stretched fine wire by lightly greasing the wire with mineral oil. From the weight of a crystal suspended on the wire, the area of the specimen image on the slit,

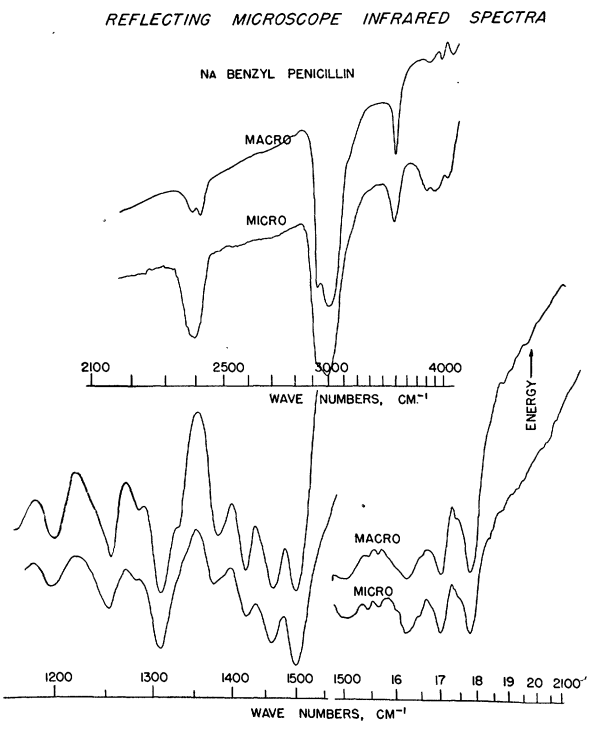

FIG. 2.

and the area of the slit it was possible to estimate the mass of the sample under observation. Inasmuch as the slit width must be increased as the wavelength is increased because of the black-body characteristics of the radiation source, the estimated mass of an observed sample varied from 0.3 μg at 4,000 cm^{-1} to 3 μg at 650 cm^{-1}.

A portion of the spectrometer recordings on macro and micro size samples of sodium benzyl penicillin are shown in Fig. 2. The effect on resolution of the wider-than-usual slit widths necessary with the microscope is observable near 2400 and 1325 cm^{-1}. The micro sample recording was made with an amplification of the thermocouple output such that 0.3 μv gave a full-scale deflection of the Brown recorder. More careful adjustment of the optical system should enable the use of smaller slit widths and less amplification.

It is obvious that the use of such totally reflecting microscope systems in connection with infrared spectrometers should permit large reductions in sample size with attendant increased applications in the biological and crystal structure fields.

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A Method for the Study of Blood Loss in Hookworm Infestation[1]

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Leichtenstern (*7*) pointed out in 1886 that the hookworm (*Ancylostoma duodenale*) was an avid consumer of blood. In 1909 Whipple (*9*) made the same observation in this species as well as in *Necator americanus.* In 1931 Wells (*8*) reported a series of observations involving *Ancylostoma caninum* while the parasites were attached to the intestinal mucosa of living dogs indicating, without a doubt, that this parasite was able to withdraw enough blood per day to account for the anemia often found associated with hookworm infestation. Work done by Cruz in Brazil, and by Rhoads and Castle and their associates in Puerto Rico has materially contributed to our knowledge of this parasitic disease. Wells (*8*) states that there is a possibility that a single worm may withdraw as much as 0.8 ml of blood in 24 hr but that there is apparently a considerable amount of variation in the activity of individual worms.

We present here a pair of preliminary studies of the average rate of blood loss in moderately infested dogs. These studies were carried out in order to determine the feasibility of the method of approach used, with the intention of extending it to a study of human hookworm infestation at a later date.

Two healthy adult mongrel dogs, 2-G and 1-J, were used in these studies. They had both been kept at an anemic level of blood hemoglobin for many months by frequent controlled hemorrhages and their reserve stores of iron were therefore depleted. At the time of application of the larvae the dogs' red cell hematocrits were 24% and 25%, respectively. About 2,000 infective larvae of *Ancylostoma caninum* suspended in saline were applied to each animal, contact being made between the toes and on the groin, where the body hair was sparse. The animals were fed a diet consisting of white bread, Klim, salmon, and cod liver oil in order to restrict iron intake (*6*), and were housed in cages with perforated metal floors. After a period of 5–6 weeks several examinations of feces showed numerous ova of the parasite. One of the dogs, 1-J, earlier had severe diarrhea, accompanied by the excretion of a moderate amount of mucus and red blood.

Four weeks following application of the larvae each animal received by vein 100 ml of blood from a donor dog. This latter animal had previously been fed large quantities of radioactive iron while anemic in order to build up red cells containing tagged iron in the hemoglobin iron (*5*). Immediately after, the diet was again allowed to consist of hospital table scraps so there was no exogenous deficiency of iron.

[1] This work was supported by a grant from the Nutrition Foundation.

Blood was collected in isotonic sodium oxalate and centrifuged at about 2,800 rpm in a type 2 International centrifuge for 35 min. The plasma was decanted and discarded. The red cells were wet ashed and their contained iron was separated by precipitation, electroplated, and examined for radioactivity content (*2*). Correction was made for decay of the isotope and for removal of the tagged iron by blood sampling as described elsewhere (*4*).

When administered by mouth to an anemic iron-depleted dog, the radioactive isotope of iron will in part be absorbed and this fraction will be incorporated for the most part in the red blood cell hemoglobin, very little being stored in other tissues. The level of the tagged iron in the red cells remains quite constant over many months, provided there is no loss by bleeding (*4*). If there is destruction of cells due to aging or hemolysis, the liberated iron is promptly reutilized to form new hemoglobin with the restoration of the original level of isotope in the circulating red cells (*4*). When the red cell isotope levels of the two dogs infested with hookworm were plotted they showed a regular drop, which appeared to be logarithmic. Plotted on semilogarithmic paper, this was borne out in the case of Dog 1-J. However, with Dog 2-G there seemed to be two periods in which the rate of blood loss was somewhat different.

In order to determine the rate of blood loss from the curves we may set up the following relations. For the sake of simplicity we shall consider only the part of the isotope curve which corresponds to the period where the hematocrit level was reasonably constant. It has been pointed out that under conditions allowing sufficient time for establishment of equilibrium in the circulation, the red cell mass is approximately a linear function of the jugular hematocrit (*3*). During the time in which there was no marked change in the hematocrit level, there was a nearly constant red cell mass in the circulation. Thus the rate of regeneration of red cells approximately equaled the rate at which they were lost to the circulation by the blood-sucking activities of the *Ancylostoma*. This may be expressed:

$$dv \longrightarrow \frac{N}{V} \longrightarrow dv$$

where v is the increment of red cell mass regenerated or lost in *ml*, N is the amount of radioactivity expressed in cpm, and V is the mass of red cells in the animal's circulation. Then

$$dN = N/V \cdot dv$$

Transposing and integrating between proper limits:

$$\int_{N_o}^{N} dN/N = 1/V \qquad \int_{O}^{v} dv$$

or $1n \frac{N}{N_o} = \frac{v}{V}$ (provided there is perfect mixing, which we are justified in assuming because of the time relations involved in the experiment). Since the concentration of radioactivity in the red cells is shown by

$$C = N/V \text{ and } C_o = N_o/V:$$

then $v = V\ 1n\ N/N_o = V\ 1n\ \frac{C}{C_o}$

or $\frac{N}{N_o} = C\ \frac{-v}{V}$.

If we take $v = V$, then $N/N_o = 1/e = 0.37$, i.e., if outflow equals the original red cell mass, the isotope concentration is 37% of its original value.

Applying this to the data on Dog 1-J we find that the initial activity was 2,000 cpm/100 ml of red cells. Then $0.37 \times N = 740$ cpm/100 ml red cells. This value for isotope level was found after 27 days. Applying the same procedure to Dog 2-G, we find the red cell mass equivalent to that originally present was lost in 16 days.

Based on body weight and hematocrits (*5*), these dogs had estimated red cell masses of 250 ml (2-G) and 275 ml (1-J). Roughly, this would indicate total blood volumes of about 1 l each. The data indicate that it required only 16 to 27 days for the loss of this amount of blood in these animals due to the proclivities of the hookworm, the animals at the same time maintaining relatively constant hematocrits. This rate of blood loss and regeneration is compatible in each case with the approximate amounts of blood it was found necessary to remove from these animals in order to maintain fairly constant hematocrits in similar periods of study prior to these experiments.

Loss of blood through hemorrhage by any route also lends itself to study by this method. It would perhaps be more direct and accurate to measure the radioactive iron in the excreta in this and any other similar type of investigation, but this is sometimes not convenient. Such direct measurements would not be subject to the difficulties encountered in total iron analyses, since the presence of interfering materials in chemical analyses may be eliminated in making the radioactivity determinations.

The use of donor tagged red cells might be questioned since their introduction has been used to study the survival time of red cells in hosts. However, in the relatively iron-free anemic dog, when the red cells disintegrate due to age or trauma, the liberated tagged iron is very rapidly reincorporated into new cells without grossly affecting the level of the isotope in the red cells (*4*). The inconvenience of preparation of such donor animals may be obviated by feeding a single dose of tagged iron to the infested subject and allowing that part which is absorbed to be synthesized by the body into hemoglobin. This method would lend itself to studies of human hookworms, especially where it was inconvenient to prepare donors. It would only require in addition to the described method that one wait until the isotope had reached a constant value in the peripheral circulation, or about 10 days.

Counts were made of ova in these dog's feces on several occasions, but it was felt that the relative variability in the degree of hydration of the stools rendered it improbable that a reliable estimate of the true worm burden was made by using these counts.

However, if the extent of blood loss were followed, employing the method herein described, for a definite period of time, and a vermifuge were then administered and the recovered worms counted, one might determine

an average value for the amount of blood consumed per day per recovered worm. Thus:

Let R = total radioactivity in excreta.
C = concentration of radioactivity in red cells.
W = total worms.
D = total days.

$$L = \left(\frac{\text{Average loss of red cells}}{\text{worm}}\right)/\text{day}.$$

Then

$$L = \frac{R}{C\,W\,D}.$$

The fact that very little iron derived from red blood cell hemoglobin is normally excreted in feces would probably make it unnecessary to correct this formula by a determination of the base line fecal iron output after vermifugation.

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Prevention of Dietary Fatty Livers by Exposure to a Cold Environment[1]

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In studies of the lesions which develop due to a deficiency of the lipotropic factors, it has been shown on numerous occasions that the development of fatty livers or of hemorrhagic kidneys is closely linked to caloric intake and to metabolic requirements. Severe lesions are more easily produced when growth is rapid and when food intake is high. Inanition may protect the liver and kidneys of an animal subsisting on a deficient diet. In an environment one or two degrees above freezing, the caloric requirement is increased greatly, as indicated by increased oxygen consumption and increased food intake. When rats weighing more than 150 g are exposed to such an environment they usually survive and some growth occurs, but at a slower rate than normal.

Two groups of ten male rats (Wistar strain), bred locally and weighing from 170 to 200 g, were given a diet ad libitum which permitted good growth but was deficient in choline and its precursors. One group was exposed to a temperature of $2.5 \pm 1°$ C for a period of two weeks, while the other was maintained for the same period under similar conditions but at a temperature of $25 \pm 2°$ C. At the end of the two-week period the animals were sacrificed, and their livers were examined chemically and histologically.

As might be expected, the fat content of the livers of the control group was high, averaging $24.8 \pm 4.90\%$ (total lipid expressed as % of wet weight). The average value found in the group maintained at 2.5° C was $7.2 \pm 1.24\%$. The average weight of the livers was approximately the same, but the weights of dry fat-free residues of the livers of rats kept in the cold environment were significantly higher than those of the control group. Although rats in the cold room ate more (average 22 g/day) than the controls kept at room temperature (average 15 g/day), their increase in body weight was less (average 1.3 g/day) than that of the control group (average 3.5 g/day).

The prevention of excessive deposition of fat in the liver in spite of increased consumption of a severely hypolipotropic diet would seem to be associated with the greatly increased total metabolic rate. The results of further study of this finding may throw light on the mechanism of action of choline, and perhaps on intermediary metabolic pathways which may be affected by exposure to a cold environment.

[1] These experiments form part of a project supported by a grant from the Defence Research Board of Canada. We are indebted to Prof. C. H. Best for his continued interest, and to Drs. Jessie Ridout and Jean Patterson for the estimations of liver lipids.

Films from Hemicellulose Acetates[1]

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Up to the present time, cellulose has been the only plant polysaccharide which has been acetylated for the production of commercial films and fibers. Commercially, hemicelluloses are separated from their natural mixture with cellulose and are regarded as undesirable impurities in pulp destined for esterification. Yet the major proportion of the hemicellulose mixture present in plants consists of xylan, a linear polysaccharide which should produce strong films. Consequently, an investigation was undertaken to obtain further information on the film-forming characteristics of hemicellulose acetates.

The hemicelluloses are sometimes isolated from crude plant material by extraction with alkaline solutions. However, lignin interferes, not only because it retards complete solution of the hemicelluloses, but also because some of it dissolves in the extract, causing difficulty in purifying hemicelluloses. These disadvantages are avoided largely through selective removal of lignin with a maximum retention of unchanged polysaccharides. Such delignified pulps are termed holocellulose (*6*).

The corncob is a typical example of hemicellulose-rich material. Approximately 80% of the corncob consists of polysaccharide material, one-half of which is cellulose, whereas the remainder is made up of a mixture of hemicelluloses. The entire polysaccharide mixture or holocellulose can be prepared by a modification (*3*) of the

[1] Contribution by Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Journal Paper No. 401.