# THE EFFECTS OF LOW ATMOSPHERIC PRESSURE ON THE FERTILITY OF MALE RABBITS

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## INTRODUCTION

The influence of high altitude upon fertility was noticed a long time ago. Monge (1943) quotes the Foundation Charter of Lima in which it is stated that in 1535 the capital of Peru was transferred from Janja (11,500 ft.) to Lima (sea-level) because horses, fowls and pigs did not reproduce there. He also recalls Father Catancha's observation (1639) that the Spanish conquerors did not have offspring at Potosi, Bolivia (14,000 ft.) until 58 years after the city was founded.

Monge (1942, 1943) reports changes in the *p*H of the semen and motility of the spermatozoa, and 50% sterility in unacclimatized rams in the Andes. The fertility of the female at high altitude is unaffected.

Dohan (1942) and Gordon, Tornetta, D'Angelo & Charipper (1943) found a decrease in the testis weight and degenerative changes in the testes of rats exposed to low atmospheric pressure.

The following experiments were designed to investigate the effect of low atmospheric pressure on spermatogenesis in rabbits, using the technique of semen collection described by Macirone & Walton (1938) so that a continuous record could be kept of sperm production, and testicular activity could be studied at all stages of treatment and recovery without having to kill the animal. This method also allows an assessment to be made of sex drive and male potency. Artificial production of low atmospheric pressure in the laboratory does not of course reproduce all the conditions of life at high altitude. In these experiments we are concerned only with the effects of lowered atmospheric pressure.

## MATERIAL AND METHODS

Seventeen sexually mature rabbits of different strains, fed on a ration of crushed oats and bran, kale, cabbage and hay *ad lib.* were kept in an air chamber at different reduced pressures for various periods of time. The air was removed from the chamber by a vacuum pump, running continuously and so supplying the animals constantly with fresh air. There was, therefore, no accumulation of $CO_2$, moisture or other gaseous products of metabolism. The desired pressure was obtained by regulating the intake through a valve. The chamber was connected with a mercury manometer. When the animal was placed in the chamber or removed, the pressure was lowered or raised at a rate of about 20 mm. Hg/min. The rabbits were weighed once weekly. Early in the experiment a marked drop of body temperature was

noticed, and from then onwards the temperature of each animal was taken just before placing it in the chamber and immediately after it was removed.

At least 2 weeks before beginning the experiment the rabbits were trained to serve an artificial vagina. Semen was collected twice weekly throughout the whole period of observation, using the technique described by Macirone & Walton (1938). Immediately after collection the ejaculate was diluted with saline (0·9% NaCl) to 10 ml. In each ejaculate, the motility, the number of spermatozoa and spermatogenetic cells, and the percentage of abnormal spermatozoa were measured. If the first ejaculate was poor a second was collected and the better of the two examined. For examining motility two drops of semen were placed on a slide covered with a cover-glass and examined under $\frac{1}{2}$ and $\frac{1}{6}$ in. objectives and recorded as follows: aspermia = 000, azoospermia = 00, necrospermia = 0, 1–10% of motile spermatozoa = 1, 11–20% = 2, and so forth.

The number of spermatozoa was estimated by means of the haemocytometer (Walton, 1927). Usually 16 squares were counted, but if these included less than 100 spermatozoa, 32 or 48 squares were counted. The standard error of a haemocytometer count is given by the relation $s = \sqrt{n}$, where $n$ = the number counted. The standard errors of the counts in this experiment were therefore approximately $s = \sqrt{100} \leqslant 10\%$. After estimation of the number of spermatozoa and spermatogenetic cells, the abnormal spermatozoa were counted in the same squares and the percentage of abnormal spermatozoa was calculated.

Abnormal spermatozoa fell into six different categories:

(1) Undifferentiated cells of testicular origin in excess of the normal 3%.

(2) 'Immature' spermatozoa, i.e. differentiated spermatozoa but with protoplasmic residues round neck and middle piece.

(3) Spermatozoa with malformed heads.

(4) Spermatozoa with malformed middle piece.

(5) Spermatozoa with malformed tails.

(6) Spermatozoa without tails.

Usually the percentage of abnormals was estimated by inspection of unstained films, but more accurate determinations were made from time to time by the following procedure. Three drops of diluted semen were smeared on a glass slide and dried in air. The cells were then fixed in 10% formol saline for 5 min. followed by hexamethyl violet for 2–3 min. They were then washed in running water, dried, and mounted in Canada balsam.

Examination of the semen of the experimental animals was begun at least 2 weeks before the exposure to low pressure, and was continued until 2 weeks after the semen had returned to normal. Some rabbits were killed after exposure and the testes, epididymides and vasa deferentia were immediately dissected out. One testis, with epididymis and vas deferens, was fixed in Bouin's solution, embedded in paraffin, sectioned, stained with haematoxylin and eosin and examined. From the other testis the epididymis and vas deferens were dissected out separately, cut in small pieces, suspended in 10 ml. saline, and the motility of spermatozoa, the number of spermatozoa and the percentage of abnormal forms were measured.

## EXPERIMENTAL RESULTS

Table 1 summarizes the experimental treatments and the main results. Animals subjected to a reduced pressure of between 380 and 400 mm. Hg for 22 hr. daily and up to 7 days' exposure showed no effects of the treatment. They maintained a normal body weight, ate well, copulated readily with the dummy rabbit, and produced ejaculations of normal semen both during the period of treatment and for some time after.

Table 1. *Summary of treatments and results*

| Pressure mm. Hg | No. of male | Hr. daily exposure | No. of days exposed | Average fall of body temp. °C. | Maximum % loss of body weight | % of normal no. of sperms* | % abnormal sperms* | Motility of sperms* |
|---|---|---|---|---|---|---|---|---|
| 380–400 | 1 | 22 | 5 | None? | None? | Normal (100%) | Normal (10–20) | Normal (9–7) |
| | 2 | 22 | 5 | ,, | ,, | ,, | ,, | ,, |
| | 5 | 22 | 6 | ,, | ,, | ,, | ,, | ,, |
| | 6 | 22 | 6 | ,, | ,, | ,, | ,, | ,, |
| | 3 | 22 | 7 | ,, | ,, | ,, | ,, | ,, |
| | 4 | 22 | 7 | ,, | ,, | ,, | ,, | ,, |
| 260–280 | 10 | 6 | 14 | −1·3 | 8·5 | 52 | 59 | 1·5 |
| | 693 | 6 | 14 | −0·4 | 7·1 | 64 | 93 | 1·8 |
| 210–260 | 11 | 16 | 3 | −2·7 | 8·7 | Normal | Normal | Normal |
| | 792 | 16 | 3 | −4·9 | 7·5 | ,, | ,, | ,, |
| | 790 | 16 | 6 | −2·6 | 23·2 | 33 | 79 | 1·2 |
| | 696 | 16 | 11 | −3·2 | 16·6 | 38 | 100 | 0·0 |
| | 793 | 16 | 11 | −3·2 | 25·8 | 18 | 97 | 0·3 |
| | | | | | | Males killed for autopsy | | |
| | | | | | | Vas | Epididymis | Testis |
| | 14 | 16 | 1 | −3·8 | 9·4 | Normal | Normal | Normal |
| | 864 | 16 | 3 | −1·8 | 20·3 | ,, | ,, | Slight effect |
| | 13 | 16 | 5 | −3·0 | 21·4 | ,, | ,, | Marked effect |
| | 12 | 16 | 6 | −2·8 | 26·3 | ,, | Few coils empty | Very marked effect |

* Mean of four recordings during period of severest symptoms.

With more severe reduction of pressure, symptoms of discomfort of the animals appeared. The chamber was cylindrical and fitted with glass ends so that the animals could be observed during exposure. When the pressure was lowered, the animals became excited at first and respiration was rapid and shallow, but later it became slow and deep. Cyanosis was observed, but it passed off soon after the animals were removed from the air chamber. On removal, the animal's temperature was taken and found to be below normal. The animals appeared sleepy. Sexual desire was slightly diminished, but all animals attempted to mate with the dummy when it was presented, although some did not ejaculate. During the period of exposure there was some loss of body weight which in one case reached 26%. In some animals there was some appearance of acclimatization, the symptoms of distress in

the chamber becoming less severe as treatment continued, but as exposures were not longer than 14 days the full effects of acclimatization were probably not seen. The effect upon the spermatozoa was most marked and appeared in about 10–14 days' time. The total number of spermatozoa in the ejaculate decreased, but an even

Table 2. *Records of individual males before, during and after treatment. Records taken twice weekly. During the fortnight when the symptoms are most severe the figures are printed in heavy type*

| Rabbit 10 | | | Rabbit 693 | | | Rabbit 696 | | | Rabbit 793 | | | Rabbit 790 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Motility | Total no. millions | % abnormals | Motility | Total no. millions | % abnormals | Motility | Total no. millions | % abnormals | Motility | Total no. millions | % abnormals | Motility | Total no. millions | % abnormals |
| 8 | 135 | 17 | 8 | 83 | 10 | 7 | 35 | 11 | 9 | 123 | 12 | 9 | 218 | 7 |
| 8 | 92 | 9 | 8 | 84 | 6 | 7 | 26 | 7 | 7 | 151 | 9 | 8 | 143 | 14 |
| 8 | 141 | 2 | 8 | 91 | 19 | 7 | 45 | 22 | 8 | 108 | 12 | 8 | 206 | 11 |
| 8 | 136 | 7 | 8 | 80 | 11 | 8 | 37 | 21 | 8 | 146 | 8 | 9 | 267 | 9 |
| Treatment started | | | | | | | | | | | | | | |
| 260–280 mm. Hg 6 hr. daily 14 days | | | 260–280 mm. Hg 6 hr. daily 14 days | | | 210–260 mm. Hg 16 hr. daily 11 days | | | 210–260 mm. Hg 16 hr. daily 11 days | | | 210–260 mm. Hg 16 hr. daily 6 days | | |
| 8 | 147 | 9 | 8 | 113 | 19 | 8 | 31 | 22 | — | — | — | — | — | — |
| 8 | 132 | 8 | 7 | 85 | 16 | — | — | — | — | — | — | — | — | — |
| 8 | 63 | 10 | 7 | 101 | 15 | — | — | — | — | — | — | Treatment stopped | | |
| 7 | 37 | 9 | 8 | 46 | 10 | Treatment stopped | | | Treatment stopped | | | 8 | 230 | 24 |
| Treatment stopped | | | Treatment stopped | | | 7 | 34 | 52 | 6 | 170 | 15 | 1 | 147 | 83 |
| 8 | 147 | 14 | 6 | 70 | 29 | 0 | 7 | 90 | 4 | 26 | 41 | 1 | **36** | **75** |
| **2** | **127** | **39** | 6 | 24 | 33 | 1 | 2 | 90 | **0** | **16** | **90** | 1 | **31** | **85** |
| **1** | **19** | **56** | 5 | 11 | 53 | 0 | 1 | 91 | **0** | **12** | **99** | **2** | **64** | **74** |
| **1** | **44** | **74** | 2 | 35 | 87 | 0 | 6 | 100 | **0** | **16** | **100** | 1 | **77** | **78** |
| **2** | **73** | **67** | **2** | **44** | **96** | 0 | 3 | 100 | 1 | **27** | **97** | 5 | 128 | 57 |
| 3 | 99 | 53 | **2** | **65** | **93** | **00** | **4** | **100** | 4 | 31 | 92 | 6 | 83 | 57 |
| 6 | 74 | 32 | **2** | **42** | **63** | **00** | **5** | **100** | 3 | 53 | 63 | 6 | 156 | 34 |
| 6 | 120 | 29 | 1 | **64** | **91** | **00** | **17** | **100** | 3 | 48 | 79 | 7 | 142 | 23 |
| 3 | 37 | 53 | 3 | 59 | 84 | **00** | **28** | **100** | 5 | 97 | 47 | 7 | 196 | 19 |
| 5 | 81 | 34 | 5 | 106 | 48 | 00 | 26 | 100 | 5 | 79 | 43 | 7 | 264 | 14 |
| 4 | 32 | 38 | 7 | 85 | 37 | 1 | 43 | 94 | 4 | 49 | 57 | 8 | 196 | 11 |
| 6 | 89 | 42 | 6 | 103 | 25 | 1 | 24 | 96 | 6 | 167 | 32 | 8 | 258 | 12 |
| 7 | 204 | 27 | 4 | 41 | 56 | 3 | 31 | 78 | 6 | 121 | 31 | 8 | 360 | 9 |
| 8 | 75 | 21 | 7 | 65 | 33 | 1 | 58 | 65 | 7 | 103 | 17 | 8 | 235 | 8 |
| 7 | 65 | 19 | 6 | 185 | 24 | 3 | 33 | 70 | 7 | 106 | 12 | 8 | 318 | 9 |
| 8 | 56 | 18 | 7 | 144 | 24 | 3 | 47 | 72 | 8 | 129 | 12 | 8 | 247 | 10 |
| 8 | 64 | 16 | 8 | 112 | 20 | 5 | 56 | 56 | 8 | 171 | 11 | | | |
| 7 | 58 | 19 | 8 | 83 | 23 | 7 | 37 | 35 | | | | | | |
| 8 | 91 | 12 | 7 | 72 | 21 | 6 | 33 | 38 | | | | | | |
| 8 | 164 | 10 | 8 | 60 | 15 | 7 | 87 | 28 | | | | | | |

more striking change was the decrease in motility and the increase in abnormal forms. Complete records of some of the more interesting examples are shown in Table 2.

After treatment was stopped all animals made complete recoveries, and after some delay the semen gradually returned to normal. Of the animals killed for autopsy

after exposure to a pressure of 210–260 mm. Hg. for 16 hr. daily, no. 14 exposed for 1 day only showed no changes in the testicular tubules. This degeneration was more marked in no. 13 exposed for 5 days and was very severe in no. 12 exposed for 6 days. In the last example, very few spermatozoa were present in the tubules, but there were many undifferentiated spermatids or spermatocytes and the germinal epithelium was reduced to a few cells in depth. These degenerative changes were more marked in the tubules in the centre of the testis and less marked at the periphery. The rest of the tract appeared normal, though some of the coils in the caput epididymis were devoid of spermatozoa.

## DISCUSSION

The experimental results show that low atmospheric pressure has a decidedly harmful effect upon the fertility of male rabbits. Degenerative changes in the testis appeared after 3 days exposure to pressure of 210–260 mm. Hg for 16 hr. daily (Table 1, no. 864), and effects were noticeable in the ejaculated spermatozoa after about a fortnight in all animals affected (Table 2). This delay in the appearance of abnormal spermatozoa in the ejaculate indicates that the spermatozoa in the vas deferens and lower sections of the epididymis were not affected, and that the harmful effect is upon the spermatozoa in the testis, either during spermatogenesis or spermiogenesis. The delay represents the time taken for the passage of spermatozoa through the epididymis when collections are taken twice weekly as in these experiments. Prolonged exposure (11 days) resulted in one animal (Table 2, no. 696) becoming completely sterile for a period of over a month. A short exposure to 210–260 mm. Hg for 3 days did not noticeably affect the spermatozoa in the ejaculate (nos. 11 and 792). A less severe reduction of pressure (260–280 mm. Hg applied for 6 hr. a day for 14 days) had a significant but less marked effect upon the spermatozoa. With pressure reduced only to 380–400 mm. Hg no abnormal symptoms appeared in animals exposed for 22 hr. daily for up to 7 days (Table 1, nos. 1–6).

From these experiments it is clear that both severity of treatment and length of exposure contribute to the production of sterility. It is also noticeable that the effect is chronic and the animal takes a fairly long time to recover, although eventually it does so completely.

The histological evidence certainly indicates that the effect is upon the spermatozoa in the testis, and mainly during the phase of spermiogenesis. This is also confirmed by the semen studies. The number of spermatozoa ejaculated falls, but is never in complete abeyance, showing that spermatogenesis continues although at a low level. The motility of the cells, however, is very markedly affected, and there is a very great increase in abnormal forms, mainly consisting of undifferentiated spermatids or spermatocytes. The hormonal function of the testis does not appear to be affected in any way. Even during the period of most extreme treatment the animals copulated readily with the dummy when it was introduced into the cage. It is true that some of them failed to ejaculate, but this may have been due to physical debility rather than to lack of sexual drive.

The most obvious explanation of the effect of low pressure is to attribute it to oxygen deprivation, acting directly upon the tissues concerned. This has some support from the histological finding that spermiogenesis is affected more severely than spermatogenesis. The cells in the centre of the tubules are farther from the blood supply than the germinal epithelium. Secondly, it was noticed that tubules in the centre of the testis itself were more affected than those nearer the peripheral arterial supply. Campbell (1935), Williams & Smith (1935), and Patterson, Smith & Pickett (1938) found a loss of fertility in animals in which anoxemia was produced by causing them to breathe air containing carbon monoxide.

A less direct mode of action is also possible. Armstrong & Heim (1938) draw a parallel between the symptoms of mountain sickness and Addison's disease, and suggest that the former may be due to cortico-adrenal insufficiency. Pincus & Hoagland (1943) report an increase in 17-ketosteroids in the urine of animals exposed to low atmospheric pressure. Gordon *et al.* (1943) found that the potency of the gonadotropic hormone of the pituitary glands of rats exposed to low atmospheric pressure is significantly greater than in normal rats. One cannot, therefore, at this stage, exclude the possibility that the effect upon the testis is an indirect one and due to hormonal disturbance.

## SUMMARY

1. Low atmospheric pressure has a detrimental effect upon the fertility of male rabbits.

2. Changes can be detected in semen samples and by histological examination of the testis.

3. The changes induced in the testis take a chronic course but are completely reversible on returning the animal to normal atmospheric pressures.

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