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# THE BLOOD VOLUME IN ANKYLOSTOMIASIS.

## WITH SOME BIOLOGICAL NOTES RELATING TO THE DISEASE.

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### *Introductory.*

THE investigations which form the subject of the present paper were carried out during my tenure of the Ernest Hart Memorial Scholarship in State Medicine of the British Medical Association. The experimental part of the work was conducted at the Lister Institute of Preventive Medicine, London.

The suggestion of the line of research came from Dr J. S. Haldane, F.R.S., who referred me to certain results obtained by Boycott and himself (1903). The essential question to be decided was the nature of the changes in the blood volume in Ankylostomiasis or hook-worm anaemia. In the paper referred to Boycott and Haldane, using the carbon monoxide method of estimation, found that in three patients suffering from hook-worm anaemia the blood volume was increased to a very considerable extent, while the total haemoglobin remained practically unchanged. The important bearing of this result upon the nature of anaemia in general appeared to demand confirmatory evidence and it was thought that such might be readily obtained by means of animal experimentation.

In setting out to deal with such a problem experimentally, several questions naturally suggested themselves. These were, in the first place, the exact nature of the disease as it is exhibited in man, its etiology, pathology and clinical identity; in the second place, the nature of the disease in the animals to be experimented upon and the question as to whether it is identical with, analogous to, or different

from the disease in man. In addition there was the question as to the possibility of accurately determining normal conditions in the experimental animals and finally various questions relating to idiosyncrasy, susceptibility and immunity.

With regard to the nature of the disease in man it is universally accepted that the prime etiological factor is the presence in the intestine of either or both of two species of parasitic worms, namely *Agchylostoma*[1] *duodenale* and *Necator americanus*. As to the direct or immediate cause of the anaemia, however, opinion is yet to a large extent empirical. From the habits of the worms of fixing on to and damaging the intestinal mucosa, and so leading to haemorrhage, it might at first sight be concluded that the causation of the anaemia could be fully explained by the resulting loss of blood. The anaemia would accordingly be of the secondary or haemorrhagic type. Against this, however, must be put certain facts of considerable weight. In the first place Looss (1911) insists that the worms do not suck blood and that their natural food is the intestinal epithelium. This view, in the latter respect, is supported by several observers, and by personal experience, to the effect that although blood is not infrequently found in the intestine of the worms the red blood corpuscles are not digested to any extent. In the second place the majority of observations point to the fact that intestinal haemorrhage is rare in cases of some duration, and thirdly the results of Boycott and Haldane give evidence that the blood content differs from that in secondary anaemias. These considerations, together with some of a more general nature, have led to the origin of the toxic theory. This in turn has of late developed along two distinct lines. On the one hand, it was believed that the worm itself secreted a toxic substance, the action of which gives rise to the anaemia; on the other hand, there is the more recent idea that the toxin is produced by some extraneous organisms such as intestinal bacteria, the products from which find their way through the damaged mucosa. Neither of these explanations, however, is entirely satisfactory. A number of attempts have been made to isolate the toxic substance and success has been reported by, amongst others, Preti (1908), Noc (1908) and Whipple (1909). The last mentioned, however, while confirming the presence of a haemolytic substance, maintains that it is too weak or present in too small quantities to be effective. Moreover the evidence that haemolysis does actually take place within the human body is conflicting and the latest observations by Ryffel are against the occurrence of any such

[1] This spelling is adopted in accordance with current zoological nomenclature.

process. Loeb and Smith (1904 and 1906), again, entirely failed to detect the presence of a haemolysin in the head of the worm, but at the same time they reported the presence of an anti-coagulating substance, which might possibly tend to favour the continuance of haemorrhage. The presence of this substance was confirmed by Noc (1908), but Liefmann (1905) could detect its presence only in a minority of cases.

The toxic theory of extraneous origin has of late been elaborated by Weinberg and Leger (1908). Their opinion is that the gravest forms of hook-worm anaemia can only be explained on the basis of a secondary microbic infection and they advance experimental proof in support of their belief. Whipple (1909), also, in discarding the haemolytic theory, comes to the conclusion that the anaemia is probably due to direct loss of blood accompanied by absorption from secondary foci of inflammation in the intestinal wall. Siccardi (1910) in a summarizing article concludes that the anaemia is chiefly of toxic origin but that the intestinal lesions and the haemorrhages are additional factors. Evidence from a different point of view is brought forward by Castellani (1910), who deals with the comparative frequence of fever in hook-worm infection in Ceylon. This fever he ascribes to the action of various intestinal, but not necessarily pathogenic, bacteria, amongst which a new form, *Bacillus asiaticus*, is particularly mentioned. The greatest objection, perhaps, to be raised against this secondary microbic theory is the rapidity with which the anaemia disappears on expulsion of the worms.

In dealing with the clinical aspects of the disease, one important point has to be borne in mind, namely, the distinction between hook-worm infection and hook-worm disease. Infection with the hook-worm, even in some cases to a very high degree, does not necessarily lead to disease. This fact has been observed by most workers who have studied the disease on a large scale and it is merely an instance of a general biological phenomenon, which is not capable of very ready explanation. Clinically the leading feature of hook-worm anaemia in man is the chronic and progressive nature of the disease, but although essentially chronic, the progress of the disease may in some cases be very rapid. The symptoms are those of severe anaemia, generally associated with some degree of gastro-enteritis. The large number of individual symptoms which may occur are not specifically characteristic of hook-worm anaemia. In the pallor and oedema, however, there is a greater resemblance to chlorosis than to pernicious anaemia. The blood shows

a more characteristic picture. The volume, according to Boycott and Haldane, may be increased by nearly 100 %, while the total oxygen capacity is only slightly decreased. The number of erythrocytes may fall as low as 1,000,000 per c. mm. while the haemoglobin percentage may be as small as 15 % or even 10 %. The colour index is generally considerably below unity. It is apparent that these facts give evidence of a close relation to secondary anaemia, the one essential difference being the fact that the total oxygen capacity is not materially diminished.

With regard to the blood cells several observers have remarked on the frequent occurrence of normoblasts and, in some cases, of megaloblasts. Boycott (1911), however, categorically denies the occurrence of such cells and argues, from their absence, that there is little regeneration of blood, "presumably because the blood contains as much haemoglobin as, and more red cells than, normal." In regard to the leucocytes the most important feature is the occurrence of eosinophilia. This was first noted in hook-worm anaemia (1891) by Mueller and Rieder, but since then it has been a matter of frequent remark, and it is now fairly well established that a high degree of eosinophilia frequently accompanies not only hook-worm anaemia but also hook-worm infection. At the same time it must be noted that the relation is not constant and that certain cases may show no eosinophilia even though heavily infected. This was pointed out by Low in the discussion on Boycott's paper in 1905 in the particular case of the natives of Uganda. It was found by Boycott (1911) that eosinophilia first became marked about two to three weeks after the initial infection and that it reached a higher degree about the time ova first appeared in the faeces, after which it tends to fall off but usually persists not only throughout the whole course of disease but even for many years after complete recovery.

Apart from bronchial asthma and scarlet fever, there are two classes of infection with which eosinophilia is particularly associated, namely parasitic worms and skin diseases. There is at present no satisfactory explanation of this remarkable association but the current theory is that the increase in the eosinophil cells betokens a reaction on the part of the marrow to toxins circulating in the blood. Such a theory is supported in the case of parasitic worms by the fact that encysted forms are frequently surrounded by a zone of cells, a large proportion of which are eosinophilic. Whatever the explanation, however, there can be no doubt that the occurrence of eosinophilia is not infrequently a useful

aid to diagnosis and it has indeed been employed by Boycott as a routine method in the case of miner's anaemia, the final and confirmatory diagnosis being made on examination of the faeces.

*The disease in dogs and other animals.*

With these facts in the case of the disease in man it is necessary to compare what is known in regard to similar conditions affecting animals, and here, as might be expected, the available facts are neither so numerous nor so carefully sifted. It is in the first place necessary to consider the class of worms to which the human hook-worms belong, for it has frequently happened that much confusion has been introduced from neglect of accurately determining the specific characters of the infective agent. In this wise epidemiological misconceptions have from time to time been promulgated, such, for instance, as the belief that dogs and other animals serve as carriers of the human hook-worms, or that the hook-worm passes through a sexual stage outside the body. *Agchylostoma duodenale* and *Necator americanus* may be characterised as bursate or Strongylid Nematodes, belonging to the family Agchylostomidae, the chief features of which, separating them from other bursate Nematodes, are the dorsal bending of the head, the presence of a large buccal capsule which is armed with symmetrical groups of teeth or cutting plates, but which lacks coronae radiatae. These characters are sufficient to differentiate the hook-worms from the Sclerostomes, on the one hand, and, on the other, from the "wire-worms" and lung worms of sheep and other Herbivora. It may be remarked that a considerable proportion of all those worms are capable of provoking haemorrhage and giving rise to anaemia and malnutrition. The Agchylostomidae, however, are regarded as particularly deserving of the title "blood suckers," although, as has already been pointed out, the term is possibly a misnomer. They are divided into two subgroups, *Agchylostominae* and *Bunostominae*, the former being parasites of Carnivora, the latter of Herbivora. The chief structural differences between these groups consist in the facts that the *Bunostominae* have an extra pair of internal teeth and an unpaired internal dorsal prominence (the so-called unpaired dorsal tooth of *Necator*) in the buccal capsule. *Agchylostoma* belongs to the first group, *Necator* to the second. As mentioned above it has from time to time been stated that *A. duodenale* occurs in the dog and other animals but it is now fairly conclusively established that this species is a specific parasite of man. The dog, the cat and the fox,

however, and probably some other Carnivores harbour a very similar species, *A. caninum*. Four additional species have been recorded from other Carnivores. Another genus, *Uncinaria*, contains a second parasite of the dog and cat, namely, *U. criniformis* and one or two other species occur in other Carnivores. *Necator americanus* is nearly as specific as *A. duodenale*, but it has been found, with certainty, in the gorilla. A second species, *N. africanus*, has been met with in the chimpanzee. The second genus of this group, namely *Bunostomum*, includes three species parasitic in cattle and sheep. Five other genera, comprising forms parasitic in various Herbivora, belong to this group. These include all the forms on which we have at present satisfactory information, but there are several others still imperfectly known.

That most of these parasites give rise to some form of anaemia is vouched for by several observers. *Bunostomum phlebotomum*, for instance, is credited (Ransom 1911) with being the cause of "salt-sickness" of cattle in Florida, which is characterised by, amongst a variety of other symptoms, progressive emaciation and pronounced anaemia, which in many cases terminates fatally. Like opinions in regard to the other species are not wanting, but it is chiefly with the disease as manifested in dogs that we are at present concerned. A form of pernicious anaemia in dogs, more particularly hunting dogs, has been known for a long time and its connection with hook-worm infection was first mooted in 1882 by Mégnin whose opinion was supported by Railliet and by Trasbot (1882), and was confirmed by further observations on the part of Mégnin (1883). From these we gather that the symptoms are as a rule much more severe than those in human hook-worm anaemia, and that the disease is on the whole less chronic and more fulminant in type. There is a similar debility, pallor, oedema and enteritis but there appears to be a greater tendency to vicarious haemorrhage especially in the form of epistaxis, and, to this, skin affections are added. That all the symptoms mentioned by these early authors constitute a single clinical entity must be a matter of some doubt. It is recognised that dogs are subject to anaemia of non-parasitic origin and it may be noted that in some of the cases of hook-worm anaemia only a few worms were found in the intestine. It is remarkable that the disease should only attack kennelled hunting dogs although it cannot be denied that the circumstances are especially favourable to continued and repeated infection. Later observations on this subject are scanty. Gray (1899) recorded the occurrence of ankylostomiasis in dogs in Assam and asserted that very profuse

haemorrhage was frequent. Powell recorded somewhat similar facts for Cochin China. Thiroux and Teppaz (1906) also record the occurrence of hook-worm disease in dogs in West Africa. It is not made clear, however, what proportion of the infected dogs the disease attacks or what the age-incidence or other epidemiological factors are. According to the observations and experimental work of Looss (1911) it would appear that only young dogs are liable to attack and Liefmann (1905) noted experimentally that older dogs resisted infection to a very considerable extent. A similar anaemic disease in cats was first observed in 1878 by Grassi.

With regard to the condition of the blood in the anaemia of dogs we have practically little or no information beyond the fact that the erythrocytes are diminished and the leucocytes and eosinophils increased.

From the foregoing remarks it is evident that idiosyncrasy and susceptibility play a certain, if little understood, part in the etiology of hook-worm infection and anaemia not only in man but also in dogs and probably other animals. In regard to hook-worm infection in man, age appears to have little importance, but in many animals and with other parasites besides hook-worms, only young animals are prone to infection. The same phenomenon is manifested in the case of the human thread-worm (*Oxyuris vermicularis*) which shows an overwhelming predilection for children and generally disappears after puberty. In the case of the human hook-worm however, idiosyncrasy is manifested in other directions in illustration of which may be quoted the fact that Boycott was apparently insusceptible to infection through the skin although he readily acquired infection through the mouth.

### *The blood-picture in normal dogs.*

There is finally the consideration of the normal condition in the experimental animals. The blood-picture of dogs and cats has been dealt with by Paton, Gulland and Fowler (1902). They found that, normally, the dog has a wide range in the number of red blood cells, namely, from 5 to 9 millions per c. mm., the leucocytes from 11,000 to 26,000, and the haemoglobin index from 80 to 110. They also found an average of 3 to 4% of eosinophils. My own observations give figures agreeing in the main with these, namely, reds 5–8 millions, leucocytes 6–24 thousand, and Hb percentage 70–105, while the eosinophils vary from 0 to 4%. With regard to the normal blood volume several authorities have made observations which show a fair

amount of uniformity but diverge in respect of the limits. Ranke arrived at an average of 1/15 of the body-weight, Jolyet and Laffont gave as limits 1/12 to 1/13; Panum found 1/12–1/15 and Heidenhain 1/12–1/18. These latter figures are quoted from Dreyer and Ray (1910), the original references not being available. More recently Abderhalden and Schmid (1910) have employed an optical method of estimation, in which they injected dextrin and determined the rotatory power of the blood before and after injection. By this means they found the blood volume to be 1/8–1/9 (11·3–12·4 %) of the body weight. Dreyer and Ray, however, maintain that the blood volume is a function not of the body weight but of the body surface (*i.e.* of the 2/3rds power of the body weight approximately). They express their results in the form B.N. = B.W. $\frac{2}{3}/k$, where $k$ is a constant determined experimentally for each species. It is evident that considerable discrepancy exists between the results of Abderhalden and Schmid and those of other observers. My own determinations on normal dogs give evidence of a comparatively wide range, the figures being 1/16–1/10 (6–10 %) of the body weight, and this apparently independent of age or size. It has been found by Boycott (1912) and also by Dreyer and Ray (1910) that the blood volume per kilo of body weight is on the whole highest in small, *i.e.* young animals, and that it tends to decrease, though not periodically, as the animal increases in size. Boycott also concludes that the total haemoglobin (*i.e.* total oxygen capacity) is highest in small animals, although the haemoglobin percentage tends to rise as the animal grows.

### *Source of culture employed.*

In view of what has been stated above and as a matter of expediency it was decided to conduct the experimental part of this investigation upon dogs, and, if possible, cats. Hook-worm disease in dogs is not known to occur naturally in this country and on that account some initial difficulty was experienced in obtaining the material for infection. This I eventually owed to the kindness of Prof. C. W. Stiles of Washington, U.S.A. A sample of egg-laden faeces from an infected dog was sent, and numerous eggs survived the journey of nearly a fortnight. From these a successful culture of infective larvae was obtained.

*Method of estimation of blood volume.*

Before proceeding to the determination of the blood volume in the infected dogs, preliminary control estimations were performed on three normal cats and five normal dogs. The method employed was a modified Welcker process, the details of which are as follows. The animal was carefully weighed, narcotised with morphine and then chloroformed. The carotid artery on one side was exposed and a three-way cannula was inserted. To this was attached a length of rubber tubing leading from the bottle containing the washing out fluid, and another clamped rubber tube by which the blood could be drawn off. The washing out fluid consisted of a 0·4% solution of potassium oxalate in 0·75% solution of sodium chloride in distilled water, which was kept at a temperature of 37° C. An accurately measured quantity of blood was then drawn off (25 c.c.), and mixed with an equal quantity of oxalate solution. This served as a standard. A further quantity of blood was then drawn off into a vessel containing oxalate solution, and this was continued for 5–10 minutes or until the flow became slow. Oxalate solution was then driven into the circulation from a pressure bottle, which was placed about six feet above the operating table. The solution was allowed to flow for 5–10 minutes, when more blood was drawn off. This was repeated several times for about an hour, by which time the heart had ceased to beat and the fluid drawn off had become somewhat pale in colour. The carotid artery was then clamped and the chest opened. A cannula was inserted into the aorta and connected with the oxalate bottle; the right auricle was incised. Oxalate solution was then perfused through the circulation and the fluid returning from the right auricle was collected in the thoracic cavity, whence it was sucked by means of a water pump into a collecting bottle. All the while the muscles and liver were firmly massaged. The lungs were completely washed out by a similar procedure. This process was generally discontinued after about two hours, by which time the fluid washed out, although it had not entirely lost colour, had become extremely pale. All the washings were then mixed and the total accurately measured. About a litre of this was retained for estimation. Meanwhile the animal was skinned and the flesh removed from the bones, along with the liver, spleen, heart and kidneys. The flesh and the bones were mashed up separately and then pressed to expel the juice which was collected and measured.

The standard sample of blood which had first been drawn off was then diluted to ·5% with distilled water to which a few drops of chloroform were added to ensure haemolysis. The washed out blood was also diluted and haemolysed, and a small quantity was passed through filter paper to remove suspended matter. By this means a clear solution was obtained without losing more than a trace of the colouring matter. The difficulty of obtaining a clear solution from the pressed muscle juice was met by saturating it with sodium chloride and filtering. Allowance was made in the calculations for the increase in volume resulting from the salt added.

Each of these solutions was compared with the standard. Three test tubes of equal calibre were employed for the estimation. Into two was placed a quantity of the standard sample and into the third a measured quantity, usually 10 c.c., of the diluted washings. To this was added distilled water from a graduated burette until the tint matched that of the standard. The amount of water added was noted and from this the strength of the solution was estimated. The total volume could then be calculated.

In some of the earlier estimations the washings were collected in two or three successive portions and each was estimated separately. It was found that the last portion contained so little haemoglobin that no material addition would have been made by continuing the washing process longer. In the case of the muscle juice the presence of muscle pigment rendered the solution brown so that it was impossible to compare it accurately with the standard solution. On that account the estimation was made by means of the spectroscope. As a standard of comparison the dilution was taken which was just sufficient to cause the *D* line of the haemoglobin spectrum to disappear. Several of the early readings were confirmed by Prof. C. J. Martin, to whom I am much indebted for help in this and other matters. Further controls of the accuracy of these readings were effected by comparing the results obtained by this with those of the ordinary colorimetric method when a comparatively strong solution of haemoglobin was used. The discrepancy was found to be of small amount.

The quantity of blood in the washings, muscle juice and bone juice having thus been obtained, the sum of these plus the 25 c.c. originally withdrawn gave the total blood volume. The details in the control experiments were:

| Cats (normal). | Weight (grams) | Blood vol. (c.c.) | Vol./Wt. (%) | Percentage washed out |
|---|---|---|---|---|
| A. | 4200 | 189·5 | 4·70 | 96·2 |
| B. | 1990 | 98·9 | 4·97 | 97·3 |
| C. | 2415 | 116·9 | 5·07 | 96·6 |
| Average | — | — | 4·91 | 96·7 |

| Dogs (normal). | Weight (grams) | Blood vol. (c.c.) | Vol./Wt. (%) | Percentage washed out |
|---|---|---|---|---|
| Z. | — | 1130·2 | — | 92·9 |
| E. | 5400 | 525·2 | 9·72 | 98·1 |
| F. | 11000 | 1120·4 | 10·19 | 92·5 |
| X. | 9100 | 545·6 | 6·00 | 91·6 |
| Y. | 9800 | 744·3 | 7·59 | 96·0 |
| Average | — | — | 8·87 | 94·2 |

The complete Welcker method is a tedious process and one which in the case of a large animal could with difficulty be completed in a day. On that account it was decided to take the amount of blood washed out as a constant percentage (94 % of the total volume). In the remaining experiments therefore only the amount of blood washed out was estimated and the volume calculated therefrom.

### *Experimental Series.*

I do not propose to enter here into the full details of each experiment, which would involve endless repetition and be of little interest. Only the outstanding data will be given, with explanatory notes wherever necessary.

*A.* (Stock.)

Apr. 18th, 1910. Infection started. Wt. 5·9 kg. Hb. 106 %. Erythrocytes 6·38 millions.
May 5th. *Agchylostoma* ova in faeces. Wt. 6·4 kg.
June 7th. Infection stopped.
July 19th. Wt. 5·8 kg. Hb. 76 %.
Oct. 7th. Hb. 96 %. Red cells 5·99 millions. Eosinophils 0 %.
,, 24th. Hb. 84 %. Red cells 7·15.
Apr. 20th, 1912. Alive and well. Several ova in faeces.
June 22nd. Killed. Hb. 96 %. Red cells 5·56. Wt. 8·3 kg. Blood volume 632 c.c. = 7·62 % of body-weight.

*B.*

Apr. 19th. Infection started.
,, 24th. Dead. Pneumonia. No hook-worms in intestine.

*C.*

Apr. 20th. Infection started.
May 5th. Dead. Pneumonia. Large number of immature hook-worms in ileum. Considerable amount of blood in intestine.

*D.* (Stock.)

| | |
|---|---|
| May 11th. | Infection started. Wt. 9·1 kg. Hb. 90 %. Red cells 5·63. |
| June 13th. | Ova in faeces. Infection stopped. |
| July 1st. | Hb. 79 %. |
| Oct. 7th. | Hb. 77 %. Eosinophils 0 %. |
| Apr. 20th, 1912. | Alive and well. Moderate number of ova in faeces. |

*G.*

| | |
|---|---|
| Feb. 28th, 1911. | Infection started. |
| Mar. 4th. | Dog ill. |
| ,, 9th. | Very ill. |
| ,, 16th. | Dead. P.M. no obvious lesions. 12 immature hook-worms in intestine. Not much blood. |

*H.*

| | |
|---|---|
| Mar. 2nd, 1911. | Infection started. |
| ,, 9th. | Very ill. |
| ,, 14th. | Dead. Pneumonia. 12 young hook-worms in intestine; also a few round-worms. Large amount of haemorrhage. |

*I.*

| | |
|---|---|
| Apr. 5th, 1911. | Infection started. Wt. 13 kg. Hb. 92 %. Red cells 6·35. |
| May 2nd. | Wt. 12·6. Hb. 68 %. Eosinophils 3½ %. |
| ,, 21st. | Wt. 12·2. Hb. 77 %. Eosinophils 1 %. |
| July 10th. | Wt. 12·3. Hb. 79 %. Eosinophils 3 %. |
| Sept. 1st. | Wt. 12·7. Hb. 98 %. Red cells 6·95. Eosinophils 0 %. Erythroblasts 650 c.mm. |
| | Killed. Blood volume 905 c.c. = 7·13 % of body-weight. P.M.: viscera practically bloodless. 28 hook-worms in intestine. Very little blood in intestine, but large number of haemorrhagic areas of considerable size. |

*J.*

| | |
|---|---|
| May 4th, 1911. | Infection started. Weight 8 kg. Hb. 88 %. Eosinophils 12½ %. Already infected with *Ascaris*. |
| ,, 29th. | No ova in faeces. Wt. 9·2 kg. Hb. 88 %. |
| June 6th. | Few ova in faeces. |
| July 10th. | Wt. 9·4 kg. Hb. 59 %. Eosinophils 2 %. |
| ,, 12th. | Hb. 75 %. Red cells 6·41. Eosinophils 5 %. |
| ,, 15th. | Wt. 10 kg. Hb. 68 %. Red cells 6·5. Eosinophils 5 %. |
| | Blood volume 729·3 c.c. = 7·29 % of body weight. P.M.: intestine contained a considerable amount of bloody mucus, with small clots of blood in places. About 170 specimens of hook-worm were met with from jejunum down to coecum, also 21 specimens of *Ascaris* in jejunum. |

*K.*

| | |
|---|---|
| May 17th. | Infection started. Wt. 15·1 kg. Eosinophils 3 %. |
| ,, 18th. | Hb. 88 %. Eosinophils 3 %. |
| ,, 29th. | Wt. 14·7 kg. Hb. 81 %. |
| June 11th. | Ova in faeces. |
| July 10th. | Infection stopped. |
| ,, 14th. | Wt. 15·8 kg. Hb. 101 %. Red cells 6·90. Numerous ova in faeces. |

Aug. 7th. Infection restarted.
,, 21st. Wt. 14·5 kg. Hb. 97 %. Eosinophils 0 %. Red cells 8·5.
Sept. 7th. Infection stopped again.
,, 29th. Wt. 17·5 kg. Hb. 97 %. Red cells 8·7.
Oct. 20th. Wt. 17·1 kg. Hb. 105 %. Eosinophils 0 %.
Killed. Blood volume 1878·4 c.c. = 10·98 % of body wt. P.M.: some injection in mesentery. Large amount of bright red blood in rectum and patches throughout the intestine, with numerous haemorrhagic points. 146 specimens of hook-worm dispersed throughout whole length of intestine, 9 being found in the rectum and 3 in the coecum. All were adult and many were full of blood.

*L.*

May 18th, 1911. Infection started. Wt. 6·6 kg. Hb. 95 %. Eosinophils 4 %.
June 11th. Numerous ova in faeces.
,, 20th. Weak. Wt. 5·6 kg. Hb. 53 %. Eosinophils 15½ %.
July 10th. Hb. 55 %. Red cells 5·94. Eosinophils 6 %.
,, 11th. Wt. 6·3 kg. Hb. 51 %. Red cells 5·98. Eosinophils 3 %.
Aug. 21st. Wt. 7·9 kg. Hb. 99 %. Red cells 8·02. Eosinophils 0 %.
Sept. 7th. Infection stopped.
Oct. 3rd. Wt. 7·0 kg. Hb. 93 %. Red cells 5·83. Eosinophils 1 %.
,, 26th. Wt. 7·1 kg. Killed.
Blood volume 427·2 c.c. = 6·02 % of body weight. P.M.: intestine full of blood. 130 hook-worms, including several in coecum and rectum.

*M.*

May 22nd. Infection started. Wt. 9·4 kg. Hb. 104 %. Eosinophils 3 %.
June 15th. Ova in the faeces.
July 14th. Wt. 8·9 kg. Hb. 81 %.
Sept. 7th. Infection stopped.
Oct. 5th. Wt. 8·1 kg. Hb. 95 %. Red cells 7·04. Eosinophils 0 %.
Dec. 5th. Wt. 8·5 kg. Hb. 98 %. Killed.
Blood volume 769·5 c.c. = 9·05 % of body weight. P.M.: very little blood or signs of haemorrhage. 30 hook-worms in intestine.

*O.*

Aug. 3rd. Infection started. Wt. 8·3 kg.
,, 17th. Wt. 7·6 kg. Hb. 86 %. Red cells 5·0. Eosinophils 0 %.
,, 24th. Very ill. Infection stopped.
,, 30th. Dead. Wt. 5·3 kg.
P.M.: very much emaciated. Mesentery greatly injected. Lower part of intestine, coecum and rectum of dark green colour from diffused blood. Intestine contained a small amount of blood. Other organs normal. 318 hook-worms found in intestine. In trachea were found two living *Agchylostoma* larvae (·62 mm. in length), one was found in oesophagus and five in stomach, measuring ·66–·74 mm. in length; also one small immature female, measuring 3·1 mm., in the stomach.

*P.*

Aug. 9th. Infection started. Wt. 5·5 kg.
,, 16th. Hb. 68 %. Red cells 4·07. Eosinophils 1 %. Ill and weak.
,, 24th. Very ill. Infection stopped. No ova in faeces.
,, 25th. Dead. Wt. 4·4 kg.

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P.M.: much emaciated. Mesentery greatly injected. Viscera very pale. Lower part of intestine and rectum of dark green colour and containing a large quantity of blood. In intestine were found 1886 hook-worms, 12 specimens of *Dipylidium caninum*, and one *Ascaris*. No larvae in lungs or trachea.

*Q.*

Sept. 26th. Infection started. Wt. 7·8 kg.
Nov. 9th. Killed. Wt. 7·8 kg. Hb. 108 %.
Blood volume 493·1 c.c. = 6·49 % of body weight. P.M.: only two hook-worms found in intestine. No blood.

*S.*

Jan. 20th, 1912. Wt. 3·0 kg.
Feb. 23rd. Wt. 5·0 kg. Hb. 70 %. Eosinophils ½ %.
Apr. 9th. Wt. 9·0 kg.
May 3rd. Wt. 6·5 kg. Hb. 86 %. Red cells 6·32. Whites 15·0. Eosinophils 4 %.
,, 7th. Wt. 6·8 kg. Hb. 76 %. Red cells 5·09. Whites 12·3. Eosinophils 2 %.
,, 10th. Wt. 6·9 kg. Hb. 80 %. Red cells 5·10. Whites 15·8.
,, 13th. Wt. 7·1 kg. Hb. 82 %. Red cells 6·19. Eosinophils 1 %.
Infected with Ankylostoma larvae by the mouth.
,, 14th. Wt. 7·7 kg.
,, 16th. Wt. 7·6 kg. Hb. 72 %. Red cells 5·15. Whites 15·7. Eosinophils 0 %. Erythroblasts 235.
,, 18th. Wt. 7·4 kg. Hb. 72 %. Red cells 5·69.
,, 22nd. Wt. 8·3 kg. Hb. 74 %. Red cells 6·50. Whites 20·1. Eosinophils 1 %. Erythroblasts 502.
,, 25th. Wt. 8·8 kg. Hb. 74 %. Red cells 5·79. Whites 11·8. Eosinophils 3 %. Erythroblasts 1121.
,, 28th. Wt. 8·9 kg. Hb. 76 %. Red cells 6·17. Whites 15·2. Eosinophils 6½ %. Erythroblasts 304.
,, 29th. Wt. 9·0 kg. Hb. 72 %. Red cells 5·90. Eosinophils 10½ %. Erythroblasts 1292.
June 1st. Wt. 9·2 kg. Whites 18·4. Eosinophils 2 %. Erythroblasts 11,960.
,, 3rd. Wt. 8·8 kg. Hb. 60 %. Red cells 4·89. Whites 9·5.
,, 6th. Wt. 8·2 kg. Hb. 52 %. Red cells 3·32. Whites 14·0.
,, 7th. Wt. 8·1 kg. Hb. 56 %. Red cells 3·57. Whites 18·8. Eosinophils 2½ %. Erythroblasts 3102.
,, 9th. Wt. 7·3 kg. Hb. 52 %. Red cells 4·05. Whites 18·7. Eosinophils 6½ %. Erythroblasts 654. Temp. 98·8. Very ill. Thin and feeble. No bleeding and apparently no pain or tenderness.
,, 10th. Wt. 7·0 kg. Hb. 50 %. Red cells 3·77.
,, 11th. Wt. 6·8 kg. Hb. 50 %. Red cells 3·29. Whites 22·7. Eosinophils 2 %. Erythroblasts 12,031.
,, 14th. Wt. 6·5 kg. Hb. 48 % Red cells 3·16. Whites 19·5. Eosinophils 4 %. Erythroblasts 1462.
,, 15th. Wt. 6·8 kg. Hb. 48 %. Red cells 2·97.
,, 18th. Wt. 6·6 kg. Hb. 40 %. Red cells 4·17. Whites 20·8.
Killed. Blood volume 490 c.c. and 7·43 %. 791 hook-worms in intestine, which contained considerable quantity of blood.

*T.*

| | |
|---|---|
| May 7th, 1912. | Wt. 4·3 kg. Hb. 78 %. Red cells 5·97. Whites 7·7. Eosinophils 2 %. Erythroblasts 422. Temp. 102·2. Already infected with *Ascaris*. |
| ,, 10th. | Wt. 4·4 kg. Eosinophils 0. Erythroblasts 38. Temp. 102·4° F. |
| ,, 14th. | Wt. 4·4 kg. Hb. 78 %. Red cells 5·67. Whites 11·9. Eosinophils 1 %. Erythroblasts 0. Temp. 101·6° F. |
| ,, 16th. | Wt. 4·2 kg. Hb. 82 %. Red cells 5·55. Whites 7·0. Eosinophils 0 %. Temp. 101·8. |
| ,, 19th. | Wt. 4·5 kg. Hb. 88 %. Red cells 6·34. Whites 8·9. Eosinophils 4 %. Erythroblasts 177. Temp. 101·3–102·7° F. |
| ,, 22nd. | Wt. 4·9 kg. Infection started. Fed by mouth. |
| ,, 24th. | Wt. 4·9 kg. Hb. 78 %. Red cells 549. Eosinophils 0 %. Erythroblasts 1327. |
| ,, 25th. | Wt. 5·0 kg. Hb. 70 %. Red cells 5·36. Whites 12·4. Eosinophils 7 %. Erythroblasts 3968. Temp. 102° F. |
| ,, 29th. | Wt. 5·4 kg. Hb. 62 %. Red cells 4·42. Temp. 101·5° F. Eosinophils 3 %. |
| ,, 31st. | Wt. 5·1 kg. Hb. 56 %. Red cells 3·98. |
| June 1st. | Wt. 5·0 kg. Hb. 52 %. Red cells 3·71. Temp. 101·8° F. Pulse 100. |
| ,, 3rd. | Wt. 4·7 kg. Hb. 16 %. Red cells 90. Whites 19·7. Eosinophils 1 %. Erythroblasts 12,903. Temp. 98·8° F. Pulse 182. No ova in faeces. Very ill. |
| ,, 4th. | Wt. 4·6 kg. Hb. 12 %. Red cells ·88. Whites 20·7. Pulse 104. Temp. 94·4° F. |
| | Killed. Blood volume 293·7 c.c. = 6·38 %. 1719 hook-worms in intestine. Very large amount of blood in ileum, much of it bright red; also in coecum and large intestine. Several punched out pits in coecum. No larvae in trachea or oesophagus, but one found in stomach. |

*U.*

| | |
|---|---|
| Aug. 8, 1912. | Wt. 2·2 kg. Hb. 70 %. Red cells 5·48. Whites 21·1. Temp. 100·9° F. Already infected with *Ascaris*. Infected by mouth with hook-worm larvae. |
| ,, 9. | Wt. 2·2 kg. Hb. 70 %. Red cells 5·75. Eosinophils 2½ %. Erythroblasts 1793. No polychromasia. Temp. 101·3° F. |
| ,, 10. | Wt. 2·2 kg. Whites 13·1. Eosinophils 2 %. Erythroblasts 1834. Temp. 101·8° F. |
| ,, 12. | Wt. 2·3 kg. Hb. 75 %. Red cells 7·11. Temp. 100·8° F. |
| ,, 13. | Wt. 2·5 kg. Whites 11·1. Eosinophils ½ %. Erythroblasts 55. |
| ,, 15. | Wt. 2·9 kg. Hb. 68 %. Red cells 6·56. Temp. 102·4° F. |
| ,, 16. | Wt. 2·9 kg. Whites 18·4. Temp. 102° F. |
| ,, 19. | Wt. 3·0 kg. Hb. 65 %. Red cells 7·72. Temp. 102·2° F. |
| , 20. | Wt. 2·9 kg. Whites 15·8. Eosinophils 0 %. Erythroblasts 553. Temp. 101·6° F. |
| ,, 21. | Wt. 3·1 kg. Whites 14·4. Eosinophils 0 %. Erythroblasts 720. Temp. 102° F. |
| ,, 22. | Wt. 3·1 kg. Hb. 64 %. Red cells 8·35. Temp. 102° F. |
| ,, 23. | Wt. 3·2 kg. Whites 11·4. Eosinophils 0 %. Temp. 101·4° F. |
| ,, 29. | Wt. 3·6 kg. Hook-worm ova in faeces. |
| Oct. 15. | Wt. 5·8 kg. Still alive and well. No appearance of anaemia. Experiment discontinued. |

*V.*

| | |
|---|---|
| Aug. 8, 1912. | Wt. 2·0 kg. Hb. 70 %. Red cells 5·40. Whites 21·5. Eosinophils 3 %. Erythroblasts 430. Temp. 101·2° F. Already infected with *Ascaris*. Infected with hook-worm larvae through skin. |
| ,, 10. | Wt. 2·0 kg. Hb. 68 %. Red cells 6·02. Temp. 102° F. |
| ,, 12. | Wt. 2·0 kg. Whites 25·9. Eosinophils 3 %. Erythroblasts 388. Temp. 100·4° F. |
| ,, 13. | Wt. 2·2 kg. Hb. 70 %. Red cells 6·86. Temp. 101·3° F. |
| ,, 15. | Wt. 2·5 kg. Whites 15·6. Eosinophils 0 %. Temp. 102·2° F. |
| ,, 16. | Wt. 2·5 kg. Hb. 66 %. Red cells 7·56. Temp. 102° F. |
| ,, 19. | Wt. 2·6 kg. Whites 19·7. Eosinophils 0 %. Erythroblasts 197. Temp. 102·3° F. |
| ,, 20. | Wt. 2·6 kg. Hb. 61 %. Red cells 6·99. Temp. 101·2° F. |
| ,, 21. | Wt. 2·9 kg. Hb. 66 %. Red cells 6·43. Temp. 102·2° F. |
| ,, 22. | Wt. 2·8 kg. Whites 14·7. Eosinophils 0 %. Erythroblasts 147. Temp. 101·3° F. |
| ,, 23. | Wt. 2·9 kg. Hb. 65 %. Red cells 6·58. Temp. 102° F. |
| ,, 29. | Wt. 3·0 kg. Hook-worm ova in faeces. |
| Oct. 15. | Wt. 4·8 kg. Still alive and well. No appearance of anaemia. Experiment discontinued. |

### *General remarks with regard to time of appearance of ova in faeces etc.*

A dog which first received larvae on April 18th, 1910, showed numerous ova in its faeces on May 5th, *i.e.* 17 days afterwards. This period corresponds with that found by other observers *e.g.* Lambinet (1905), Looss (1911), who have experimented with the dog hook-worm. A second dog, infected on April 19th, died on April 24th from pneumonia. No worms or larvae were found in the intestine. A third dog, infected on April 25th, died on May 5th from the same cause. Over 100 specimens of *Agchylostoma caninum* were found in the ileum. They were adult but none of the females contained mature ova. There had been a considerable amount of haemorrhage in the intestine, bright red blood being found as far down as the coecum. The death of these two dogs was almost certainly not to be ascribed to the infection with *Agchylostoma* for two other dogs in the same batch died from pneumonia before infection was started. From the lungs of these dogs an organism of the *Pasteurella* group was isolated, but whether this was the cause of death or not was not determined.

The first dog escaped the pneumonic infection, and small quantities of larvae were administered to it regularly every second day for about a month. It remained alive and healthy for over two years, and continued to show a moderate number of ova in its faeces during the

whole of that period. From it, chiefly, the remaining dogs of the series have been infected. At no time did it display any very marked signs of anaemia, although the haemoglobin percentage was at one time as low as 76. Blood examinations were made only at irregular intervals on this dog. On one occasion, during an attack of mange, the Hb percentage was as high as 96. Later, however, on treatment and recovery, it fell to 84. The erythrocytes rarely fell below six million per c.mm., while the leucocytes varied from 14–25 thousand, the higher figures being recorded during the early stages of infection. At no time was there any degree of eosinophilia, eosinophils being frequently entirely absent.

A fourth dog was infected on May 11th. No ova could be found in the faeces up to the 20th day. They were first seen on June 13th (33 days), but were probably present some days earlier as examination was intermitted for about a week. The weight of this dog, which was full grown, remained practically constant at about nine kilograms. The haemoglobin percentage, which was at first 95–100, had fallen to 80 when infection was established, *i.e.* when ova were demonstrable in the faeces, and remained about that figure for a year. The red cells did not fall below five millions per c.mm., while the leucocytes were generally about 20,000. In this case, again, no evidence of eosinophilia was obtained, but in the faeces there were distinct signs of intestinal haemorrhage. This dog is still alive and well and its faeces still contain moderate numbers of hook-worm ova. It has been maintained as a reserve stock.

As already remarked no pronounced symptoms of anaemia were observed in these dogs even after a month's constant infection. Similar infection was prolonged for periods of three, six, and more months in the case of other dogs, but in no case could a severe chronic anaemia, similar to that occurring in man, be produced. These dogs were all reputably under one year old and some were believed to be not much over six months. With still younger dogs there was the constant difficulty of intercurrent affections, such as pneumonia and distemper, to which in the majority of cases they succumbed soon after infection was started. These difficulties delayed the progress of the experiments very considerably, and it was at last decided to examine the blood volume of such dogs as had been infected and had shown signs of anaemia even in a minor degree. The general course of infection in these dogs was the advent of a slight though distinct degree of anaemia about three to four weeks after infection was started, this continuing for

varying periods of a month or longer, and being followed by gradual recovery, and this in spite of continued infection. The Hb percentage in some cases sank as low as 50, but the red cells were rarely under 5,000,000. The Hb index was therefore usually decidedly under unity. In the young dogs which did not succumb to intercurrent affections the symptoms were much more severe and the disease rapidly terminated in death. It is to be regretted that the blood volume of these dogs was not estimated, but this, unfortunately, was deferred in the hope of obtaining a chronic affection, a hope, however, which was in no case realised.

The results of the blood volume estimations may be tabulated as follows:

| | Weight (gms.) | Blood washed out (c.c.) | Total vol. (calculated) | Blood vol. body wt. (%) | Hb % | No. of worms | Length of infection (days) |
|---|---|---|---|---|---|---|---|
| *A.* | 8300 | 595·0 | 632·0 | 7·62 | — | 12 | 796 |
| *I.* | 12700 | 851·2 | 905·0 | 7·13 | 96 | 28 | 150 |
| *J.* | 10000 | 685·6 | 729·3 | 7·29 | 68 | 170 | 72 |
| *K.* | 17100 | 1766·5 | 1878·4 | 10·98 | 105 | 146 | 129 |
| *L.* | 7100 | 401·7 | 427·2 | 6·02 | 95 | 130 | 134 |
| *M.* | 8500 | 723·4 | 769·5 | 9·05 | 98 | 30 | 170 |
| *N.* | 9800 | 714·7 | 744·3 | 7·59 | — | 2 | 264 |
| *Q.* | 7800 | 463·5 | 493·1 | 6·49 | 108 | 2 | 44 |
| *S.* | 6600 | 461·0 | 490·4 | 7·43 | 40 | 791 | 36 |
| *T.* | 4600 | 276·1 | 293·7 | 6·38 | 12 | 1719 | 13 |
| Aver. | 9230 | | | 7·60 (1/13) | | | |

On comparing this table with that on page 379 it is evident that on the average the blood volume per kilog. is distinctly less in the infected dogs than in the normal, although at the same time it must be noted that the infected dogs were on the whole a somewhat heavier lot.

Calculating the blood volume according to the formula of Dreyer and Ray, and taking as the observed volume only the amount washed out, we obtain the following values of the constant "*k*."

| Normal | | Infected | |
|---|---|---|---|
| *E.* | 0·746 | *I.* | 0·638 |
| *F.* | 0·873 | *J.* | 0·672 |
| *X.* | 0·599 | *K.* | 0·386 |
| *Y.* | 0·477 | *L.* | 0·922 |
| | | *M.* | 0·572 |
| | | *N.* | 0·642 |
| | | *Q.* | 0·836 |
| | | *S.* | 0·763 |
| | | *T.* | 0·927 |
| Average | 0·674 | | 0·706 |

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From this it would appear that if the blood volume is a function of the surface and not of the body weight there is on the average little difference between the infected dogs and the normal, but that if anything the volume is somewhat decreased; individually there are three very marked deviations, namely in the case of dogs *K* and *L* and *T*, the former of which has a decidedly increased volume and the latter a markedly diminished volume.

The oxygen capacity of the blood per kilo of body weight has also been calculated, in this case on the estimated total volume, and the figures are as follows:

Normal:—*E*. 14·6. *F*. 10·8. *X*. 19·0. *Y*. 16·7. Average 15·3.
Infected:—*I*. 12·1. *J*. 9·4. *K*. 21·9. *L*. 10·6. *M*. 16·4. *N*. 14·6. *Q*. 13·3. *S*. 5·5. *T*. 2·4. Average 11·8.

From this we see that on the average the oxygen capacity per kilo of weight is diminished in the infected dogs, but that at the same time there is a considerable amount of variation even in the normal dogs. We notice the very high figure given by *K*, and the very low figures of *S* and *T*, in which there was massive infection and considerable bleeding into the intestine.

These results are so variable, even in the case of normal animals, that it is difficult to draw any very definite conclusions from them. One fact would appear, namely, that normal dogs show wide variation in the factors on which the results of these experiments are based and on that account it would be necessary to obtain some very wide deviations in order to arrive at any definite conclusion.

A further endeavour was made to deduce some relation between the change in blood volume and the number of worms present, and the length of infection, but as will be seen from the table above no constant relation is obvious.

There are two additional matters which must be discussed before attempting to draw any final conclusion. These are the absence of eosinophilia and the presence of erythroblasts. The diagnostic significance of eosinophilia has already been mentioned in the earlier part of this paper, and it is somewhat remarkable to find it so constantly absent in the animals under investigation. The only animals in which it was at any time noted were *J* and *L*. In the former it was present before infection was started, and from the fact that a large number of round worms were found in the intestine after death, it is not improbable that they had some connection with the eosinophilia. In the case of *L* the eosinophilia made its first appearance about a month after infection was

started, but it fell off considerably during the course of the succeeding month, and eventually disappeared. No ready explanation of this curious circumstance presents itself. It must serve, however, to emphasise the fact that eosinophilia is not by any means a constant or invariable accompaniment of infection with intestinal worms.

The marked occurrence of erythroblasts in some cases is also worthy of note. In the earlier of these observations no account was taken of these cells, and it is possible that they escaped notice. Latterly, however, they were looked for and their numbers estimated. They did not occur in every case, but were particularly noted in dogs *I* and *R*. In the former they were found in the very considerable number of 650 per c.mm. They were also noted, however, in the young uninfected pup *R* to the extent of 100 per c.mm. Later, after infection, the number rose to 300 and eventually to 1150 per c.mm., after which it again fell to 300. These cells were usually of the normoblastic type and no megaloblasts were seen. The majority had a central nucleus, an evidence of their recent formation, but all stages were seen up to those in which the nucleus was quite peripheral. No rosette-shaped or dividing nuclei were observed. To me it appears there can be little question that the presence of these cells gives evidence of a distinct and active regeneration of blood cells to meet, presumably, the loss by haemorrhage, and this lends support to the view that the intestinal haemorrhage plays some part in the causation of the anaemia, at any rate in the early part of the infection. The occurrence of erythroblasts in the young normal pup is not, I believe, an abnormal circumstance, as these cells are not infrequently observed in the blood of children and of young animals for a few months after birth.

Other features in regard to the leucocytes were noted during the course of these experiments, the most interesting of which was the occurrence of intense basophilia during attacks of mange.

In addition to these observations, others, of biological rather than clinical interest, may be mentioned. Some of these have already been remarked upon in the opening part of this paper. There is first of all the remarkable fact that while young dogs may be infected to an extent only limited by death, older dogs, on the other hand, appear to be susceptible only to a very restricted degree. This is a circumstance which led to very great delay in the present research. It was always found possible to infect dogs, irrespective of age, and in the course of two or three weeks ova were found in the faeces. It was a natural assumption that by continuing the administration of infective material,

a very gross infection would eventually supervene, but such, as has already been stated, did not prove to be the case. It is impossible to imagine what factor enabled them to withstand further infection and the problem is one which undoubtedly possesses considerable biological interest.

Of a similar nature were the facts derived from attempts to infect cats and monkeys with the dog hook-worm. The cat, as might be expected from zoological considerations, is to a certain extent susceptible to infection with parasites of the dog. Thus, we find the common tape-worm, *Dipylidium caninum*, as frequent in cats as in dogs. The round-worms ("*Ascaris canis*" and "*Ascaris mystax*") also appear, to a certain extent, to be common to both, although this is a matter for further and more accurate investigation. On the other hand, the tape-worms of the genus *Taenia* appear to be extremely specific. The cat has its own species, *T. crassicollis*, which is never found in the dog, and conversely, the numerous species of *Taenia*, met with in the dog, are never found in the cat. The hook-worms appear to occupy an intermediate position as regards specificity. One species, *Uncinaria criniformis*, has been recorded only from the dog, while *Agchylostoma caninum* has been recorded from both, although much more frequent in the dog.

I attempted a considerable number of infection experiments with kittens, about two to four months old, but the results were vitiated by the "distemper" which almost invariably followed. This "distemper" occurred at two well-marked periods, namely, about the third and sixth months. In a litter of half a dozen, half would succumb at the third month, the others recovering only to have a second attack at the sixth month, from which there would be a solitary survivor. The disease was usually of the broncho-pneumonic type but not infrequently accompanied by gastro-enteritis. In only three cases did the kittens live long enough to display hook-worm eggs in their faeces. Only one survived much beyond the sixth month and this animal continued to pass eggs for about six months, but at the end of nine months it had apparently got rid of infection for no eggs could be detected after this period. Of two adult cats, one became slightly infected, the other was absolutely insusceptible.

Similar experiments were tried with three monkeys (*Macacus*) but in no case did infection take place, although they were fed with infective material continuously for three months.

Finally the experiment was tried of personal infection, but this also

was unsuccessful, and although infective cultures have been handled both by myself and by my assistant very frequently during the last two years, without any stringent precaution, no infection has taken place in either. This fact lends support to the belief that the dog hook-worm cannot infect man, and disposes of the idea that the dog can act as the carrier of hook-worm infection in man, either by harbouring the human form or by spreading its own particular species.

Owing to my departure for Australia these experiments have had to be discontinued and some of the points which might have required further investigation have had to be left incomplete.

## Summary and Conclusions.

In these experiments the hook-worm anaemia of dogs does not appear to be exactly analogous to the corresponding disease in man, but differs from it in two essential particulars, namely, that only young animals suffer and that in them its course progresses much more rapidly to a fatal termination.

Older dogs, although not altogether insusceptible, acquire infection only to a moderate extent, which gives rise to a minor degree of anaemia. From this they gradually recover, even in spite of repeated and continued attempts at re-infection.

The anaemia in young dogs was characterised by great loss of weight, emaciation, prostration and intestinal haemorrhage, but in no case was epistaxis observed.

The blood volume of dogs suffering from the minor degree of hook-worm anaemia is not materially altered, but if anything is somewhat diminished. The oxygen capacity of the blood per unit of body weight is also, on the average, somewhat decreased.

Infection is generally accompanied by distinct though not profuse haemorrhage, which is most marked in the early stages, but tends to disappear.

Eosinophilia was not a constant sign either of infection or of disease.

Evidence of blood regeneration was furnished by the appearance of large numbers of erythroblasts (normoblasts) which increased with the progress of the disease.

Cats are much less easily infected than dogs, and monkeys are altogether insusceptible. Man, also, were found to be insusceptible to infection with the dog hook-worm.

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