THE RESISTANCE OF RATS TO DIPHThERIA
TOXIN.By LOUIS COBBETT, M.A., M.B.CANTAB., F.R.C.S.ENG.
(From the Pathological Laboratory, Cambridge.)

I.—It is well known that rats and mice will tolerate large doses of diphtheria toxin. Roux and Yersin in 1888¹ injected 2.0 c.cm. of a toxin (of which 0.15 c.cm. sufficed to kill a guinea-pig of 350 grams) into a rat, and the same quantity into a mouse, and these animals remained well and no necrosis occurred at the seat of inoculation; and they state² that rats and mice may receive without harm quantities of diphtheria toxin which would kill a dog of moderate size.

With the diphtheria toxins of those days it was impossible to kill a rat without injecting enormous quantities, in volume almost equal to that of the animal itself, but they are now so much increased in strength that a few cubic centimetres are fatal to these animals.

I do not know that anyone has actually measured the power of these naturally immune animals to resist the toxin, if not the following experimental determination of the relative susceptibility of the rat and the guinea-pig may be of interest.

The filtrate of an eleven-day culture of *B. diphtheriæ* in 2 per cent. bouillon (Spronck) was obtained, and its minimal fatal dose for guinea-pigs and rats determined.

Note.—Since the two series of experiments were not quite simultaneous, it should be added that the filtrate was some two months old at the time the experiments were made, and had therefore reached a fairly stable condition.³ Experimental confirmation of this belief is afforded by the fact that on September 28th and October 19th the same dose (0.01 c.cm.) proved fatal to guinea-pigs of nearly the same weight within the same space of time, namely, four days. The guinea-pigs and rats used were young animals, the former weighing from 250 to 280, the latter 75 to 110 grams.

TABLE I.—Determination of the Minimal Fatal Dose of Filtrate
No. 1.

Date.	Weight of Guinea-pig in Grams.	Filtrate in c.cm.	Result.
September 28th ...	280	0.0100	† 4th day.
October 19th ...	265	0.0100	† 4th day.
" 4th ...	250	0.0095	† 7th day.
" 4th ...	250	0.0090	† 4th day.
" 4th ...	250	0.0085	† 2nd day.
" 7th ...	250	0.0080	† 3rd day.
September 28th ...	280	0.0075	† 9th day.

Date.	Weight of Rat in Grams.	Filtrate in c.cm.	Result.
October 20th ...	110	5.0	† 5th day.
November 3rd ...	100	3.0	Small swelling, hair fell out, no necrosis, lived.
October 25th ...	100	2.0	Nil, lived.
" 15th ...	78	1.0	Nil, lived.

Remarks as to size of swelling, shedding of hair, presence, or absence of necrosis refer to the seat of inoculation.
† = Death of the animal.

From these experiments it appears that to kill a white or black and white rat of 100 grams weight by subcutaneous injection of the filtrate of a culture of *B. diphtheriæ*, it is necessary to use about 600 or 700 times as much as would kill a guinea-pig of 250 grams weight. Or—to express it in terms similar to those used by Knorr when speaking of the relative susceptibility of various kinds of animals to tetanus toxin—it takes between 1,500 and 1,750 times as much diphtheria toxin to kill unit weight of rat as it does to kill an equal quantity of guinea-pig. Thus it may be said that the susceptibility of the guinea-pig to this poison is from 1,500 to 1,750 times as great as that of the rat.

A second series of observations was made with another

filtrate,⁴ and this time the minimal fatal doses for the guinea-pig and for the rat were determined simultaneously.

TABLE II.—Determination of the Minimal Fatal Dose of Filtrate No. 2.

Date.	Weight of Guinea-pig in Grams.	Filtrate in c.cm.	Result.
November 3rd	230	0.00900	† 2nd day.
" 3rd	230	0.00800	† 4th day.
" 10th	220	0.00750	† 2nd day.
" 3rd	230	0.00700	† 5th day.
" 10th	220	0.00650	† 2nd day.
" 15th	280	0.00600	† 8th day.
" 15th	245	0.00550	† 4th day.
" 15th	280	0.00500	† 8th day.
" 25th	240	0.00475	† 7th day.
" 25th	240	0.00450	Large swelling, necrosis, lived.
" 25th	230	0.00425	" " "

Date.	Weight of Rat in Grams.	Filtrate in c.cm.	Result.
November 18th	75	4.00	† 5th day.
" 15th	105	3.75	† 6th day.
" 25th	112	3.65	† 4th day.
" 25th	95	3.60	Small swelling, no necrosis, lived.
" 25th	104	3.55	† 5th day.
" 10th	100	3.50	Small swelling, no necrosis, lived.
" 29th	115	3.45	Small swelling, no necrosis, lived.

† Death of the animal.

From these experiments it appears that to kill a guinea-pig of 250 grams it takes about 0.005 c.cm. of the filtrate, while 3.55 is necessary to kill a rat of 100 grams weight. Now, $0.005 : 3.55 = 1 : 710$, therefore it takes 710 times as much filtrate to kill a rat of 100 grams as to kill a guinea-pig of 250 grams, or $1,775 (= 2\frac{1}{2} \times 710)$ as much filtrate to kill unit weight of rat—if the expression may be allowed—as it does to kill unit weight of guinea-pig. Thus both series of experiments concur in showing that the susceptibility of the rat to diphtheria toxin is only $\frac{1}{1,500}$ to $\frac{1}{1,750}$ as great as that of the guinea-pig.

It is worth noting that the enormous doses of toxin which may be injected into the rat without causing death produce but little local disturbance in that animal. In guinea-pigs doses which are very much smaller than the minimal fatal cause widespread oedema, and often necrosis of large areas of skin; and I have found that if these animals be protected by an antecedent or simultaneous injection into the peritoneal cavity of a unit of antitoxin, the subcutaneous injection of considerable quantities of toxin may produce gangrene of a large portion of the skin covering the abdominal and thoracic walls, without causing any apparent disturbance of general health or loss of weight. In the rat, on the other hand, the immensely larger doses which approximate to the minimal fatal quantity for that animal, cause either no apparent local lesion, or at most a small swelling, followed by the falling out of a little hair, necrosis I have never seen in these animals. I have seen the injection of a quantity of filtrate, which would have sufficed to kill several hundred guinea-pigs, produce in the rat no discoverable lesion and no loss of weight. The resistance of the rat to diphtheria toxin therefore differs from that of the guinea-pig protected by antitoxin in that the tissues are far better able to resist the local action of quantities of poison which are but little smaller than that which would have caused death.

II.—The comparative insusceptibility of these animals is not due to any antitoxic action of their blood serum, for the latter fails to protect guinea-pigs in doses of 1.0 c.cm. against quantities of filtrate which are but little larger than the minimal fatal; and this is the case both when the serum is mixed with the toxin before injection, and when it is injected into one part of the body and the toxin into another several hours later.

TABLE III.—To Determine whether the Serum of Rats has any Power of Protecting more Susceptible Animals against Diphtheria Toxin.

—	Date.	Weight of Guinea-pig in grams.	Diphtheria filtrate in c.cm.	Rat's serum.	Result.
Serum of white rat mixed with the toxin before injection.	Oct. 18th.	300	0.30	1.0	† In 2 days.
	" "	290	0.03	1.0	† In 2 days.
	" 19th.	260	0.01	1.0	† In 2 days.
Serum of white rat subcutaneous, toxin 20 hours later in another place.	Oct. 18th.	275	0.024	1.0	† In 24 hours.
	" "	280	0.010	1.0	† In 3 days.
Serum of brown rat mixed with the toxin.	Oct. 25th.	235	0.001	2.25	† In 3 days.

For control experiments with this toxin (No. 1) alone, see Table I.
† = Death of the animal.

The filtrate used in this experiment was the same as used in the first series of experiments made to compare the susceptibility of the rat and guinea-pig. The smallest quantity fatal within four days to a guinea-pig of about 250 grams was 0.008 c.cm. When mixed with 1.0 c.cm. of rat's serum and injected, or when injected eighteen hours after 1.0 c.cm. of rat's serum, 0.01 c.cm. of this filtrate (= $1\frac{1}{4}$ minimal fatal dose) proved fatal to guinea-pigs in less time than did the same dose of the filtrate alone. Thus it may be that the serum even shortened life. The serum alone, however, seemed to have at least no marked toxic action, for no immediate ill-effects (as is usually the case when one animal's serum which is toxic for another is injected into the latter) followed the injections.

The serum used in all cases but one was obtained from white rats; in the exceptional instance 2.25 c.cm. of the serum of a brown (wild) rat was mixed with 0.01 c.cm. of the filtrate and injected into a guinea-pig of 235 grams, with the result that no immediate ill-effect was observed, and the animal died in three days.

The comparative insusceptibility of the rat to diphtheria toxin is, therefore, like that of the fowl to tetanus toxin, not due to an antitoxic action of its blood serum.³ And in these respects it differs from the acquired immunity of animals which have been immunised to these poisons.

CONCLUSIONS.

1. The white or black and white rat of 100 grams weight is only relatively insusceptible to the action of the products of the diphtheria bacillus, and succumbs to the subcutaneous injection of filtered cultures, in quantities which are, weight for weight, from 1,500 to 1,800 times as great as those which suffice to kill guinea-pigs of 250 grams.

2. Its tissues are but little affected locally by the injection of large quantities of filtrate, and have not been observed to suffer necrosis.

3. The serum of these refractory animals, in doses of 1.0 c.cm., does not protect guinea-pigs against quantities of filtrate which are little greater than the minimal fatal.

NOTES AND REFERENCES.

¹ *Annales de l'Institut Pasteur*, vol. ii, p. 661. ² *Ibid.*, vol. iii, p. 278. ³ This filtrate contained 119.4 equivalents of protoxoid and syntoxoid, 56 units of toxin, and 24.6 equivalents of epitoxoid in the quantity (0.45 c.cm.) which was exactly neutralised by 1 unit of standard serum; compare Ehrlich, *Die Wertbemessung des Diphtherieheilserums*, *Klinisches Jahrbuch*, Jena, 1897. ⁴ This filtrate contained 97.5 equivalents of protoxoid and syntoxoid, 70 units of toxin, and 32.5 equivalents of epitoxoid in the quantity (0.45 c.cm.), which was exactly neutralised by 1 unit of standard serum. ⁵ Kuprianow found that rat's serum did not protect guinea-pigs against living cultures of *B. diphtheriæ*; *Centralb. f. Bakt. u. Parasit.*, Bd. xvi, p. 415.

Two municipal institutes for the gratuitous examination and treatment of cases of diphtheria have recently been established in Moscow.

MEMORANDA:

MEDICAL, SURGICAL, OBSTETRICAL, THERAPEUTICAL, PATHOLOGICAL, Etc.

IPPECACUANHA IN DYSENTERY.

In the *BRITISH MEDICAL JOURNAL* of February 4th Dr. Day of the Niger Protectorate compares his results obtained with magnesium sulphate and with ipecacuanha in the treatment of dysentery. He concludes that magnesium is much the superior drug. I think that some grave objections can be raised against this deduction from his cases.

1. He gave the ipecacuanha in the form of tablets. Is he quite sure that these tablets were thoroughly broken up in the intestinal tract? Ready-made pills, tablets, and the multitude of similar preparations have many drawbacks, and not the least of these is their tendency to get hard. It is no uncommon thing to find a case of malarial fever which seems to resist quinine, the patient declaring that he is taking large doses daily, but on inquiry one finds that the quinine is in the pilular form. Such pills, if got from home, are usually like little bullets in consistence, and it is little wonder that the disordered stomach is unable to disintegrate them; hence they are passed only partially dissolved, doing little good and leading one to a fallacious conclusion. Might not the same apply to the tablets of ipecacuanha given by Dr. Day?

2. He gave ipecacuanha *sine emetine*. Is ipecacuanha any use without its emetine? Emetine is said to be the active principle of the ipecacuanha root, and it is perhaps exactly this emetine which is the antagonist to the *materies morbi* of dysentery. At any rate it is well known that vomiting is not readily caused by ipecacuanha when it is given for dysentery.

3. He gave two 5-grain tablets every two hours. This dose is too small to do justice to the drug. Nothing less than 40 grains should be given as the first dose. Quinine in malaria fever shows the great advantage of a single large dose over several small doses. It seems to require a thorough saturation of the system, so to speak, to destroy the morbid factor at work. There is little fear of emesis occurring if the ipecacuanha is properly given. Amongst the thousands of coolies on tea gardens dysentery is constantly present, and the plan I always adopt is this:

1. Apply a large mustard blister over the stomach.
2. Give 20 minims of tincture of opium.
3. Ten minutes afterwards give 40 gr. pulv. ipecac. in four 10-grain pills freshly made up.
4. The horizontal position to be kept for two hours.
5. No food nor drink to be allowed within that time. This treatment to be repeated in eight hours if no improvement has taken place; if there has then a smaller dose with the same precautions.

The result will almost invariably justify the name—*radix antidysenterica*.

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CHLOROFORM PARALYSING THE RESPIRATORY CENTRE BEFORE THE HEART.

The following case is a good illustration of the Edinburgh teaching as regards the action of chloroform in causing paralysis of the respiratory centre before cardiac paralysis occurs:

A Chinese coolie, aged 51, had for the last fifteen years suffered from intractable supraorbital neuralgia. After finding treatment by sedatives ineffectual, we decided to resect the supraorbital nerve. The operation could not be performed at his own residence, so we arranged to do it in our surgery. The patient was emaciated, but the heart was quite sound, and there was no evidence of any organic disease. He was placed on his back, and chloroform was administered on a piece of lint. He took the anæsthetic well, and there was no struggling or excitement. The corneal reflex had just disappeared, and the skin incision was being made, when he suddenly stopped breathing, and his lips became livid. The pupils were not markedly dilated. The radial pulse could still be felt, but the heart sounds were feeble and the cardiac impulse weak.

Artificial respiration was at once begun, and a hypodermic injection of ether given. The air could be heard freely entering and leaving the lungs with each movement of the arms,