Montl	Deaths
	from March 1928 to April 1929	deaths	Deaths from pneu- mococcus	Total deaths as %	from pneu- mococcus as %
Sows: irradiated Sows: control	177 154	5·9 8·6	4·1 3·4	3·3 5·6	$2 \cdot 3 \\ 2 \cdot 2$

Fertility data. The data under this heading will be found in Table IV. In two instances the average size of the litters is higher in the irradiated guineapigs, and in the remaining two groups it is higher in the control guinea-pigs, but Dr McKinlay's calculations show that in no one of these is the difference statistically significant (Table V). The conclusion may be drawn that the average size of the litter is not affected to any appreciable extent by irradiation. In Table IV there are also shown the number of young per 100 breeding sows in each group. In two instances the figures are in favour of the irradiated guinea-pigs, and in two in favour of the control guinea-pigs.

		•						Weig	Weight of young	ng		
Z a	No. of sows (Average irradia- tion (minutes)	Average age on 14. ii. 28 (months)	No. of young per 100 sows		Average no. of litters	Average size of litters	No.	Average weight (grm.)	ht Total	Births Survivors al as %	
Survivors of initial stock: irradiated Survivors of initial stock: control Survivors of replacements: irradiated	40 38 71	906 791	13-3 13-0 	$1112 \\1187 \\768$		ლ.ლ.ღ. ლ.∔.ლ	$3.32 \\ 3.44 \\ 3.26$	$189 \\ 234 \\ 147$	91.0 86.1			
Survivors of replacements: control Initial stock dead during experiment: irradiated Initial stock dead during experiment: control	96 93 96	471	13.7 15.0	664 669 581		2:0 1:9 1:8	3·30 3·49 3·28	$111 \\ 346 \\ 285 \\ 285$	97.2 85.2 88.3		1 82.0 2 56.1 57.9	
Replacements dead during experiment: irradiated Replacements dead during experiment: control	53 45	555		-408 516 of young	in first	1.1 1.5 t 4 week			82·6 91·7		6 54-7 2 54-7	
ļ			Nos.		ł			%		fron	% mortality from pneumo-	
(-	12 hr.	lst week	2nd week	3rd week	4th week	12 hr.	lst week	2nd week	3rd 4th week week	ſ	during first 4 weeks	
d :: :: :: ed	$50^{(1)}_{27}$ 27 ⁽²⁾ 26 ⁽⁸⁾	17 ⁽⁴⁾ 9 22 ⁽⁸⁾	7(4) 5 12(1)	$ \begin{array}{c} 3 \\ 3 \\ 6 \\ \begin{array}{c} 3 \\ 3 \\ \begin{array}{c} 3 \\ 3 \\ \end{array} \end{array} $	¢1 — 61	11-2 6-0 4-8	8 0 0 9 0 8	$2.2 \stackrel{1.6}{\cdot}$	0.5 0.7 1.1 0	0.5 0.2 4	11-5 6-6 26-5	
: irradiated] : control 1 it: irradiated 1 it: control	$60^{(3)}$ $168^{(25)}$ $149^{(6)}$ $40^{(7)}$	23 (3) 54 (14) 63 (17) 33 (6) 20 (1)	11 24 ⁽⁴⁾ 14 ⁽²⁾ 20 ⁽¹⁾	or − 10 or 10	0-0-0	$\begin{array}{c} 11.0\\ 27.0\\ 26.7\\ 18.5\\ 26.7\\ 26.7\end{array}$	4-2 8-7 15-3 8-6 8-6	0,0,0,0,0 0,0,0,0,0,0,0,0,0,0,0,0,0,0,0	4.0000 4.000 4.000 00000	0 0 0 0 0 0 0 0 0 0 0	6.2 17.1 7.4 8.5	

Table IV. Comparing (1) the fertility of the irradiated and non-irradiated breeding sows in the period 14. ii. 28 to 15. v. 29,

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Table V. Showing the probable errors of data relating to the average size of litters produced in the two groups, as set forth in Table IV.

Average size of litters.

	Irradiated	Control
Survivors of initial stock	3.32 ± 0.07	3.44 ± 0.06
Initial stock dead during experiment	3.49 ± 0.06	$3\cdot28\pm0\cdot06$
Survivors of replacements	3.26 ± 0.05	$3\cdot 30\pm 0\cdot 05$
Replacements dead during experiment	3.72 ± 0.10	3.41 ± 0.08

THE INFLUENCE OF IRRADIATION UPON THE BREEDING BOARS.

The figures for the boars are too small to warrant any definite conclusion, but there is little reason to believe that there is any notable difference between those that were irradiated and those in the control group. With an average monthly population of 26 boars in the irradiated stock there were three deaths from pneumococcal infection in 14 months, whereas with an average monthly population of 27 in the control group there were four deaths from this infection in 14 months.

ANALYSIS OF DATA RELATING TO THE PROGENY OF THE IRRADIATED AND CONTROL ANIMALS.

Survival-rate of the young. In Table IV, which summarises the results of the whole period of the experiment, it will be seen that in three out of four instances the survival-rate of the young is higher among the control groups than among the irradiated groups, but in only one of these can the difference be regarded as trustworthy (Table VI). In the remaining group ("survivors of

Table VI. Showing the probable errors for survival-rate differences, as set forth in Table IV.

Survival-rate differences.

Survivors of initial stock Initial stock dead during experiment Survivors of replacements	$\begin{array}{r} -7.5 \pm 1.6 \\ -1.8 \pm 2.0 \\ +5.3 \pm 1.5 \\ 2.8 \pm 2.2 \end{array}$
Replacements dead during experiment	-3.8 ± 3.2
+ indicates difference in favour of irradiat	ted animals.

indicates difference in favour of control animals.

replacements: irradiated"), in which the survival-rate is higher in the irradiated guinea-pigs than in the control group, the difference is probably significant. With regard to Table VII, which arranges the data according to four periods, Dr McKinlay reports as follows:

1. In the first period, none of the differences in survival-rates is found to be significant.

2. In the second period the significant differences are: "Survivors of initial stock"-in favour of control guinea-pigs. "Initial stock dead during experiment"-in favour of irradiated guinea-pigs. "Survivors of replacements"--in favour of irradiated guinea-pigs. The remaining one ("replacements dead during experiment") is insignificant.

3. In the third period the only significant difference is in the group "survivors of initial stock," and it is in favour of the control animals.

 Table VII.
 Comparing (1) the survival-rate, and (2) the death-rate during the first 6 weeks of life of the young guinea-pigs

 born of irradiated and non-irradiated mothers.
 The data are similar to those in Table VI, but have been divided into

 four groups, the second of which corresponds to the rise in the mortality curve (see Chart I).

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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	vivors of initial stock: control	23	1	11.8	I	١	75	92.0	2.7	5.3	ł	ļ	ļ
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	ed	74	712		I	١	220	90·5	5.0	3. 5 7	17	6.0	ļ
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	VIVORS OF replacements: control	55	18	ן ן		1	20 20 20 20 20 20 20 20 20 20 20 20 20 2	82.4	13.8	Т.ż	ŀ	0-0]
$\begin{bmatrix} 66 & 5 & 614 & & & 14 & 50 & 50 & 0 \\ 7 & & & 21 & 85 & 7 & 14 & 3 & \\ & & & & & & & & \\ \end{bmatrix}$	ual stock dead during experiment: irradiated tial stock dead during exnemment: control	0 00	89 8	10. 1 9-2			56 76	19.2	1.4.1	3.8 8			
	placements dead during experiment: irradiated	5	614		I	I	14	50-0	50.0	I	I	I	ł
	placements dead during experiment: control	-	Í		i	I	77	80-7	14.3	I	١.		ļ

Influence of Irradiation

4. In the fourth period the groups "survivors of replacements" and "replacements dead during experiment" show substantial differences, one in favour of the treated and the other in favour of the untreated guinea-pigs. The differences in the two remaining groups are probably of no significance.

In other words, although several of the apparent differences are very unlikely to have arisen by chance, the differences in favour of the untreated and treated guinea-pigs are about equally balanced, and there is therefore little evidence that irradiation has any effect on the survival of the offspring.

Death-rate from pneumococcal infection among the young. The figures will be found in Table IV, and they show that the percentage of deaths from pneumococcal infection in the young in the first 4 weeks of life is higher among the irradiated than among the control animals, except in one group ("replacements dead during experiment"). Dr McKinlay comments that each of these three differences is more than three times the probable error involved, and must be regarded as significant. In the remaining group, in which the irradiated animals show the more favourable experience, the difference is less than the probable error involved and cannot therefore be regarded as substantial (Table VIII).

Table VIII. Showing the probable errors for differences in the death-rate of the young from the guinea-pig pneumococcus, as set forth in Table IV.

Pneumococcal mortality differences.

Survivors of initial stock	$- 4.9 \pm 1.3$
Initial stock dead during experiment	$- 6.2 \pm 1.4$
Survivors of replacements	-20.3 ± 1.5
Replacements dead during experiment	$+ 0.9 \pm 1.7$

+ indicates difference in favour of irradiated animals.

- indicates difference in favour of control animals.

Weight at birth in the irradiated and control groups. Table IX compares the average weight of guinea-pigs at birth in relation to (1) the size of the litters and (2) the irradiation or non-irradiation of the mother. The figures show that, in accordance with expectation, as the size of the litter increases the average

Table IX. Comparing the average weight of guinea-pigs at birth in relation to (1) the size of the litter, and (2) the irradiation or non-irradiation of the mother.

	Litters of sows sur- viving experiment: Irradiated		viving e	f sows sur- xperiment: ntrol	during e	f sows dead xperiment: diated	during e	f sows dead xperiment: ntrol
No. in litter	No. of litters weighed	Average weight per guinea-pig (grm.)	No. of litters weighed	Average weight per guinea-pig (grm.)	No. of litters weighed	Average weight per guinea-pig (grm.)	No. of litters weighed	Average weight per guinea-pig (grm.)
1 2 3 4 5 6	4 17 43 26 9 3	$ \begin{array}{r} 111.0\\ 114.0\\ 95.4\\ 83.2\\ 71.7\\ 72.5 \end{array} $	5 17 52 24 9 1	127.0 109.0 92.6 87.6 80.8 70.0	3 14 52 40 14 1	120·0 104·4 91·0 80·0 71·6 55·8	$egin{array}{c} 4\\ 30\\ 42\\ 28\\ 6\\ 2\end{array}$	115·0 103·0 94·0 83·0 77·2 66·6
	102	91.3	108	94.7	124	87.1	112	89.8

Influence of Irradiation

weight decreases; and further, that treatment of the mother with ultraviolet light has no apparent influence upon the nutrition of the foetus.

CONCLUSION.

The experiment furnishes no evidence that irradiation of a breeding stock of guinea-pigs with ultraviolet light from a mercury vapour lamp exerts any favourable influence upon (1) the general mortality, the susceptibility to a spontaneous pneumococcal infection, and the fertility of the adult animals; and (2) the survival-rate of the young in the early weeks of life, their nutrition *in utero* as indicated by the weight at birth, and their susceptibility to the pneumococcal infection.

In view of this conclusion the two sets of observations may be regarded as homogeneous material. They represent as a whole a biometrical analysis of a breeding stock of guinea-pigs, the only one apparently that has hitherto appeared.

I wish to thank Dr P. L. McKinlay for his help, not only in estimating the statistical significance of the results, but in furnishing references to experiments of a similar kind; these are given below¹. My thanks, too, are due to my colleague, Dr W. T. J. Morgan, and to Mr F. K. Fox, for valuable assistance in the laborious work of reducing the data to tabular form.

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¹ These references are to articles describing the effects of irradiation on children and experimental animals and are appended for readers interested in this subject. As the results and methods are not readily comparable with each other, it has not been thought necessary to refer to these articles in the text.

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