disease may slay its tens of thousands and seriously deplete the fighting resources of a nation; and that the maintenance of health concerns both military and civilian populations, for disease and epidemics may spread from one to the other. It follows, therefore, that all measures directed towards the maintenance of national health and the prevention of disease bulk largely in the war effort of this country.

Fortunately, also, in successive years of peace we have learned that the attack on disease is more than a question of doctor and patient. It comprehends a thousand matters of environment and social reform in which many agencies play their part. All this complex organization is bent now to health problems in a country at war, and our experience of co-operative effort is of considerable aid in this unprecedented work. The civil authority has the responsibility of hospital provision for civilians and for men of the fighting services so far as they are not provided for by their own hospitals; it has organized a vast emergency medical and nursing service, and has coped with countless medical problems of the evacuation scheme.

The civilian arm has yet to receive its baptism of fire. If that stern ordeal comes I have little doubt it will endeavour to keep the flag of national health flying in the storm of war as zealously as it did in the sunshine of peace.

INFLUENCE OF LOW OXYGEN PRESSURE IN THE ATMOSPHERE UPON INCIDENCE OF PRIMARY LUNG TUMOURS IN MICE

BY

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So far as I know no previous information has been available regarding the influence of low oxygen pressure in the atmosphere upon the incidence of primary lung tumours. With respect to cancer in general several observers have suggested that tumours originate in cells suffering from oxygen deficiency. On the other hand, some observers have found that general oxygen deficiency inhibits growth of tumours but does not destroy the cancer cells completely (Warburg and co-workers, 1926; Campbell and Cramer, 1928; Campbell, 1932, 1933). Of course, the origin of the cancer cell may be controlled by factors differing from those controlling the rate of growth of the resulting tumour. Most of the evidence, but not all, indicates that a tumour grows more rapidly in a host with favourable conditions as to general nutrition.

If low oxygen pressure plays a part in the production of so-called spontaneous tumours, exposure of animals for long periods to an atmosphere with an abnormally low oxygen pressure should increase the incidence of the tumours. The term "spontaneous" does not seem to be very suitable, at any rate so far as lung tumours are concerned. Most experiments with mice indicate that some "irritant" from the outside atmosphere acts as the trigger in the starting off of lung tumours.

During an investigation of the powers of acclimatization of mice to low oxygen pressure an opportunity was obtained to study the incidence of primary lung tumours under subnormal oxygen pressure. Lung tumours in mice often appear to originate in a collapsed or thickened area, and presumably the cells in the centre of such an area might suffer from some degree of anoxia. This will

be enhanced if the animal breathes oxygen at lowered pressure. Our stock of mice is on the whole not very susceptible to tumour growth; about 10 per cent. of control mice of all ages reveal tumours of any organ at death. This is not unlike the condition for man; for him about one out of seven or ten deaths is due to cancer. In the mice of our stock, however, the most common tumour is that of the lung; mammary or other tumours are rare. Age has an effect on these lung tumours, which are rare in mice under 10 months old. This is similar to the position with regard to human lung cancer, where the mean age at death is 55 years. Obviously a man who dies of cancer of the lung is susceptible to it, and it serves no good purpose to use non-susceptible animals for experiment. Therefore, in experiments with mice most attention should be directed to the incidence of lung tumours in those older than 10 months.

Methods

For the experiment twenty-five male and twenty-five female mice about 3 months old were enclosed in a respiration chamber of the type already described (Campbell, 1927). In this the carbon dioxide produced by the mice is absorbed by soda lime, and the oxygen used is replaced automatically from a gas container. The desired oxygen pressure in the chamber is attained, under normal atmospheric pressure, by passing nitrogen in appropriate quantity into the chamber immediately after closing it. The atmosphere in the chamber is kept under constant observation by analysis and the gases inside the chamber are well mixed by means of a fan. The chamber is opened to the air for only about twenty minutes every three or four days for the cleansing of boxes and replacement of food and water. Food consists of moistened stale brown bread, milk, cod-liver oil, oats, wheat germ, hemp, canary seed, linseed, and sprouted oats. The zinc boxes, which have perforated lids and a bedding of sawdust, are large enough to hold up to ten adult mice.

The experiment began on December 9, 1936, and continued until September 2, 1939, when unfortunately it was stopped on the outbreak of the war, owing to danger of fire from the oxygen. By this time, however, some useful information had been obtained, and is here recorded. The surviving mice are still under observation, and as most were born in conditions of low oxygen pressure they and their descendants may provide some interesting data; breeding is being continued under normal oxygen pressure again.

The low-oxygen-pressure experiment was fully controlled by a batch of mice, five males and thirteen females, placed in a similar chamber with soda lime, etc., but exposed to oxygen at normal pressure—namely, 20.9 per cent. of an atmosphere. Temperature was kept about 20° C.

Periods of Subnormal Oxygen Pressure and Breeding of Mice

Table I records the duration in days of each stage in the lowering of the oxygen pressure within the chamber. Mating of the mice was allowed, and it was found that on first lowering the oxygen pressure to 14 per cent. of an atmosphere the females became pregnant but refused to rear their young. Therefore, on August 31, 1937, the oxygen pressure was raised to 15 per cent. again and kept at this level for a further 314 days. This difficulty in breeding has also been observed in man at high altitudes (McFarland, 1937; Keys, 1938) and in animals suffering from anoxia due to carbon monoxide (Camp-

TABLE I.—*Duration and Percentages of O₂ Pressure in the Chamber with Subnormal Oxygen Pressure*

Date	Duration (Days)	Approx. Oxygen Pressure in Chamber (per cent. atmosphere)
9/12/36 to 18/12/36	9	21
18/12/36 to 25/1/37	38	17
25/1/37 to 26/2/37	32	16
26/2/37 to 12/3/37	14	15
12/3/37 to 31/8/37	172	14
31/8/37 to 11/7/38	314	15
11/7/38 to 19/8/38	39	14
19/8/38 to 2/9/39	379	13
Total	997	

bell, 1934). When they had been under 15 per cent. of oxygen for some time the mice reared their young, and on July 11, 1938, the oxygen pressure was lowered again to 14 per cent. for thirty-nine days, after which, on August 19, 1938, it was further lowered to 13 per cent. It was kept at this level for 379 days—that is, until the experiment was stopped.

The data for the young born and reared under the various oxygen pressures are given in Table II. Fourteen

TABLE II.—*Low O₂ Pressure Experiment: Numbers of Mice, Original and Born in Chamber*

Mice	No. of Adult Mice			No. of Females		O ₂ Pressure (per cent. atmosphere)
	Males	Females	Total	Pregnant	Rearing Young	
Original ..	25	25	50	14	3	14-17
1st generation	7	1	8	1	1	13-15
2nd ..	14	11	25	10	6	13-15
3rd ..	14	8	22	4	3	13-15
4th ..	2	8	10	5	2	13
5th ..	2	2	4	—	—	13

of the original twenty-five females became pregnant, but only three of them reared their young. These three between them reared seven males and one female. This single female (Mouse No. 52) of the first generation born in the chamber is responsible for the success of the experiment, as she had sixty-one descendants—thirty-two males and twenty-nine females; she survived the experiment, living in all 778 days, and she herself had reared twenty-five young, composed of fourteen males and eleven females. She was born under a pressure of 15 per cent. oxygen and lived thus for the first 290 days of her existence (Table III). Her mother (Mouse No. 31) had lived

TABLE III.—*Duration under Low O₂ Pressure of Two Female Mice, Nos. 31 and 52*

Mouse	Duration (Days)	O ₂ Pressure (per cent. atmosphere)
Original, No. 31 ..	70	16-17
	299	14-15
	290	15
1st generation, No. 52 ..	39	14
	379	13

under an oxygen pressure of between 16 and 17 per cent. for seventy days and under 14 to 15 per cent. for 206 days before she (No. 52) was born. Before the experiment was stopped four mice of the fifth generation were

born under low oxygen pressure. These, as well as some mice of the earlier generations born under low oxygen pressure, are still living.

The descendants of the control mice enclosed in the chamber under normal oxygen pressure are arranged in the order of succeeding generations in Table IV.

TABLE IV.—*Numbers of Mice: Original and Born in Chamber with Normal Oxygen Pressure, 20.9 per cent. Atmosphere*

Mice	No. of Adult Mice			No. of Females Pregnant
	Males	Females	Total	
Original	5	13	18	8
1st generation	9	15	24	12
2nd	31	26	57	5
3rd	11	14	25	5
4th	8	20	28	10
5th	10	12	22	6
Total born in chamber ..	69	87	156	38

They have also bred to the fifth generation while enclosed within the chamber. The numbers reared in this case do not refer to the maximum of breeding possible, because the females had little difficulty in rearing their young under normal oxygen pressure. The numbers reared were controlled merely by the capacity of the chamber, and the females could have bred beyond this limit if required. On the other hand, as already pointed out, under low oxygen pressure the females did not attain this limit. The main object of the breeding of the control mice was to obtain for comparison a sufficient number of mice born inside the chamber under conditions similar to those for the mice under low oxygen pressure except as regards the oxygen percentage in the air. The total number of control mice born in the chamber was 156—sixty-nine males and eighty-seven females.

Rate of Growth of Mice

The rate of growth of the mice born under 13 per cent. of oxygen was similar to that for the mice born in the chamber under normal oxygen pressure (see Diagram).

Diagram showing the rate of growth of male mice born in the respiratory chambers.

Normal mice born outside the chamber show a similar rate of growth. In this respect, then, the mice may be said to have become acclimatized to the degree of lowered oxygen pressure in the atmosphere breathed. As already recorded in Table II, the mice were also able to breed

and rear young under 13 per cent. pressure of oxygen. They had apparently become fully acclimatized to these conditions.

Incidence of Primary Lung Tumours

Mice of All Ages.—Of the 106 control chamber mice that have died twelve, or 11.3 per cent., possessed primary tumours of the lung, in five cases of which the tumours were malignant, while the remaining seven were simple papillary adenomata (Table V); one of the tumours had a metastasis or extension in the lymph nodes. Of the mice exposed to low oxygen pressure sixty-nine have died, and of these twelve, or 17.4 per cent., possessed tumours of the lung, seven of which were malignant (Table VI), one of them with a metastasis or extension in the lymph nodes. This is a small increase in incidence over the control figure, but for judicial comparison some attention must be paid to age and to length of time of exposure to the low oxygen pressure. For this we may take the mice younger and older than 10 months.

TABLE V.—Incidence of Primary Lung Tumours in Mice of All Ages in Control Chamber

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
Original.. .. .	18	0	1
1st generation.. .. .	16	1	1
2nd	41	1	2
3rd	12	1	1
4th	13	3	0
5th	6	1	0
Totals	106	7 (12=11.3%)	5

TABLE VI.—Incidence of Primary Lung Tumours in Mice of All Ages in Chamber with Subnormal O₂ Pressure

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
Original.. .. .	36	2	3
1st generation	7	1	0
2nd	17	2	3
3rd	9	0	1
Totals	69	5 (12=17.4%)	7

Mice Younger than 10 Months (300 Days).—Only three out of the fifty-one (5.8 per cent.) control mice (Table VII) under 10 months possessed primary lung tumours, compared with two out of thirty-seven, or 5.4 per cent., of the mice (Table VIII) exposed to low oxygen pressure. Thus, as might be expected, short exposures and exposures to the lesser degrees of lowered oxygen pressure produced no change in incidence of primary lung tumours. As a rule it is very rare for any mice of our stock under 10 months of age to possess primary lung tumours, and it might be considered that some common factor in both chambers—for example, the soda lime dust—may have stimulated the growth of the five tumours, but the total figures are too small for final conclusions to be drawn.

Mice Older than 10 Months.—There were fifty-five control mice above this age which died, and nine of these, or 16.3 per cent., possessed primary lung tumours (Tables VII and IX). On the other hand, thirty-two mice older

TABLE VII.—Influence of Age on Incidence of Primary Lung Tumours in Mice in Control Chamber

Age of Mice (Days)	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
100-200.. .. .	26	2	0
201-300.. .. .	25	0	1
301-400.. .. .	30	2	1
401-500.. .. .	12	0	0
501-600.. .. .	9	2	2
601-700.. .. .	3	1	1
701-800.. .. .	1	0	0
Totals	106	7 (12=11.3%)	5

TABLE VIII.—Influence of Age on Incidence of Primary Lung Tumours in Mice in Chamber with Subnormal O₂ Pressure

Age of Mice (Days)	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
100-200.. .. .	19	0	0
201-300.. .. .	18	1	1
301-400.. .. .	11	1	0
401-500.. .. .	7	0	1
501-600.. .. .	11	2	4
601-700.. .. .	2	0	1
701-800.. .. .	1	1	0
Totals	69	5 (12=17.4%)	7

than 10 months died under low oxygen pressure; ten, or 31.2 per cent., of these possessed primary lung tumours (Tables VIII and X). It appears from this method of examination that the anoxia doubled the incidence of primary lung tumours, and the increase is present among both the original mice and their descendants.

TABLE IX.—Incidence of Primary Tumours in Mice over 10 Months of Age in Control Chamber

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
Original.. .. .	13	0	1
1st generation	7	1	1
2nd	23	1	1
3rd	6	1	1
4th	6	2	0
Totals	55	5 (9=16.3%)	4

TABLE X.—Incidence of Primary Lung Tumours in Mice over 10 Months of Age in Chamber with Subnormal O₂ Pressure

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
Original.. .. .	19	2	3
1st generation	4	1	0
2nd	6	1	2
3rd	3	0	1
Totals	32	4 (10=31.2%)	6

Mice which had Breathed Oxygen at 13 per cent. Pressure.—There were thirty dead mice of all ages which had breathed the lowest pressure of oxygen tested—namely, 13 per cent. Seven, or 23.3 per cent., of them revealed primary lung tumours (Table XI). This is double the control figure of 11.3 per cent. (Table V) for all ages. If we take only the thirteen dead mice older than 10 months which had breathed 13 per cent. of oxygen we find that five, or 38.4 per cent., possessed lung tumours (Table XII) compared with 16.3 per cent. of the controls (Table IX). This is further evidence in favour of anoxia increasing the incidence.

TABLE XI.—Incidence of Primary Lung Tumours in Mice of All Ages Breathing O₂ at 13 per cent. Atmosphere

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
1st generation	4	1	0
2nd „	17	2	3
3rd „	9	0	1
Totals	30	3 (7=23.3%)	4

TABLE XII.—Incidence of Primary Lung Tumours in Mice over 10 Months of Age Breathing O₂ at 13 per cent. Atmosphere

Mice	No. of Mice	No. of Mice with Lung Tumours	
		Simple	Malignant
1st generation	4	1	0
2nd „	6	1	2
3rd „	3	0	1
Totals	13	2 (5=38.4%)	3

Tumours of Other Organs

There were no mammary tumours among the mice in the control chamber; but in the low-oxygen-pressure chamber one of the mice (first generation, aged 465 days) had a typical mammary carcinoma with metastases in the lung, and another mouse (original, aged 636 days) had a small mammary tumour. One of the control mice (second generation, aged 455 days) had a carcinoma involving the liver and mesentery, while one of the experimental mice had a fibroma of the subcutaneous tissue near an ulcer. In both groups of mice there were four with some hypertrophy of the mediastinal lymph tissue.

Summary and Conclusion

Heredity (see Campbell, 1937) may play an important part in the incidence of lung tumours in mice. However, we have no reason to believe that heredity has influenced the results for our experimental mice to any greater degree than the results for our control mice. In both cases the mice were from the same stock. Many of the mice considered in the tables were descended from one female mouse—namely, No. 52. Her parents, however, did not possess tumours of the lung.

The results, of course, are rather few in number, but, so far as they go, they indicate that anoxia increases the incidence of primary lung tumours in mice older than 10 months, and may therefore be a factor in the origination of tumour cells.

The results also prove that it is possible to acclimatize mice to 13 per cent. of oxygen—equivalent to an altitude

of about 15,000 feet—so that they breed and rear young which grow at a normal rate under this low oxygen pressure. Previous experiments indicate that 10 per cent. of oxygen, corresponding to an altitude of 20,000 feet, is beyond the limit for full acclimatization for mice and other mammals tested (Campbell, 1935); this apparently holds also for man (McFarland, 1937; Keys, 1938).

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WOUNDS PENETRATING THE KNEE-JOINT

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The object of this paper is to record our experience of the treatment of wounds due to penetration of the knee-joint by rock occurring in natives employed in gold-mining operations in South Africa. The conditions are not exactly comparable to those met with on the European battlefields (Frankau, 1939), as the wounds, though they are always contaminated, seldom develop tetanus or gas gangrene. "Penetration of the knee-joint" includes injuries of the large suprapatellar bursa, which is involved in the majority of these accidents.

The condition is of such frequent occurrence that for the past few years the mines have each adopted some form of preventive—for example, various types of knee-guards, of hard or soft crêpe rubber, sometimes combined with a shin-guard resembling a cricket pad. This provision has reduced the incidence of penetrating wounds considerably; but rolling or falling rocks, together with neglect in wearing the guards provided or their improper application, still cause a large number of cases. Knee-guards were introduced to the mine under consideration in December, 1936.

Treatment

First Aid.—It must be insisted that all wounds in the neighbourhood of the knee, no matter how small, be immediately covered with a large sterile dressing and the limb splinted. The patient must then be placed on a stretcher and taken to hospital. Prevention of the use of the limb after injury lessens the blood effusion into the joint and increases the chances of a perfect result.

Diagnosis.—The diagnosis of a penetrating wound is made when synovial fluid is found escaping or is being forced out through the wound on fully flexing the injured knee, which is done by the nursing staff. The patient is then prepared for immediate operation.

Pre-operative Measures.—The wound is covered with an acriflavine dressing and the rest of the limb, from toe