extent, to avoid the dangers referred to through the use of the common adhesive tape. The tape was used in the form of small strips, and the whole manipulation was carried on in the following way. A tiny drop of water was put on the gummed surface of the tape, and the fossil placed on the wet spot in a desirable position. After a few minutes the glue was dried and held the fossil firmly enough to allow of its preparation. When the preparation was finished, the strip of the tape was plunged into water, the fossil removed from the tape with a soft brush and the adherent remains of glue washed off. It is very important to take as little water as possible for fixing fossils on the tape. If the drops were too large, it would not only take too much time before they would dry out sufficiently to give a good hold to fossils, but the latter might sink into the glue which, dried on their surface, would interfere with the work of the needle. The most difficult part of the operation is the fixing of the fossils on the gummed surface in exactly the desired position.

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SPECIAL ARTICLES

AN OBSERVATION WHICH SUGGESTS AN EXPLANATION OF THE ANEMIA IN HOOKWORM DISEASE

During the course of an experiment on absorption from the small intestine of the dog in which the mucosal surface of the gut was exposed in a special device for observation of the movements of the villi, the author's attention was attracted by the activity of several hookworms (*Ancylostoma caninum*) attached to the mucosa. While watching one of them through a binocular microscope, a large droplet of blood was seen to emerge suddenly from its anal orifice. Within the next few minutes a considerable number of droplets had been ejected by the same worm. Eight or ten other worms present were all seen to be passing blood in the same manner.

The passing of blood in such quantities naturally aroused our interest, as it seems to suggest a plausible explanation of the anemia in hookworm disease. For, although anemia has long been recognized as the essential feature of the disease, its cause has remained obscure. Hemorrhagic areas, due to the bites of the worms, have been noted, and blood has frequently been found in the digestive tracts of the canine and the human forms of the parasites. Indeed, the worms have been seen to eject blood, both from the mouth and from the anus, on placing them in water after removal from the dead host. But no direct observations on the blood-sucking activities in the living host have been made heretofore, so far as the author is aware. All evidence of the presence of blood in the tracts of the worms has been considered merely as showing that blood may be the principal food of the animals. It has naturally been assumed that, as the worms can not require much blood for food and as the loss to the host by hemorrhage from the mucosa is seldom very great, there must be some other reason for the anemia. Toxins, including agents acting on the blood-forming organs or on the blood cells directly, have been postulated as possible causes. But aside from the finding of a hemolytic agent in extracts from dead worms, the evidence for such toxins has never been convincing. The observations here reported show that, in the case of the dog hookworm at least, blood may be removed from the host to a degree hitherto unsuspected, which indicates that it may be necessary to reconsider the factor of blood-sucking in relation to the causation of anemia.

Although a rather elaborate apparatus was used in the original observations, this is not necessary. The procedure may be successfully carried out as follows. A dog having a fairly heavy infestation, as shown by examination of a fecal smear for ova, is anesthetized by the administration of 0.35 gram of sodium barbital per kilo in approximately 10 per cent. solution, either by mouth or intravenously. In an hour or less the abdomen may be incised in the midline and a loop of bowel pulled out. With sharp scissors the wall of the gut is cut longitudinally on a line opposite the attachment of the mesentery. Hemorrhage from the cut borders of the segment should be checked by the application of spring paper clamps. The activity of the worms may be observed with the unaided eye and with ordinary illumination, but better results can be obtained with a Greenough type binocular microscope of low power and with the field illuminated by means of an arc or other strong source. It is advisable to keep the worms submerged in warm isotonic saline solution to prevent them from drying.

To date, several dogs have been used and some fifty worms examined, all of which were seen to be passing blood as in the first experiment. The frequency of ejection of the droplets varied considerably. In the first two dogs studied, the intervals between ejections varied from 2 seconds to 10 or 15 minutes. However, during several active periods extending over 20 minutes or more, the intervals were never longer than one minute, the average being for two such extended periods 15 and 22 seconds, respectively. During the periods of rapid ejection the tract of the worm remains more or less distended with blood. One gains the impression that the worm gorges itself with blood before starting to eject. The red material which gradually fills the intestine may be readily seen through the transparent tissues of the worm. The anal end becomes dilated and immediately there occurs a spasmodic contraction, often of sufficient force to move the whole posterior part of the worm. A droplet appears with great suddenness from the anal orifice. It was noticed in the case of some worms that blood may finally cease to replace that ejected and that the worm may gradually become almost white or colorless. When a worm was seen to move to a new point of attachment or when it disengaged itself from a part of the mucosa to which the blood supply had been cut off it was usually white, with little or no blood visible in its tract. Some worms were observed which never became colorless during the entire day. But in these as in the others there were periods when no blood was emitted. In the case of one dog the worms observed were relatively inactive. Periods during which little or no blood was ejected lasted for half an hour or more, and both the frequency and the size of droplets during the active periods tended to be less than in the first experiments. But even in this instance there were occasionally short periods during which large droplets were emitted at intervals of less than a minute.

The color of the blood emitted was sometimes purplish like venous blood, sometimes bright red or arterial in hue. The color seemed to be a characteristic peculiar to the individual worm and hence probably dependent on the local nature of the blood supply at the point of attachment. There was no distinct evidence of any change of color, from red to blue, as the blood passed through the worm. This point may need further study, for one is impressed with the possibility that the enormous amount of blood ingested by the parasite may serve a respiratory function.

The size of the ejected droplet was estimated by collecting a single emission in a capillary pipette containing isotonic salt solution. The blood was mixed and made to a definite volume of 0.5 or 1.0 cc. The red corpuscles were counted in the usual manner. Calculation gives the total number of red cells emitted per drop. In the case of two drops, each from a different worm, there were 1,168,000 and 1,268,000, respectively. Assuming the erythrocyte count of the dog to have been 5,000,000 per cu mm, the droplets would therefore each represent the red cells from approximately 0.25 cu mm of the dog's blood. Knowing the size of the droplet one could easily estimate the daily loss of blood incurred at the expense of the host provided one knew the average rate of ejection by each worm and the number of worms present. Although the normal average rate of ejection is not known at present, one can gain an idea of the possibilities by assuming a rate of, let us say, one ejection per minute per worm, which is certainly not an excessive maximum, as judged by two experiments lasting for over seven hours. On this assumption there will be removed from a dog in this manner 1 cu mm in 4 minutes, 15 cu mm per hour or 360 cu mm per day by a single worm. With 1,000 worms present and similarly active the loss to the host would be 360 cc per day. In this calculation no allowance is made for blood cells digested by the worm, nor, of course, for blood that may be lost by direct hemorrhage at the point of attachment to the mucosa.

Although at present one can only speculate as to the actual amount of blood discharged by the worms under normal conditions, there is every reason to believe that the parasites not only can but do take in and expel much more blood than could be accounted for by their food requirements alone. As to what biological purpose is served by this process, so wasteful of the blood of the host, it is impossible at present to judge.

Although these observations do not tell us anything of the behavior of those forms of the parasite which infest man, they do suggest that the factor of blood-sucking should be reconsidered, on the possibility that this activity of the worms may be found to play a part more important than formerly suspected in the production of anemia in human cases.

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A RELATION BETWEEN ROTENONE, DEGUELIN AND TEPHROSIN

In a previous communication to this journal[1] it was stated that apparently the principal toxic constituents of derris and cubé roots, namely, rotenone, toxicarol, deguelin and tephrosin, were, from a chemical standpoint, more or less closely related. At the time the report referred to was made, only indirect evidence supporting the assumption was available. This consisted of the similar solubilities of the compounds in many solvents, the identity or close similarity of their empirical formulas, the fact that all contained two methoxyl groups, and, finally, their general behavior toward certain reagents.

As the study of the chemistry of these materials has

[1] E. P. Clark, Science, 71: 396, April 11, 1930.