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INFECTIVE METHAEMOGLOBINAEMIA IN RATS CAUSED BY GAERTNER'S BACILLUS[1].

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IN the course of some experiments designed to test the influence of feeding with oleic acid on the blood[2], in which rats were used, it was found that in a certain number of animals the blood was more or less brown. The brown colour was found to be due to the presence of methaemoglobin. It soon appeared that oleic acid had nothing to do with the matter since rats with brown blood were found among the control animals as well as in those which had been subjected to special feeding. The spleens of the affected rats were noticeably enlarged and from them were obtained pure cultures of an organism which afterwards proved to be Gaertner's bacillus. Inoculation of fresh rats with these cultures in appropriate doses produced in a proportion of animals all the symptoms and changes of the natural disease.

The spontaneous occurrence of methaemoglobinaemia in animals and the fact that the condition may be produced by artificial bacillary infection have not, as far as I am aware, been previously described. The affected rats, at any rate when a considerable proportion of the haemoglobin is changed in albino animals, present rather a striking picture. The tail, feet and ears, and to a less extent the whole surface of the body, look pale, livid and blue[3], and the normal bright pink of

[1] Towards the expenses of this research grants were made by the British Medical Association and by the Royal Society.

[2] *British Medical Journal*, 5 November, 1910.

[3] The bacillus which turned canaries blue described by H. G. Wells some years since was, so the author informs me, entirely imaginary.

the eyes is changed to a pale brownish red. These changes are particularly noticeable if normal albino rats are put side by side with the affected animals, and in this way comparatively slight degrees of methaemoglobinaemia may be detected. They are however not very obvious if pigmented rats are used, nor is anything characteristic necessarily found on post-mortem examination since the methaemoglobin is soon reduced after death. Hence the condition may easily have been missed in the absence of any blood examination before death, though the chocolate-coloured blood of a blue rat during life is strange enough to attract immediate notice.

The condition was first noticed in May 1910, and between that date and January 1911 ten well-marked cases were found among about 1000 tame rats which were used in the laboratory. Along with these there occurred a number of rats which, though not showing methaemoglobinaemia, were found to be infected with Gaertner's bacillus; some of these were suffering from obvious general illness, some were anaemic, some were apparently normal as regards their health and blood condition. A certain number of rats found dead among the stock were also probably examples of the disease. For about nine months therefore a smouldering epidemic prevailed among the rats. These rats came from a number of different places and no connection could be traced between their place of origin and the prevalence of infection. Infected animals occurred pretty continuously among the various lots of rats which formed the constantly changing population of the animal houses. Repeated disinfection did not prevent the occurrence of fresh cases, and in November 1910 an entirely fresh animal house was brought into use without producing any effect. It was therefore improbable that infection was in all cases contracted by the rats after arriving at this laboratory, a conclusion substantiated by the discovery of infected animals among batches of rats which were killed and examined immediately after arrival. It appears therefore that infection with Gaertner's bacillus is, or was, widespread among the ordinary stocks of laboratory rats. In the present case no very great mortality was caused. Epidemics associated with acute fatal illness due to Gaertner's bacillus and killing most of the animals exposed to infection are however known among rats. Such an epidemic occurred among tame rats in this laboratory in 1908, and about the same time Bainbridge investigated a similar outbreak among wild rats in confinement[1]; he

[1] *Journal of Pathology*, Vol. XIII. (1909) p. 342.

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has also recently (1911) met with another acute Gaertner epidemic with great mortality among tame rats. Petrie and Macalister[1] found organisms belonging to the Gaertner group in wild rats in Suffolk, and Bainbridge and O'Brien[2] have lately shown that epidemics among guinea-pigs may be due to Gaertner's bacillus as well as to *B. suipestifer*[3]. The rat is therefore probably an important reservoir of Gaertner's bacillus in this country—perhaps in part owing to the use of bacillary rat poisons which, as Bainbridge showed[4], often contain or consist of that organism.

Methaemoglobinaemia is a well-known result of some forms of poisoning in man and animals (nitrites and allied bodies, aniline, chlorates, acetanilide, etc.). There have also occurred in man a series of cases of cryptogenetic cyanosis in which the blood is more or less brown. This condition may persist, more or less continuously, for years, and is usually associated with either diarrhoea or extravagant constipation. There is however no absolute justification for the term "enterogenous cyanosis", which is often used. In all the earlier cases the brown pigment in the blood was described as methaemoglobin, but, since the recognition of sulphaemoglobin[5], the majority have been found to be due to the presence of that substance[6] and cases of true methaemoglobinaemia must be regarded as being decidedly rare. One such case was pretty fully investigated by Gibson and Douglas[7] who, having on one occasion found what appears to have been a typical *B. coli* in a blood culture, named the condition "microbic cyanosis." Methaemoglobinaemia also appears to occur occasionally in the ill-defined group of diseases of young children known as Buhl's and Winckel's diseases[8]. Most of these seem to be severe infections of one kind or another; in

[1] Reports to the Local Government Board on public health and medical subjects, New Series, No. 52 (1911), p. 59. Dr G. H. K. Macalister informs me that these organisms have since been identified as genuine Gaertner strains.

[2] *Journal of Pathology*, Vol. XVI. (1911) p. 145.

[3] *Journal of Hygiene*, Vol. X. (1910) pp. 231, 287.

[4] *Journal of Pathology*, Vol. XIII. (1909) p. 457.

[5] See T. W. Clarke and W. H. Hurtley, *Journal of Physiology*, Vol. XXXVI. (1907) p. 62.

[6] S. West and T. W. Clarke, *Lancet*, 1907, Vol. I. p. 272; A. E. Russell, *Trans. Path. Soc.*, London, Vol. LVIII. (1907) p. 177; W. E. Wynter, *Proc. Roy. Soc. Med.* Vol. I. 1908, Clinical Section, pp. 48, 197; T. W. Clarke and R. M. Curtis, *Medical Record*, Vol. LXXVIII. (1910) p. 987.

[7] G. A. Gibson and C. C. Douglas, *Lancet*, 1906, Vol. II. p. 72; *Quart. Journ. Med.* Vol. I. (1907) p. 29.

[8] Knöpfelmacher, in Pfaundler and Schlossmann's *Hand. d. Kinderheilkunde*, Vol. I. (1910) p. 386.

a recent case, in which methaemoglobinaemia was observed, Röthler[1] isolated a staphylococcus. It is however by no means established that these obscure cases of human methaemoglobinaemia are infective in origin.

(1) *Changes in the blood of naturally infected rats.*

(*a*) *Methaemoglobin.* The brown colour of the blood is undoubtedly due to methaemoglobin and no evidence of the presence of any other brown or yellow pigment was obtained. The band in the red, which alone is clearly visible in the presence of oxyhaemoglobin, occupied the same place (620–640) in the spectrum as in solutions of methaemoglobin prepared by any of the ordinary methods; it remained unaltered on saturating with carbon monoxide and immediately disappeared on reduction. It was therefore certainly not sulphaemoglobin[2], in which the band which is conspicuous in the presence of oxyhaemoglobin lies more blueward (610–630), moves further blueward[3] on saturation with CO and is, for some time at any rate, unaltered by the addition of reducing agents.

The presence of methaemoglobin (or any other brown pigment) is best ascertained by examining the blood in the ordinary way with the Gowers-Haldane haemoglobinometer. Very small quantities, which would show nothing abnormal on spectroscopic examination, may be detected after saturation with carbon monoxide since the full pink tint of the pure CO-haemoglobin of the haemoglobinometer standard will not be realised. In examining such blood it is best to first saturate with CO[4]; the degree by which the sample fails to be as pink as the standard solution gives one a rough idea of the relative amounts of haemoglobin and methaemoglobin present. If a grain or two of sodium hydrosulphite ($Na_2S_2O_4$) are then added, the methaemoglobin is at once reduced to haemoglobin and this turned to CO-haemoglobin: the

[1] *Deutsche med. Woch.* 1911, p. 545.

[2] See T. W. Clarke and W. H. Hurtley, *Journal of Physiology*, Vol. xxxvi. (1907) p. 62; S. West and T. W. Clarke, *Lancet*, 1907, Vol. i. p. 272.

[3] As with the pigment which may appear in rabbit's blood in aniline poisoning (C. Price Jones and A. E. Boycott, *Guy's Hospital Reports*, Vol. lxiii. (1909) p. 313), and which may be sulphaemoglobin. In man aniline poisoning produces real methaemoglobinaemia.

[4] Owing to the possibility of the presence of cyanogen compounds in illuminating gas, it is best to use pure CO rather than coal gas to avoid difficulties of colour which might arise from the formation of cyanmethaemoglobin (see J. S. Haldane, *Journal of Physiology*, Vol. xxv. (1900) p. 230; A. S. Grünbaum, *ib.* Vol. xxxvi. (1907), *Proceedings*, p. iv).

percentage of haemoglobin in the sample is in this way readily ascertained. If, after reduction, the full pink of the standard cannot be obtained after repeated saturation with CO, it may be assumed that some brown pigment other than methaemoglobin is present.

The methaemoglobin is contained within the corpuscles; none is present in plasma or serum. No histological changes were found in the red cells which appeared to correspond to the presence of methaemoglobin, nor indeed do I know whether the change involved all the haemoglobin in some cells or some of the haemoglobin in all the cells. The proportion of haemoglobin changed was determined in but one spontaneous case; this animal was comparatively slightly affected and only 12 per cent. was methaemoglobin. In a number of inoculated rats however more than half the blood pigment was methaemoglobin, the highest figure observed being 68 per cent. (see Table IV below, p. 454). As might be expected, such animals showed marked dyspnoea.

After death the methaemoglobin is quickly reduced and disappears. In several cases in which the time of death of blue rats was accurately observed, the heart blood contained no methaemoglobin when the animal was examined as soon as one hour later. In no case was methaemoglobin present in affected rats which were found dead in the morning. As will be noted below, in many of the animals methaemoglobinaemia was associated with more or less profound anaemia and various forms of young red cells were abundantly present in the blood. It has been shown by Douglas[1] and by Morawitz and Pratt[2] that such anaemic blood consumes oxygen very much faster than normal blood, and that the effective agents are immature red cells which continue to be more or less actively metabolic after entering the circulating blood. The disappearance of methaemoglobin after death is presumably accelerated under these circumstances which may also operate compensatorily by way of limiting to some extent the conversion of haemoglobin during life.

(*b*) Apart from the presence of methaemoglobin, the blood of most of the blue rats was distinctly abnormal in other respects, more especially in the sense that more or less anaemia was present with some increase of blood volume. Neither the methaemoglobinaemia nor these other changes are constant in infected rats: the blood may be brown

[1] C. G. Douglas, *Journal of Physiology*, Vol. xxxix. (1910) p. 453.

[2] *München. med. Woch.* Vol. lv. (1908) p. 1817; Schmiedeberg's *Archiv*, Vol. lx. (1909) p. 298. See also M. Onaka, *Zeitschr. f. physiol. Chemie*, Vol. lxxi. (1911) p. 193, who attributes the chief action to the platelets.

but otherwise not abnormal (*e.g.* rat 1, Table II) or the blood pigment may be normal in quality but very deficient in quantity (*e.g.* rats 12 and 13, Table III). Infection may indeed be present with the usual pathological changes in the liver and spleen without any definite abnormality in the red corpuscles (*e.g.* rats 2, 3 and 4, Table III).

In examining the blood, samples for the estimation of the haemoglobin percentage and for the enumeration of red cells were obtained from the heart under chloroform anaesthesia. All the haemoglobin was then collected by washing out the circulation with citrated saline, the washings laked, mixed and titrated against standardised dilutions of rat or human blood. In titrating brown blood it is convenient to reduce both the sample and the standard with hydrosulphite immediately before titration and work with reduced haemoglobin, or, if the tint be preferred, saturate with carbon monoxide as well and titrate as CO-haemoglobin.

The distribution of the various values for normal rats has been described before for a small number of animals[1] and has been recently dealt with at length by Chisolm[2] on the basis of an extended series of measurements. His results as far as they are necessary for comparison with the present series of animals are as follows:

TABLE I. *Blood data for normal rats between 50 and 150 grammes body weight.*

	Average	Range of variation* 95 per cent. of normal rats are within the limits	Range of variation* All normal rats are within the limits
Total haemoglobin per kilo body weight expressed as cub. cent. of oxygen capacity	10·0	8·2— 11·7	7·3— 12·6
Blood volume per kilo body weight	63	49— 76	43— 83
Haemoglobin per cent. on human scale	88	71—104	63—113
Red cells in millions per cub. mill.	8·8	6·8— 10·8	5·8— 11·8

* The two standards of normality are defined by the means ± twice and three times the standard deviations respectively. These limits naturally apply only to individuals, not to the means of series.

For the purpose of calculating the colour index, the normal figures have been taken as nine million red cells and 88 per cent. haemoglobin, and in the tables the indices are given on this rat scale: on the human scale the normal colour index of the rat is 0·5. Stained blood films were also examined. In normal rats, especially young animals, a slight

[1] *British Medical Journal*, 5 November, 1910.

[2] *Quart. Journ. Exper. Physiol.* Vol. IV. (1911) p. 208.

degree of polychromasia is often present, but much polychromasia or the presence of nucleated red cells indicates active blood formation; from this, in the absence of any excess of haemoglobin, we may assume considerable blood destruction.

The quantities of haemoglobin and blood are usually expressed in terms of body weight. In sick animals however the weight may be abnormal owing to partial starvation, oedema or the like. To exclude this source of fallacy, it is desirable to have some independent criterion of bigness, the most convenient being the body length. The relation between body weight and body length in normal rats, according to Chisolm's observations, is given in Fig. 1, and the length of the

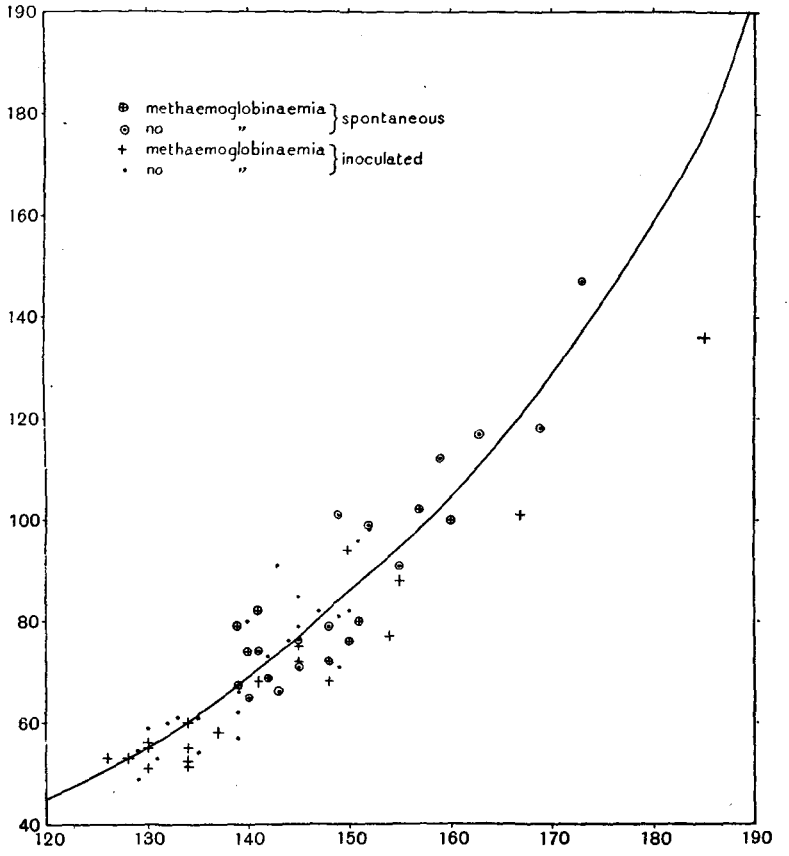

Fig. 1. Relation between body weight and body length. The normal relation is given by the continuous line. There is a tendency for the infected rats to be too light for their body length.

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individual animals is shown in the tables. After giving due weight to the consideration that an animal of abnormal body weight will, in adjusting its blood to pathological circumstances, probably take some note of the actual mass of tissue subsisting at the moment, it appears likely that it is on the whole better to refer the blood data to body length as well as body weight; the quantities of haemoglobin and blood in the present series of animals calculated on this basis also are therefore given in Tables VI and VII below.

TABLE II. *Blood data for spontaneous cases of methaemoglobinaemia. Liver necrosis present and spleen cultures positive in all cases.*

Number	Sex	Body weight (grammes)	Body length (mm.)	Haemoglobin (per cent.)	Total oxygen capacity per kilo body weight* (c.c.)	Blood volume per kilo body weight (c.c.)	Polychromasia	Nucleated red cells	Colour index
1	M	102	157	71	9·9	77	+	0	1·2
2	F	67	139	63	8·5	73	+	0	1·0
3	M	79	139	59	8·2	75	+ + +	0	—
4	F	69	142	58	7·0	66	0	0	1·1
5	M	76	150	57	11·3	107	+ +	0	—
6	M	80	151	55	7·9	78	+ + +	+ + +	—
7	F	100	160	49	7·6	84	+ + +	0	—
8	F	72	148	48	7·8	88	+	0	—
9	M	81	141	25	4·2	91	+ + +	+ + +	—
10	M	74	140	12	3·4	158	lost		—

* In these figures the methaemoglobin is reckoned as if it had the oxygen capacity of normal haemoglobin.

Table II shows that most of the blue rats were also anaemic in the sense that the percentage of haemoglobin was too low; many of them also had too little total haemoglobin, and five of them had very large blood volumes. Rats 9 and 10 show very profound degrees of anaemia; the latter animal had one-third the normal amount of haemoglobin and $2\frac{1}{2}$ times the proper quantity of blood. In no case is there any evidence of any compensatory production of normal haemoglobin to replace that which was put out of action by being converted into methaemoglobin.

In the infected rats which did not have brown blood (Table III) the changes are less pronounced. There is the same general tendency for the haemoglobin percentage to be low, the total haemoglobin to be low and the blood volume to be high, but only two (12 and 13) were severely anaemic.

(2) *Changes in other organs.*

The most marked changes are seen in the spleen and liver. The spleen is much enlarged, firm, rather pale and mottled over with white or pale yellow flecks. Histologically the pale flecks are found to be patches of necrosis; elsewhere the pulp is stuffed with blood, leucocytes and proliferating endothelial cells: in some cases, especially immediately round the necrotic areas, plasma cells are abundant; a good many are sometimes present in spleens which are apparently normal obtained from uninfected rats. The liver is rather pale and

TABLE III. *Blood data for spontaneous infected rats which did not have methaemoglobinaemia when examined. Liver necrosis present and spleen cultures positive in all cases.*

Number	Sex	Body weight (grammes)	Body length (mm.)	Haemoglobin (per cent.)	Total oxygen capacity per kilo body weight (c.c.)	Blood volume per kilo body weight (c.c.)	Polychromasia	Nucleated red cells	Colour index
1	M	147	173	103	9·2	48	0	0	1·1
2	F	65	140	80	10·4	70	0	0	0·7
3	M	101	149	78	10·2	71	+	0	—
4	F	91	155	70	9·6	74	+	0	—
5	M	118	169	70	10·7	83	+	0	1·0
6	M	76	145	67	7·7	62	—	—	—
7	F	79	148	66	9·3	76	+	0	—
8	M	117	163	65	7·4	61	+	+	1·2
9	F	63	143	60	8·7	78	+	0	0·8
10	M	74	141	58	7·6	71	+ +	0	—
11	F	71	145	56	8·5	81	+	0	0·8
12	M	99	152	47	6·0	69	+ +	+ + +	—
13	M	112	159	42	5·1	66	+ + +	0	—

sometimes definitely mottled on close examination but the white flecks are never so large as in the spleen. Histologically there are abundant focal necroses. There is nothing characteristic in these lesions; they are exactly the same as those described by Ledingham[1] in rats infected with plague, and his description will stand as a more ample account of the changes found in the animals, blue or not, infected with Gaertner. It is possible, however, as will be seen below, that the

[1] *Journal of Hygiene*, Vol. VII. (1907) p. 359. I noticed the same lesions in the acute Gaertner rat epidemic which occurred here in 1908.

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liver necrosis has some connection with the methaemoglobinaemia; its presence denotes at any rate a definite and effective infection as compared with an invasion of the body with bacilli which gives rise only to something akin to the "carrier" state.

(3) *Distribution of the bacilli.*

In blue rats there is always a septicaemia. Exact measurements were not made, but one drop of blood (about 0·02 c.c.) spread over an agar slope gave a continuous growth in all cases. In infected rats which were not blue, no septicaemia was generally found unless the animals were obviously ill. Cultures were usually made from the spleen whence the bacillus is readily obtained pure. Cultures from intestinal contents and scrapings of the walls of the bowels sometimes gave pure cultures but were more often negative as regards Gaertner's bacillus[1]. Faeces gave the same results; the animals nearly always had more or less profuse diarrhoea.

(4) *Results of inoculation with the rat bacillus*[2].

In order to reproduce the features of the natural disease by artificial inoculation, it is necessary to use appropriate doses of bacilli. If rats are given large doses (*e.g.* 1 c.c. 20 hours' broth culture intraperitoneally) of a recently isolated strain, they die within the next 48 hours and their blood is very seldom, and never more than a little, brown. After various trials a dose of about 0·2 c.c. of a 20 hours' broth culture given subcutaneously was found to give pretty good results and was generally used. Many experiments showed that with this dose some six or seven rats out of ten would generally develope methaemoglobinaemia to some extent. The results of two typical experiments are as follows:

Exp. 1. June 21. 10 rats inoculated subcutaneously with strain B (from rat 5, Table II), 0·2 c.c. 20 hours' broth culture.
,, 23. 1 ill.
,, 25. 2 found dead, 2 blue which were killed.
,, 26. 3 blue, killed.
July 1. 1 found dead.
,, 4. 2 survivors seem well; killed; spleen cultures negative, no liver necrosis.

[1] Non-lactose fermenters other than Gaertner's bacillus are so abundant in rats' intestines that, using as I did McConkey lactose-bile-salt-agar plates, it would be easy to miss a specific organism: no exhaustive search was made.

[2] Albino rats were always used in order that the changes in the general appearance of the animal might be as obvious as possible.

EXP. 2. October 20. 19 rats inoculated subcutaneously with strain D (from rat 13, Table III), 0·2 c.c. 20 hours' broth culture.
,, 22. 3 blue.
,, 24. 1 dead, 7 blue.
,, 25. 4 dead, 4 blue and killed.
,, 28. 1 dead, 2 blue, one of which was killed.
,, 29. 2 blue, one of which was killed.
,, 31. 1 blue.
November 2. Blue one recovered, killed; 6 others seem well, killed; of these two gave positive spleen cultures, one of which also had necrosis of liver.

In the first case therefore of ten rats, three died without any proof that they had had methaemoglobinaemia, five became blue, and two survived. In the second experiment, in which the bacillus used had been sub-cultured on artificial media for five months, of 19 rats one died, 12 became blue, one blue one recovered (a very exceptional occurrence) and six survived. Of the eight survivors (out of 29 rats) which had never appeared ill in the two experiments, the bacillus was isolated from the spleen in only two, and in only one was necrosis of the liver present. There is therefore no evidence in six of these animals that the inoculation had given rise to any generalised infection though they were all infected in the sense that each one had the small abscess at the site of injection which is the almost constant result of subcutaneous inoculation.

Similar results were obtained with 15 different strains of Gaertner's bacillus isolated from rats which were blue, anaemic but not blue or apparently healthy. After some months' cultivation their efficiency showed some signs of falling off, but the change was not very definite. There can therefore be little doubt that *methaemoglobinaemia in rats can be produced and is directly caused by Gaertner infection.* To satisfy the possible criticism which is suggested by the fact already noted that Gaertner's bacillus was rather common among the experimental rats, three sets of experiments were done on 34 rats, in which the organs of blue rats were ground up with saline, filtered through a Berkefeld filter and inoculated in large and small doses. No illness or blueness resulted and no evidence of a "filter passer" was obtained. The rats were subsequently tested by subcutaneous inoculation of live broth cultures of the rat bacillus (strains D and 41); they proved to be susceptible to a normal degree.

The changes in the liver and spleen were just the same as in the naturally-infected animals. It was ascertained however that at least four or five days must elapse after inoculation before they reached the stage

usually found in natural infections. In five animals killed 13 or 14 days after inoculation, one of them having been blue and recovered and the other four having shown no signs of illness, a localised purulent myocarditis of the left ventricle was found—a lesion not noted in the spontaneous cases. With regard to the septicaemia, inoculation enabled one to ascertain that a slight septicaemia (two to five colonies from 0·02 c.c. of blood) was present when the animal first began to look blue or show any signs of general illness. Afterwards the septicaemia increased to the stage found in the naturally infected blue rats.

The blood was examined quantitatively in a number of inoculated animals. Table IV shows the results in a series of animals which had brown blood. The figures differ from those obtained from naturally infected blue rats chiefly in that the total oxygen capacity is not so much reduced and histological signs of blood regeneration were less marked and not infrequently absent. In two animals however—numbers 17 and 18—a notable degree of haemoglobin deficiency was achieved,

TABLE IV. *Blood data for cases of methaemoglobinaemia produced by inoculation of bacillus from rats. Liver necrosis present and spleen cultures positive in all cases.*

Number	Sex	Body weight (grms.)	Body length (mm.)	Haemoglobin (per cent.)	Total oxygen capacity per kilo body weight (c.c.)	Percentage of haemoglobin changed to methaemoglobin	Effective oxygen capacity per kilo body weight (c.c.)	Blood volume per kilo body weight (c.c.)	Polychromasia	Nucleated red cells	Colour index	Days after inoculation
1	F	136	185	72	10·7	**37**	**6·8**	81	0	0	1·1	4
2	F	55	130	63	10·8	—	—	93	+	0	1·0	6
3	F	52	134	63	10·5	**61**	**4·1**	91	+	0	1·0	5
4	M	68	141	58	10·8	**68**	**3·4**	100	0	0	1·1	5
5	F	101	167	57	10·4	**45**	**5·7**	99	0	0	1·3	4
6	F	55	134	56	10·0	**56**	**4·4**	96	0	0	1·1	5
7	M	75	145	55	10·2	**55**	**4·6**	100	+ +	+	0·9	9
8	F	56	130	54	9·9	**45**	**5·5**	99	+	0	1·1	5
9	M	72	145	52	10·3	**59**	**4·2**	108	0	0	1·3	5
10	M	53	126	51	8·7	**60**	**3·5**	93	+	0	0·8	9
11	F	88	153	51	8·1	**32**	**5·5**	87	0	0	0·9	7
12	M	60	134	50	8·1	**51**	**4·0**	88	+	+	1·1	9
13	M	94	150	50	6·9	trace	**6·9**	74	+ +	0	1·2	18 (?)
14	M	52	134	43	8·3	**18**	**6·8**	105	0	0	1·0	5
15	F	58	137	42	7·6	**36**	**4·9**	98	+ +	+	1·1	5
16*	F	77	154	41	7·5	**58**	**3·1**	99	+ +	0	1·1	14 (?)
17	F	68	148	28	5·4	**54**	**2·5**	105	0	0	1·0	6
18	M	51	130	26	5·1	**15**	**4·4**	107	+	0	1·1	6

* This rat was found naturally infected with *Trypanosoma lewisi*.

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and if allowance is made for wasting and the figures calculated on the weight normal for the observed body length rather than on the observed weight at the time of death most of the rats prove to be short of haemoglobin; this is shown graphically in Fig. 2. In other respects the results closely resemble those found in spontaneous blue rats. Fifteen of the eighteen animals have a low percentage of haemoglobin outside the normal limits and sixteen have an equally abnormal high blood volume. On the average the blood volume exceeds the normal by about 50 per cent.

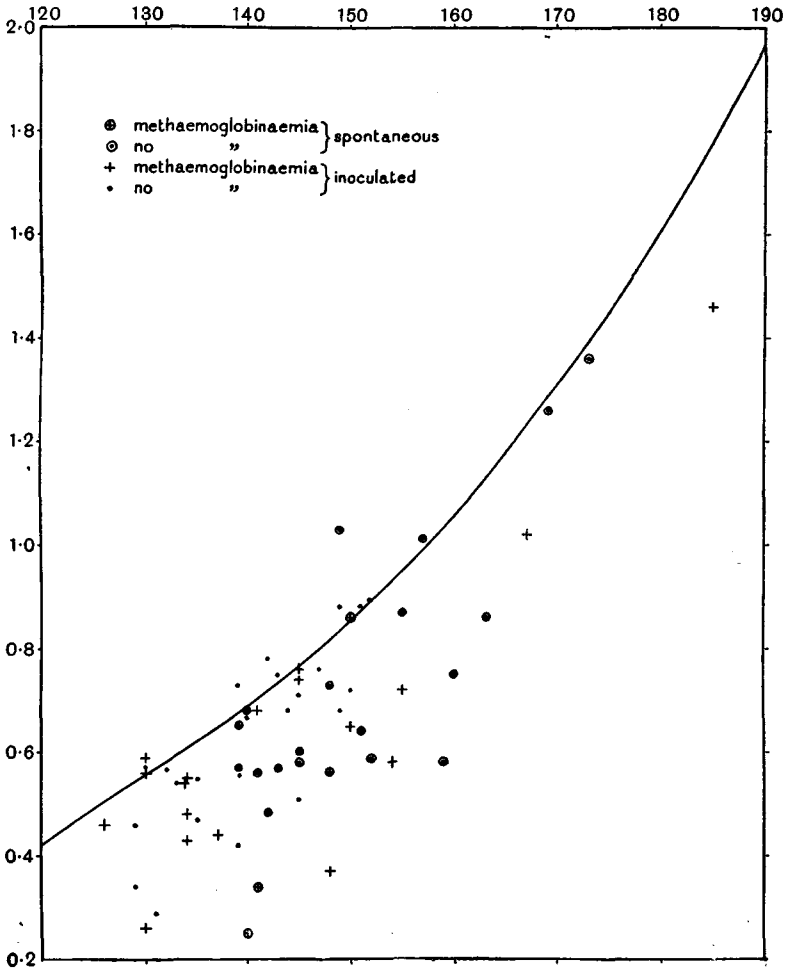

Fig. 2. Relation between body length and total oxygen capacity. The normal relation is given by the continuous line. Most of the animals are anaemic.

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Information was obtained from this series of animals with regard to the proportion of haemoglobin converted into methaemoglobin. At first various colorimetric methods were tried[1]: they proved unsatisfactory. Good results were however obtained as follows. About 2 c.c. of blood was obtained from the heart and the oxygen capacity of the haemoglobin and the methaemoglobin together was determined, after saturation with CO and reduction, with the haemoglobinometer in the usual way. After saturation with air the oxygen capacity was directly determined by the ferricyanide method in the Barcroft-Haldane-Brodie apparatus[2].

TABLE V. *Blood data for animals inoculated with the rat bacillus which did not develop methaemoglobinaemia.*

Number	Sex	Body weight (grms.)	Body length (mm.)	Haemoglobin (per cent.)	Total oxygen capacity per kilo body weight (c.c.)	Blood volume per kilo body weight (c.c.)	Polychromasia	Nucleated red cells	Colour index	Days after inoculation
Group A. Animals showing signs of general infection when killed.										
1	F	57	139	77	10·0	70	+	0	1·0	13
2	M	82	150	76	8·8	76	0	0	1·0	13
3	M	66	139	73	11·0	81	0	0	1·0	13
4*	F	73	142	64	10·7	91	+ +	+	1·0	13
5	F	76	144	60	8·9	81	+	0	1·0	13
6	F	85	145	60	8·4	75	0	0	1·0	12
7	M	61	135	56	9·0	87	+ +	+	0·9	10
8	M	49	129	53	9·5	96	+ +	+	1·0	12
9	F	54	135	46	8·8	103	+	+	0·9	10
10	F	54	129	40	6·3	85	+ +	+	1·3	9
11	M	79	145	37	6·4	95	+ + +	+	1·1	12
Group B. Animals showing no signs of infection when killed.										
1	F	71	149	97	12·4	69	0	0	1·0	16
2	M	91	143	82	8·2	54	+	0	1·1	18
3	M	96	151	77	9·1	64	+ +	0	1·0	14
4	M	82	147	75	9·2	67	0	0	1·0	12
5	M	98	152	72	9·0	68	0	0	1·0	13
6	M	81	149	69	8·4	66	0	0	0·9	14
7†	F	60	132	61	9·6	85	+	+	1·0	9

* This rat had had well-marked methaemoglobinaemia but had recovered.

† This rat was found naturally infected with *Trypanosoma lewisi*.

[1] See J. S. Haldane, R. H. Makgill and A. E. Mavrogordato, *Journal of Physiology*, Vol. xxi. (1897) p. 169.

[2] *Journal of Physiology*, Vol. xxviii. (1902) p. 232. The present much improved form may be obtained from F. P. Rittershaus, 47 Gray's Inn Road, W.C.

The whole procedure occupies only a few minutes and the results seem quite satisfactory if care is taken to obtain the maximum reading before oxygen begins to be absorbed in appreciable quantities: this is especially liable to happen if the blood contains immature red cells[1]. The difference between the two determinations gives the amount of methaemoglobin. For example, the haemoglobinometer readings were 44 and 45 per cent., mean 44·5 per cent., giving an oxygen capacity of $\frac{44{\cdot}5 \times 18{\cdot}5}{100} = 8{\cdot}23$ c.c. per cent. The same blood gave by direct experiment an actual oxygen capacity of 3·29 per cent. The methaemoglobin was therefore $8{\cdot}23 - 3{\cdot}29 = 4{\cdot}94$ or 60 per cent. of the whole. The data given in Table IV were obtained in this way. From there it appears that in an average case about half the haemoglobin is converted; in no case was a conversion of more than two-thirds observed.

A number of the rats inoculated with the rat bacillus did not develop obvious methaemoglobinaemia[2]. On some of these blood examinations were made. The results are shown in Table V which includes only the survivors from batches of inoculated rats, some of each lot having developed methaemoglobinaemia. The first eleven animals were found post-mortem to have definite signs of general infection—liver necrosis, myocarditis or the characteristic spleen containing the bacillus. These show much the same blood changes as were found in the blue animals but to a less marked degree; the severe anaemias observed in what may be regarded as the corresponding group of naturally infected animals (see Table III) were not reproduced. In the remaining rats there was a small abscess at the site of inoculation but no signs of general infection, nor was the organism isolated from the spleen. The blood data show only a somewhat doubtful tendency towards slight anaemia.

In considering the significance of these various changes in the blood it is necessary to separate, as far as may be, those which may be due to the presence of methaemoglobin from those due to the infection. The diminution of haemoglobin percentage, the increase in blood volume, the diminished total oxygen capacity and the histological signs of

[1] C. G. Douglas, *Journal of Physiology*, Vol. xxxix. (1910) p. 458.

[2] That is, did not appear blue or anaemic on careful comparison with healthy white rats. It did not seem necessary to obtain actual samples of blood in all cases, and indeed it is hardly fair to the experiment to do so daily. The presence of small degrees of methaemoglobinaemia cannot therefore be positively affirmed to have been absent in all cases though, in the rats which were examined, no methaemoglobin was ever found unless a moderate suspicion of its presence had been aroused by the general appearance of the animal.

regeneration in the blood are probably due to the infection alone. Chisolm[1] has pointed out that these changes may occur also in rats bearing transplanted sarcomata, as well as in those infected with Gaertner's bacillus, *Bacillus suipestifer* (*B. aertryck*), or rat scabies. In some cases the only change is a diminution of haemoglobin percentage with an increase of blood volume, in other words an anaemic plethora rather than a true anaemia. As has been shown, the most constant change in

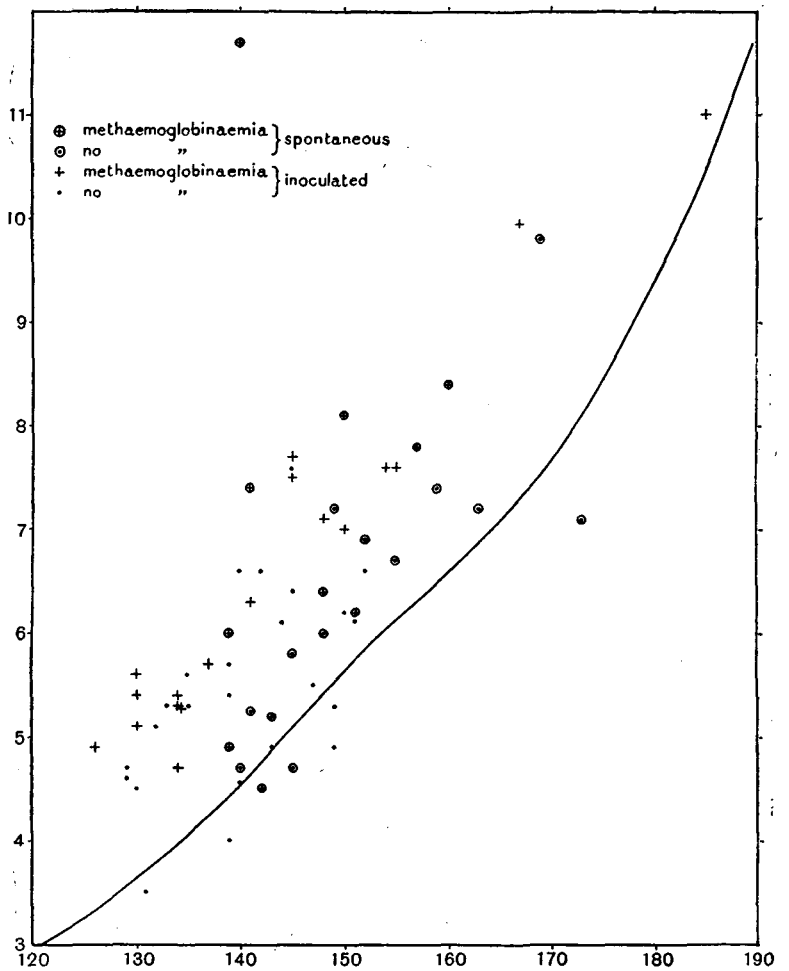

Fig. 3. Relation between body length and blood volume. The normal relation is given by the continuous line. Most of the animals have too much blood.

[1] *Journal of Pathology*, Vol. xv. (1911) p. 358; Vol. xvi. (1911) p. 152.

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the Gaertner-infected rats has been a tendency towards the same condition, and this therefore is probably attributable to general illness of almost any kind. Table VIII gives the blood changes found in a few animals inoculated with *B. suipestifer*, showing that much the same condition is produced as by inoculation with Gaertner's bacillus.

TABLE VI. *Showing the total haemoglobin in terms of the normal values for rats of the given body lengths: normal* = 100*.

Naturally infected rats				Inoculated rats			
blue (Table II)		not blue (Table III)		blue (Table IV)		not blue (Table V)	
Number		Number		Number		Number	
1	103	1	99	1	83	A 1	84
2	84	2	98	2	106	2	83
3	96	3	104	3	91	3	107
4	67	4	134	4	95	4	109
5	100	5	69	5	85	5	89
6	72	6	76	6	92	6	92
7	73	7	73	7	99	7	89
8	68	8	77	8	101	8	86
9	48	9	77	9	96	9	76
10	37	10	80	10	90	10	63
		11	78	11	79	11	66
		12	67	12	81	B 1	105
		13	56	13	75	2	101
				14	72	3	100
				15	68	4	94
				16	62	5	100
				17	45	6	81
				18	48	7	100

* The limits of normality for individuals are (Table I) 82–117 and 73–126.

Owing to the presence of methaemoglobin any anaemia is in effect exaggerated and there may be an extreme defect in the actual oxygen-carrying power of the blood. Thus in rat 17 of Table IV, the blood per unit volume had an actual and effective oxygen capacity of only one-quarter of the normal, and in rats 9 and 10 of Table II this may be presumed to have been reduced to about one-fifth. Rats with the full normal quantum of red blood corpuscles also become effectively anaemic; thus in Table IV there are seven animals (1, 3, 4, 5, 6, 7 and 9) with more than 10 c.c. total oxygen capacity per kilo, the mean being 10·4 c.c. On the average 54 per cent. of haemoglobin is methaemoglobin, and the effective oxygen capacity varies from 3·4 c.c.

to 6·8 c.c. per kilo, mean 4·7 c.c. Under these circumstances it might be expected that some over-production of haemoglobin would occur in an attempt to compensate for that put out of action as happens in animals in which a proportion of the haemoglobin is rendered useless

TABLE VII. *Showing the blood volumes in terms of the normal values for rats of the given body lengths: normal* = 100*.

Naturally infected rats				Inoculated rats			
blue (Table II)		not blue (Table III)		blue (Table IV)		not blue (Table V)	
Number		Number		Number		Number	
1	126	1	82	1	99	A 1	94
2	114	2	107	2	148	2	115
3	139	3	128	3	125	3	125
4	100	4	166	4	141	4	146
5	150	5	85	5	131	5	128
6	112	6	97	6	140	6	132
7	128	7	96	7	154	7	135
8	121	8	102	8	161	8	139
9	165	9	111	9	160	9	143
10	269	10	118	10	153	10	135
		11	119	11	133	11	154
		12	122	12	139	B 1	93
		13	116	13	129	2	105
				14	143	3	111
				15	138	4	107
				16	129	5	119
				17	136	6	100
				18	157	7	141

* The limits of normality for individuals (Table I) are 78–121 and 68–132.

TABLE VIII. *Blood data for animals inoculated with* Bacillus suipestifer.

Number	Sex	Body weight (grms.)	Body length (mm.)	Haemoglobin (per cent.)	Total oxygen capacity per kilo body weight (c.c.)	Blood volume per kilo body weight (c.c.)	Polychromasia	Nucleated red cells	Colour index	Days after inoculation
1	F	59	130	68	9·6	76	0	0	0·8	12
2	F	61	133	55	8·9	87	+	0	0·9	8
3	M	80	140	55	8·4	82	+ +	+	1·1	12
4	F	53	131	45	5·5	66	+ +	+	1·2	8
5	F	62	139	40	6·8	92	+	0	1·1	8

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by chronic CO-poisoning[1]. There is however no evidence that this occurs except that histological signs of active blood formation may occasionally (rat 5, Table II, rat 7, Table IV, rat 4, Table V) be present in animals which already have, for their weight and length, fully the normal amount of haemoglobin—an observation which becomes perhaps less significant when it is noted that these animals also had blood volumes much too big, and bigger than similar rats in which no signs of regeneration were present. The time during which a substantial degree of methaemoglobinaemia prevails might appear too short for compensatory production of red cells: this however is not the case since experiments show that after haemorrhage[2] or under the influence of diminished atmospheric pressure a rat may generate an amount of haemoglobin equal to half its normal quantum within a week. The failure of compensation may therefore be attributed to the sickness of the animal. In general we may note that any production of fresh haemoglobin would not necessarily be effective since there are grounds for supposing that the amount converted into methaemoglobin is not determined by the absolute activity of the methaemoglobin-producing mechanism but in a proportion which is a product of the balance between this mechanism and the reducing substances present in the blood.

(5) *The occurrence of the bacillus in rats.*

The same bacillus was invariably obtained from naturally infected rats with methaemoglobinaemia. Of these during nine months ten were identified out of rather more than 1000 rats which were used in the laboratory during that time. No doubt more occurred without being noticed, though the absence of any obviously excessive mortality among the stock rats excludes any considerable prevalence of methaemoglobinaemia at any time. The numerical frequency of infected rats which did not show methaemoglobinaemia cannot be definitely stated, since at first only minute fragments of splenic tissue were cultivated. Later on the whole spleen was incubated in dulcite bile-salt broth[3], and if acid and gas developed sub-cultured in the same medium before

[1] G. G. Nasmith and D. A. L. Graham, *Journal of Physiology*, Vol. xxxv. (1906) p. 32. Polycythaemia is described in some human cases of chronic methaemoglobinaemia from poisoning with *e.g.* acetanilide, but the total haemoglobin does not appear to have been determined.

[2] *Journal of Pathology*, Vol. xvi. (1911) p. 269.

[3] On the advantages of preliminary cultivation in dulcite media for the isolation of organisms of this group, see Boycott, *Journal of Hygiene*, Vol. vi. (1906) p. 35.

plating. In this way it may more fairly be assumed that any small number of bacilli in the spleen would be found; using only such bits of splenic pulp as may be dug out with a platinum needle, it is doubtful whether very much weight ought to be attached to negative results. Working in this way, 50 consecutive apparently normal rats were examined, most of which had been killed for other purposes. The bacillus was isolated from the spleen in eight and from the intestines once. Of these nine rats, liver necrosis was present in five and absent in four. In another series of 27 rats which died after inoculation with pneumococci, six were found infected by spleen culture, in five of which liver necrosis was present. Roughly speaking therefore about 20 per cent. of the animals seem to have harboured the bacillus.

During the course of the experiments, cultures were made from ten wild rats (*Mus decumanus* sp. *norvegicus*) caught in the animal houses and the laboratory: Gaertner's bacillus was isolated from the spleen of one of them.

(6) *Modes of infection.*

Infection is conveyed by feeding but not apparently with any great certainty. Of four rats fed with half a 20 hours' agar culture, one became blue; of six rats fed with two old agar cultures and 20 c.c. of four days old broth culture, two became blue; and of four fed on the corpse of a blue rat, none gave positive results.

Infection also results by living together[1]. Thus six rats, which had been under observation for eight days, had added to their cage three rats blue from inoculation; these died on the first, second and eighth days respectively, their bodies being removed uneaten. Of the normal rats two developed methaemoglobinaemia and died on the eleventh day, and another on the sixteenth day: the other three survived.

If contact and feeding are combined, infection may spread more widely. Thus, in one experiment, to a cage of nine normal rats were added, over a space of 13 days, seven rats which had developed methaemoglobinaemia in sequence to inoculation; their bodies were all more or less eaten after death. One normal rat died on the eighth day, but the spleen and liver were normal and the spleen culture negative. On the thirteenth day the other eight were removed to a clean cage, and

[1] As already noted, the bacillus is not readily obtained from the bowels. Considering however the facts that the evacuations are very often blood-stained and that the organism is present in the blood in large numbers, this is probably due to defective technique.

during the next four days five of them developed methaemoglobinaemia and died. As in the previous experiment, there was some lag in the development of infection.

Fleas were never found on the stock rats. Infection was however to some extent present in the wild rats living in the neighbourhood and these harbour both *Ceratophyllus fasciatus* and *Pulex cheopis* in considerable numbers[1]. Several experiments with both parasites were done[2] to see whether infection would spread from rat to rat by contiguity without contact in the presence of abundant fleas and whether infection could be transferred by transferring fleas which had sucked the blood of blue rats. They were all negative. By cultivating fleas which have fed on blue rats, the bacillus may be recovered pretty easily on the first and second day, but on the fifth day and later the results were uniformly negative.

(7) *The identity of the causative organism*[3].

The organism isolated corresponds culturally with *B. enteritidis* of Gaertner. It is an actively motile, Gram-negative bacillus growing freely on ordinary media at room temperature and in the incubator and, though rather less luxuriantly, anaerobically: no indol: no liquefaction of gelatin: litmus milk acid for the first two or three days, later becoming markedly alkaline. Grown in broth, it ferments and produces gas with dextrose, laevulose, galactose, maltose, dulcite, mannite, sorbite and arabinose, but gives no change with lactose, cane-sugar, glycerin, dextrin, raffinose, inulin, adonite, amygdalin or erythrite. A good deal of confusion arose at first from the fact that strains freshly isolated from rats generally produced acid only, and never more than a bubble or two of gas, when grown in maltose broth in the usual way with Durham's tubes. Bainbridge has noted that laboratory cultures of

[1] *Guy's Hospital Gazette*, Vol. xxv. (1911) pp. 73, 318.

[2] These experiments were done at the time of year when the original epidemic occurred; this may be an important point as has been shown in the case of plague transmission (*Journal of Hygiene*, Vol. viii. (1908) p. 279).

[3] I am very much indebted to Dr F. A. Bainbridge who furnished me with the standard cultures of Gaertner's bacillus, *B. aertryck*, paratyphoid A, paratyphoid B, and *B. suipestifer*, which he used in his enquiry into this group of organisms (*Journal of Pathology*, Vol. xiii. (1909) p. 443), and was also good enough to give me agglutinating rabbit sera prepared with them. See also on the differentiation of these Gaertner strains H. R. Dean, *Proc. Roy. Soc. Med.* 1911, Vol. iv. Path. Sect. p. 251; G. Sobernheim and E. Seligmann, *Zeitschr. f. Immunitätsf.* Vol. vii. (1910) p. 342.

Gaertner's bacillus and the allied organisms produce less gas from maltose than from the other fermentable sugars, and in my own strains definite, though always small, gas production appeared after several sub-cultures outside the body. In isodulcite (rhamnose) the standard Gaertner produced acid and a little gas in 24 hours, whereas the rat bacilli caused no change for a week and then only acid appeared.

Culturally therefore the organism belongs to one of the three groups, (1) Gaertner's bacillus, (2) *B. aertryck* and *B. suipestifer* or (3) paratyphoid B. That the rat bacillus is a true Gaertner is shown by agglutination and absorption tests.

TABLE IX. *Agglutination with standard Gaertner serum (Bainbridge).*

	1/500	1/1000	1/2000	1/5000	1/10,000
Standard Gaertner	+ + +	+ + +	+ + +	+ +	0
Rat bacillus A	+ + +	+ + +	+ + +	+ +	0
,, D	+ + +	+ + +	+ + +	+ +	0
,, M	+ + +	+ + +	+ + +	+ +	+
,, 16	+ + +	+ + +	+ + +	+	0
,, 9	+ + +	+ + +	+ + +	+	0
Suipestifer	0	0			
Paratyphoid B	0	0			
Suipestifer E	0				
Gaertner 2	+ + +	+ + +	+ + +	+ +	+
Suipestifer 4	0	0			
Gaertner R	+ + +	+ + +	+ + +	+ +	+
Suipestifer M	0	0			

The Gaertner serum was then absorbed with excess of standard Gaertner, rat bacillus M, 16 and 9, suipestifer and paratyphoid B, the growth off two 48 hours' agar slopes being mixed with 0·2 c.c. serum diluted $\frac{1}{50}$ for two hours at 37°. The concentration of serum in the test was therefore between $\frac{1}{100}$ and $\frac{1}{200}$.

TABLE X.

Absorbed with:—	Standard Gaertner	M	16	9	Suipestifer	Paratyphoid B
Standard Gaertner	0	0	0	0	+ + +	+ + +
Rat bacillus M	0	0	0	0	+ + +	+ + +
,, 16	0	0	0	0	+ + +	+ + +
,, 9	0	0	0	0	+ + +	+ + +

With Aertryck (*suipestifer*) agglutinating serum, the homologous organisms reacted $\frac{1}{2000}$ to $\frac{1}{5000}$; rat bacillus A, D and M gave no agglutination at $\frac{1}{200}$. Further experiments were made with the sera of

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rabbits repeatedly inoculated with dead, and later living, cultures of rat bacillus D and M. Both sera gave the same result: those for strain D were as follows:

TABLE XI. *Agglutination with rat bacillus serum, strain D.*

	1/20	1/100	1/500	1/1000	1/2000	1/5000	1/10,000	1/20,000
Rat bacillus D	+++	+++	+++	+++	+++	++	++	+
,, 19	+++	+++	+++	+++	+++	++	++	+
,, B	+++	+++	+++	+++	++	++	++	+
,, Y	+++	+++	+++	+++	+++	+++	+++	++
Standard Gaertner	+++	+++	+++	+++	+++	+++	+++	++
Suipestifer	+++	0	0	0	—	—	—	—
Paratyphoid B	+++	++	+	0	—	—	—	—

After absorption (diluted $\frac{1}{500}$) the results were:

TABLE XII.

Absorbed with :—	Paratyphoid B	Suipestifer	D	B	Gaertner
Rat bacillus D	+++	+++	0	0	0
,, 19	+++	+++	0	0	0
,, B	+++	+++	0	0	0
,, Y	+++	+++	0	0	0
Standard Gaertner	+++	+++	0	0	0
Suipestifer	0	0	0	0	0
Paratyphoid B	0	0	0	0	0

These results show clearly therefore that *the rat bacillus is Gaertner's bacillus* in the sense that it is indistinguishable from Bainbridge's standard strain of Gaertner, *i.e.* from the original strain isolated by Gaertner. They also demonstrate that the various strains of the rat bacillus isolated at different times are identical. Strains A, B, 16 and 19 were isolated from rats with spontaneous methaemoglobinaemia on 13th May, 18th May, 30th November and 8th December respectively; D, M, Y and 9 from rats without methaemoglobinaemia, and in the case of 9 without signs of illness, on 24th May, 29th June, 2nd November, and 22nd November respectively.

(8) *Inoculation with other strains of Gaertner's bacillus.*

Experiments were made with five other strains of Gaertner's bacillus, all of which had been identified by agglutination and absorption as well as by cultural tests.

Of strains isolated from rats I had two. One of these (Gaertner R, Table IX) had been obtained from the acute epidemic here in 1908.

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I am indebted to Dr Eyre for its preservation. With doses of 0·2 c.c. 20 hours' broth culture subcutaneously, of ten rats, five died without developing methaemoglobinaemia (two on the first day, one on the second, two on the fourth) and one was blue and died on the fifth day. For the other rat strain I have to thank Dr Bainbridge who isolated it recently (1911) from another acute epidemic. With the same dose, of ten rats, four showed well-marked methaemoglobinaemia, four died and two survived.

I have also to thank Dr Bainbridge and Dr O'Brien for a Gaertner culture recently isolated from a natural epidemic among guinea-pigs[1]. Ten rats received 0·5 c.c. 20 hours' broth subcutaneously; they all had exceptionally large lesions at the site of inoculation but only one became blue and died, the other nine showing no signs of illness.

Of old laboratory strains, incomplete experiments have been made with two. The original standard strain of Gaertner gave negative results, but after six quick passages through the peritoneal cavity of rats, two animals out of ten developed methaemoglobinaemia. Another old strain (Gaertner 2, Table IX) from a human source given me by Dr Eyre was also negative at first, but after passage gave positive results in six out of eight rats.

In the present instance there is of course more than the usual possibility that the organism recovered from the passage animals was not the same as that put into them. Assuming however that no change occurred, all five strains of Gaertner, from rats, guinea-pigs and men, produced methaemoglobinaemia under appropriate circumstances.

(9) *Vaccination experiments.*

Further evidence with regard to the relationship of the rat bacillus and Gaertner's bacillus to the disease was obtained by vaccination experiments.

(*a*) Ten rats received subcutaneously 0·3 c.c. 20 hours' broth culture of the rat bacillus (strain D) heated to 60° C. for 1¼ hours: fifteen days later they were inoculated with 0·2 c.c. live culture (strain D). Two died, seven went blue and one survived. Of 19 controls six died, seven went blue and six survived.

(*b*) 17 rats had 0·2 c.c. subcutaneously of a 20 hours' broth culture of the rat bacillus (strain D) heated to 55° C. for 1½ hours, and twelve

[1] *Journal of Pathology*, Vol. xvi. (1911) p. 145.

days later a second dose of 0·5 c.c. of a similar preparation. Twelve days afterwards, eight of them were inoculated with 0·5 c.c. live culture (strain 41) subcutaneously: five went blue, three survived; of ten controls all died in seven days, nine being found blue before death. The other nine vaccinated rats were exposed to contact with blue rats (strain D): one died, four went blue and four survived.

(*c*) 17 rats were inoculated subcutaneously with 0·2 c.c. 20 hours' broth culture of the original Gaertner strain which was relatively avirulent: three died. The remaining 14 animals, 12 days later, received 0·5 c.c. 20 hours' broth culture of rat bacillus (strain 41) and two died; after further 11 days the 12 survivors had 1 c.c. subcutaneously and all survived.

Vaccination with dead cultures seemed therefore to confer no protection. With a live culture however 12 of 17 rats resisted a dose which killed all of ten controls.

(10) *Experiments with animals other than rats.*

A number of experiments were made with rabbits, guinea-pigs and mice, but in no case was methaemoglobinaemia produced by inoculation with any strain of Gaertner's bacillus. The inoculated animals either presented no signs of illness or developed a septicaemia and died; in no case was any necrosis of liver found similar to that which appears to be constant in infected rats. Particular attention was paid to mice, and the dose given carefully adjusted so that they died in about five days; no trace of brownness could however be found in the blood of 40 animals. Guinea-pigs inoculated with the Gaertner strain derived from a guinea-pig epidemic did not show methaemoglobinaemia.

(11) *Experiments on rats with other organisms.*

As regards other members of the Gaertner group, rats were inoculated with the standard strains of paratyphoid A, paratyphoid B, *B. aertryck* and *B. suipestifer* as well as with three other laboratory strains which proved to be *suipestifer* (*suipestifer* E, 4 and M, Table IX). One rat inoculated with paratyphoid A developed a slight degree of methaemoglobinaemia and from the spleen paratyphoid A alone was recovered; the possibility of this case being a natural infection with Gaertner's bacillus cannot be altogether excluded. Otherwise the results were all negative. The organisms are all more or less pathogenic for rats, cause

a large local lesion at the site of inoculation and in a proportion of cases liver necrosis also. Experiments after passage through rats have not yet been made.

Many inoculations were done with the pneumococcus, three strains recently isolated from human beings by inoculation of rabbits being employed. This organism was used because rats can be killed by it with a sub-acute illness and because it is *in vitro* exceptionally active in producing methaemoglobin from blood. No cases of methaemoglobinaemia arose in the inoculated rats and, in the absence of Gaertner infection, liver necrosis was not found. As it happened, among the rats dying of pneumococcus infection there were several which proved on spleen culture to be examples of spontaneous Gaertner infection.

(12) *Pathogenesis of methaemoglobinaemia.*

The nature of methaemoglobin and its relation to haemoglobin are unfortunately not understood. It seems however clear that in methaemoglobin and in oxyhaemoglobin the oxygen is joined on to the rest of the molecule in different ways, the amount of oxygen being the same in both cases. Maethaemoglobin may be produced from oxyhaemoglobin by allowing sterile blood to stand, especially at body temperature, and also by a large number of oxidising agents; it is perhaps open to doubt whether the "reducing" agents (*e.g.* nitrites) which make methaemoglobin do so by actual reduction. By reducing agents methaemoglobin is immediately converted to reduced haemoglobin and to oxyhaemoglobin if free oxygen is available[1].

How the methaemoglobin is produced in rats by Gaertner infection I have not been able to discover. There are however a variety of considerations which may be noted with a view to limiting the discussion of the problem.

The methaemoglobin is in the rats entirely within the corpuscles. Hence the agent which produces it may be presumed to be one which can penetrate the red corpuscles. Potassium ferricyanide for example does not cause methaemoglobinaemia when injected directly into the circulation since the corpuscles are impermeable to it; for the same reason chlorates do not produce methaemoglobinaemia in the rabbit though they do in man. It is conceivable that the corpuscles containing methaemoglobin have been so made in the bone-marrow; the rapidity

[1] I have been over most of the ground with rats' blood and haemoglobin without finding that they differ from those more commonly employed.

with which more than half the haemoglobin may be altered and the absence from the blood, in some cases, of any marked signs of marrow activity render this view hardly possible.

In the next place the persistence of marked methaemoglobinaemia for two, three or even four days before death shows that the substance or mechanism which produces methaemoglobin must be in continued action during that period. At all times in the circulating blood the proportion of haemoglobin converted must be the result of a balance between the methaemoglobin-forming activity and the reducing activity. All the circulating haemoglobin cannot be turned into methaemoglobin because of the reducing substances present in the blood. After death, when the supply of oxygen fails, these reducing substances quickly cause the disappearance of all the methaemoglobin. The same happens during life if methaemoglobin is formed as the result of the administration of a single dose of a suitable poison. Thus a rabbit may be very nearly killed by a dose of sodium nitrite[1] or of the very active phenyl-hydroxylamine[2], but if it can tide over the worst period of oxygen deficiency the methaemoglobin is soon reduced and in a few hours the animal is practically well again.

It appears that the agent which produces methaemoglobin is not in the blood. If one makes mixtures of normal blood and blood from a blue rat there is no increase in the proportion of methaemoglobin in the mixture either at once or after incubating at 37° for six or eight hours with or without a stream of oxygen bubbling through.

The bacilli themselves are not direct producers of methaemoglobin. Animals inoculated with large doses of dead bacilli do not develop methaemoglobinaemia. If the bacilli are grown on blood-agar or in solutions of blood (either from rabbits or rats), methaemoglobin may be produced, but not invariably, after three or four days' incubation. The same result may however be obtained with many different organisms or even with sterile blood. In the same way neither live nor dead bacilli would produce methaemoglobin within a few hours from whole or laked blood if the mixture was constantly aerated with a stream of oxygen. Experiments with media of which rat formed the basis instead of cow were also negative, as were also those in which the organisms were grown in fresh or heated rat serum mixtures.

[1] Haldane, Makgill and Mavrogordato, *Journal of Physiology*, Vol. xxi. (1897) p. 171.

[2] Lewin, Schmiedeberg's *Archiv*, Vol. xxxv. (1895) p. 401. I am much indebted to Mr W. C. Ball who made some of this unstable substance for me: it produces nothing else but methaemoglobin in the blood of rabbits.

No extract—watery, alcoholic or chloroformic—could be obtained from blue rats which would *in vitro* produce methaemoglobin.

A series of experiments was also made in which the organs of blue rats were washed free of blood, pounded up, suspended in normal rats' blood and incubated with a stream of oxygen bubbling through. In these the liver in some experiments gave very definite results, a large proportion of the haemoglobin being converted to methaemoglobin in one hour. Controls with the livers of normal rats, with the livers of both blue and normal rats which had been killed by immersion in alcohol for several days and with muscle, kidney[1], etc., also usually gave a slight production of methaemoglobin, which was absent from the tubes containing blood alone. Though there was therefore no absolute distinction between blue and normal animals, the quantitative difference seemed pretty clear.

These experiments suggest at once that there is some connection between liver necrosis and methaemoglobinaemia. It will be remembered that extensive liver necrosis was an invariable accompaniment of methaemoglobinaemia both in the naturally and experimentally infected rats, and that it was, equally with the methaemoglobinaemia, absent in mice, guinea-pigs and rabbits killed with the rat bacillus. On the other hand, but clearly with a significance not necessarily as great, liver necrosis was present in a certain number of rats infected with Gaertner's bacillus, and in some infected with other members of the Gaertner group, in which methaemoglobinaemia was not observed[2]. I am inclined therefore at present to attach to the liver necrosis some aetiological connection with the methaemoglobinaemia.

In their human case of idiopathic methaemoglobinaemia ("microbic cyanosis") Gibson and Douglas[3] report the finding of *nitrites* in the blood serum. It seemed therefore desirable to consider the possibility of the rat methaemoglobinaemia being due to nitrite poisoning[4]. The changes in the blood pigment in poisoning by nitrites and allied substances are fully described by Haldane, Makgill and Mavrogordato[5]; they point

[1] The spleen cannot be washed free of blood and no experiments were made with it.

[2] Dr Rowland tells me that he has not noticed methaemoglobinaemia in plague-infected rats in which liver necrosis is generally extensive.

[3] *Lancet*, 1906, Vol. II. p. 72.

[4] Cf. the suggestion, strange enough, that cholera is fatal through nitrite poisoning; see R. Emmerich, A. A. Hymans, van den Bergh and A. Grutterink in *Berliner klin. Woch.* 1909, pp. 2008, 2229; and 1910, pp. 779 and 1320; J. Choukewitch, *Ann. Inst. Past.* Vol. xxv. (1911) p. 433.

[5] *Journal of Physiology*, Vol. xxi. (1897) p. 160.

out that nitrites produce NO-haemoglobin as well as methaemoglobin. The presence of the former may be detected by the fact that it persists after reduction and that therefore if a mixture of methaemoglobin and NO-haemoglobin is treated with reducing agents the continuous band of reduced haemoglobin will be intensified at two points corresponding to the position of the two prominent bands of NO-haemoglobin. Since NO-haemoglobin is pink (though not so pink as CO-haemoglobin) such a mixture will also differ in colour from a solution of methaemoglobin, both before and after reduction and saturation with carbon monoxide.

Further, if the body of an animal killed by sodium nitrite be left till putrefaction begins, the tissues are distinctly reddish; in the same way the ultimate product of reducing agents is NO-haemoglobin if the blood contains an excess of nitrites. In none of these points did the blood of the blue rats resemble that of nitrite poisoning. It should however be pointed out that the presence of NO-haemoglobin is at least not easy to detect if such a minimal dose of nitrite is administered to a rat that about two-thirds of the haemoglobin remains unchanged. My experiments indicated however that the difference could be detected with fair certainty (especially by putting the animal in the incubator over-night) if as much as half the haemoglobin was altered. As already shown (Table IV), in many blue rats more than 50 per cent. of the blood pigment is methaemoglobin.

The quantity of nitrite required to keep rats blue for two days would also be considerable. If given by the mouth, about 0·5 grammes is wanted about every 8 hours to produce definite lividity in a rat of about 100 grammes. More definite experiments in which sodium nitrite was injected intraperitoneally were made on a series of rats: the animals all received the same dose and were killed at intervals afterwards, the proportion of altered haemoglobin in their heart blood being estimated as in the blue Gaertner rats (see above, p. 456). Table XIII

TABLE XIII.

Dose of sodium nitrite per kilo	Killed, hours after	Proportion of unaltered haemoglobin in heart blood	Dose of sodium nitrite per kilo	Killed, hours after	Proportion of unaltered haemoglobin in heart blood
0·02 gms.	½	74 %	0·05 gms.	½	44 %
,,	1	76	,,	1	58
,,	2	74	,,	2	44 44
,	3	92	,,	2½	54
				3	67
				4	84

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shows the results from which it appears that a rat of 100 grammes would require about ten doses of 0·005 gms. sodium nitrite to keep its blood approximately half converted for twenty-four hours. It is not easy to imagine where such a quantity of nitrite as this could come from. The rat bacillus does not appear to produce nitrites and it actually destroys traces of nitrites added to, or present in, ordinary broth or peptone water[1]. The nitrite being used up in producing methaemoglobin, it is perhaps not conclusive evidence that no clear proof of the presence of nitrites in the blood or organs of blue rats could be obtained. Serum and plasma were tested directly; the other organs and whole rats by mincing them up, acidifying with phosphoric acid and distilling, the distillate being tested with α-naphthylamine and sulphanilic acid. On a few occasions a slight reaction was obtained by this very delicate reagent, but equally positive results were given by normal rats treated in the same way. I conclude that this infective methaemoglobinaemia in rats is not directly due to nitrite poisoning.

Summary.

1. A spontaneous epidemic of Gaertner infection among rats was found associated with methaemoglobinaemia and, in some cases, anaemia.

2. Strains of Gaertner's bacillus isolated from these rats reproduced methaemoglobinaemia in fresh rats but not in rabbits, guinea-pigs or mice.

3. Other strains of Gaertner's bacillus from rats, guinea-pigs and human sources also caused methaemoglobinaemia either before or after passage through rats.

4. Other organisms pathogenic for rats did not produce methaemoglobinaemia.

[1] Cf. W. J. Logie, *Journal of Pathology*, Vol. xv. (1910) p. 146; *Journal of Hygiene*, Vol. x. (1910) p. 143; Vol. xi. (1911) p. 361; E. Pelz, *Cent. f. Bakt.* Orig. Vol. lvii. (1910) p. 1; P. Mazé, *Compt. Rend.* Vol. cliii. (1911) p. 1624.