in harmony with the observations made on rabbits, in that it shows that the embryo of the larger breed grows faster when other conditions are equal.

Another and even clearer indication that breed (genetic constitution) affects the rate of growth of the embryo throughout the entire period of incubation (even before endocrine organs are established) seems to have been overlooked by Byerly. This is the more rapid growth of crossed as compared with uncrossed embryos. The difference in blastoderm composition in the two breeds prior to incubation, which obviously influences embryo weight up to the ninth day of incubation, may be completely eliminated by confining the comparison to the eggs of one breed at a time, comparing the size of embryos produced in White eggs fertilized by White males with that of embryos produced in White eggs fertilized by Red males, and also comparing the size of embryos produced in Red eggs fertilized by Red males with that of embryos produced in Red eggs fertilized by White males. In both cases Byerly's observations show the cross-bred embryos to be preponderantly heavier, whether the mother was White or Red.

The White eggs opened each day (Table I) range in number from 10 to 75 in each series (pure-bred and cross-bred). The pure-breds average heavier on 4 of the 19 days of incubation, *viz.*, the 2nd, 8th, 16th and 18th. On the 15 remaining days, including both the first and the last, the cross-bred embryos are heavier.

The observations made on Red eggs are less numerous but point to the same conclusion. The cross-bred embryo is in general heavier. The number of cross-bred embryos studied is smaller and does not cover every day of the incubation period, ranging from 4 to 11 embryos per day, but its indications are clear. The period covered is from the 2nd to the 19th days of incubation, omitting the 6th and 7th, and the 13th, 14th and 15th. Cross-bred embryos are heavier on all except two (the 9th and 18th) of the 13 days sampled.

As to the hatching weight, that of the cross-breds is slightly greater in the Red series and slightly less in the White series. Here available nourishment within the egg comes in as a limiting factor. If this were removed, by taking body weights a few weeks subsequent to hatching, cross-breds would undoubtedly be found again heavier, as is well known from other observations.

The specially controlled series of embryos produced by Byerly, from eggs of the same size incubated simultaneously side by side, summarized in his Table 3, confirms the conclusions based on his more general series summarized in Table I. The number of embryos studied is smaller, ranging from 2 to 14 per day in each of the four series, but the conditions under which they were produced make their evidence particularly important. Embryos from the eggs of White hens mated with White males are heavier on 3 of the 12 days sampled; *viz.*, the 2nd, 3d and 12th; cross-bred embryos from the eggs of White hens mated with Red males are heavier on the other nine days (4, 5, 8, 9, 10, 11, 16, 17 and 19). Embryos from the eggs of Red hens mated with Red males are heavier on 4 of the 12 days sampled; *viz.*, 9, 11, 16 and 19; cross-bred embryos from the eggs of Red hens mated with White males are heavier on the other eight days (2, 3, 4, 5, 8, 10, 12 and 17). With the small number of embryos examined, it is evident that random sampling affects the results here more than in Table I, which included larger numbers; nevertheless the general trend of the observations is clear and consistent with the results of Table I. *Other things being equal, a cross-bred embryo grows faster than one not cross-bred.*

There is, we think, no escaping the conclusion based on Byerly's own observations that breed (genetic constitution) does influence growth rate and through it body size. Embryos of the larger breed grow faster as soon as they have attained an even start. Also eggs of the same breed laid by the same flock of hens under identical conditions, if fertilized by males of their own breed, produce smaller embryos than are produced if fertilization is accomplished by males of the other breed. Are the cross-bred embryos heavier because they contain more cells or larger cells? We may take our choice of these alternatives. If they contain more cells, then cell multiplication must occur more rapidly in the larger embryo, exactly as it does in rabbits. If one chooses to assume that the cells are larger rather than more numerous in cross-bred embryos, the burden of proof rests with him, for Painter has not found it so in rabbits, but in any case it is obvious that a cross-bred embryo grows faster than one not cross-bred in birds as well as in rabbits.

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THE EFFECT OF DIET ON HOOKWORM INFESTATION IN DOGS[1]

THE investigations summarized in this brief preliminary report give an experimental demonstration

[1] From the department of helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. This work was made possible by the aid of the International Health Division of the Rockefeller Foundation.

of a definite correlation in dogs between undernourishment and susceptibility to infection with the common dog hookworm, *Ancylostoma caninum*. They also show that hookworm infestations which develop in dogs on a deficient diet can be practically eliminated by placing them on an adequate diet.

The routine dog diet used in our laboratory consists of pig lungs, milk, bread and water. Small amounts of cod liver oil are added to this diet for puppies and for the experimental dogs when changed from a deficient diet. The deficient diet used, which was suggested by Dr. H. D. Kruse of the department of chemical hygiene of this institution, consists of 35 per cent. of corn starch by weight, 35 per cent. of dried ground peas, 29 per cent. of Mazola oil, 1 per cent. of NaCl (C. P.) and an abundant supply of water. This food was always given in sufficient quantities to satisfy hunger, the significant fact being that it is very deficient in vitamines and important minerals.

The course of the infestations in the dogs was carefully followed by fecal examinations by the Lane method and the Stoll dilution egg counting technique, and the total fecal output of each animal was routinely screened for the recovery of any worms passed.

In the first experiment, five dogs were used. Two of them had been born in the laboratory and the other three had been in the laboratory since they were only a few weeks old. These five dogs had been used by Dr. O. R. McCoy[2] for studies of resistance to hookworms in animals which had been given repeated infections. When they were turned over to us for the diet experiments, they were all full grown, 9 to 16 months old and had very slight infestations. They were practically immune to further infection with the dog hookworm due to age and the long series of previous infections to which they had been subjected. In all five cases, the resistance was so pronounced that doses of 4,000 or more infective hookworm larvae produced no increase in their worm burden, as measured by eggs in the feces. In one case a single dose of 500,000 infective larvae resulted in no increase in egg production at a time when eggs in the feces could only be detected at all by the most careful examinations.

These five animals were all placed on the deficient diet at the same time. In spite of treatment, they all harbored a few worms at the beginning of the experiment. Two of these dogs with no additional infections began to show a considerable increase in daily output of hookworm eggs in their feces after about ten weeks on the poor diet. This was interpreted to mean that worms already present were enabled to produce more eggs as the host was affected by the poor diet. This same thing was shown by three other dogs in a later experiment not included in this paper. These dogs had been given infestations while on a good diet. After the curves of the egg counts had gone down to a low level in the natural course of the infestation, they were placed on the deficient diet and were given no further doses of larvae. Not long afterwards their egg counts increased very considerably indicating an increased egg production in the worms that remained. This finding fits in well with some of McCoy's results in which he found that the egg production of worms in resistant dogs was about one third of those in susceptible dogs.

After these first two dogs had shown the increased egg production on the deficient diet, they were treated until negative. Then after twenty weeks on this diet they were each given 500 infective hookworm larvae by mouth. After a normal prepatent period, they became positive and their egg counts rose rapidly reaching peaks of egg production comparable to those previously reached when they were susceptible puppies. In fact in one case the egg production was much greater than that produced by the earlier infections. These two dogs were transferred to the good diet after 149 days on the deficient diet with the egg output still at the peak. They rapidly regained their weight and general health and there was a rapid reduction in daily egg production, the egg count curves falling almost to zero in a period of only four weeks on the good diet. Numbers of worms were spontaneously lost after the egg production had dropped to a few thousand eggs per day. This phenomenon is suggestive of a dietary cure of hookworm in dogs. Following this spontaneous cure, repeated doses of larvae failed to produce any significant infestations showing that resistance to hookworms was regained.

The other three dogs on the first experiment were handled somewhat differently. Although previously treated, they still harbored a few worms when placed on the deficient diet. After they had been on this diet for ten weeks, they were each given a single dose of 500 infective hookworm larvae. Again after the normal prepatent period they became positive and the egg counts rose rapidly reaching peaks of egg production comparable to those produced by infections when they were susceptible puppies. These dogs were then treated until they were negative for hookworms before being returned to the good diet. This change of diet soon increased their weight and also restored their resistance since repeated doses of hookworm larvae failed to produce significant infestations.

The point to be emphasized is that all five of the dogs in the first experiment, which had been

[2] O. R. McCoy, *Am. Jour. Hygiene*, 1931 (in press).

shown to be resistant to enormous doses of infective hookworm larvae while on an adequate diet, quickly lost their resistance and developed rather heavy infestations when given 500 larvae each after ten or twenty weeks on the deficient diet. Two of them when transferred to the good diet while still harboring large numbers of worms expelled them in a short time.

A second experiment with two dogs, which were estimated to be about two years old, was carried out in a somewhat different way. These two dogs were brought into the laboratory as pregnant females and were kept on the good diet about two months before the puppies were born and while they were being nursed. During this period, they were treated and later found to be negative to hookworms by repeated examination. While still on the good diet, they were each given 500 infective hookworm larvae by mouth. Both remained negative to repeated examinations for a period of six weeks, which indicated that they were very resistant to the hookworm. At this time, they were put on the poor diet and given repeated doses of 500 larvae at intervals of two weeks, the first dose being given two weeks after they had been placed on the deficient diet. After prepatent periods of 19 and 17 days, respectively, or after 33 and 31 days on the poor diet, they became positive. The egg counts increased with each subsequent infection until the number of eggs given off per day was about 700,000 for one dog and 900,000 for the other. At this time, 90 days after they had been placed on the deficient diet, they were transferred to the good diet. The doses of larvae at the two-week intervals were continued as before. But in spite of these constant doses of larvae, the egg counts in both dogs came steadily down until they reached a low level. In each case numbers of worms were lost after the egg counts had been greatly reduced. These two dogs are of especial interest since they show first the rapid breaking of the resistance in old dogs on the poor diet, and then a cure and a regaining of the resistance when they were placed on the good diet in spite of continued doses of infective larvae.

In the experiments outlined above, a definite correlation is demonstrated between deficiency in diet in dogs and susceptibility to infection with the dog hookworm, *Ancylostoma caninum*. The undernourished condition is characterized by lowered resistance to infection, increased rate of development of the worms and increased egg production per worm. When the dogs that had acquired an infestation while on the deficient diet were transferred to the good diet their recovery of resistance was indicated by a reduced egg production of the worms present, a spontaneous loss of worms and a resistance to further infection. It seems possible from this and other evidence that a similar relation may exist between the human hookworms and their hosts. We suspect that it will be found that heavy infestations are more easily built up in people on poor diets, and that not only the effects of the worms on the hosts may be reduced, but also the worms themselves may be partially or wholly eliminated by improvement in diet alone.

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FURTHER STUDIES ON THE ADRENAL CORTICAL HORMONE[1]

THE work herewith reported was done upon dogs with the adrenal cortical hormone prepared according to the method of Swingle and Pfiffner.[2] The material does not contain adrenalin in excess of 1 to 1,500,000. A series of bilaterally adrenalectomized dogs maintained in good health for considerable periods with the cortical hormone were then injected with gradually decreasing doses of extract (subcutaneous, one dose each day), the dose being changed at five-day intervals. One dog was maintained for a period of five days on 1/6 cc per kg weight per day, the others on 1/4 cc per kg weight per day without symptoms of insufficiency. We regard 1/4 cc therefore as the minimal maintenance dose for a single injection per day in dogs weighing 10 to 15 kg.

Extensive experiments have been made on the blood concentration and urinary excretion of various inorganic substances and of the nitrogenous compounds, following the injection of cortical extract into normal dogs, and into adrenalectomized animals. In the normal dogs we have been entirely unable to detect characteristic changes in the blood constituents which we have followed. Carbon dioxide content and capacity (alkaline reserve), oxygen capacity, nonprotein and urea nitrogen, creatinine, sugar, calcium, potassium and magnesium, cholesterol, lactic acid, plasma chlorides, hematocrit and plasma proteins, examined in arterial blood samples are not altered in any definite or quantitative manner. The determinations have been made at hourly intervals following injection, up to five hours and at the end of twenty-four and forty-eight hours. Stress should be laid, we believe, on the fact that we have used trained animals at rest, strictly in fasting condition. There was no change in the respiratory metabolism (oxygen consumption, or R.Q.) in a normal animal so injected within five hours or at the end of twenty-four hours. The amount of extract injected has varied

[1] Aided in part by a grant from the Josiah Macy, Jr., Foundation of New York.

[2] W. W. Swingle and J. J. Pfiffner, *Anat. Record*, xliv, 225, 1929; *Am. Jour. Physiol*, Vol. 96, 1931.