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OCLC 22257407

Issued April 20, 1911.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 135.

A. D. MELVIN, CHIEF OF BUREAU.

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COMPARATIVE STUDY OF METHODS OF EXAMINING FECES FOR EVIDENCES OF PARASITISM.

BY

MAURICE C. HALL,

Assistant Zoologist, Zoological Division.

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LETTER OF TRANSMITTAL.

UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., January 17, 1911.

SIR: I have the honor to transmit the accompanying manuscript of an article entitled "A Comparative Study of Methods of Examining Feces for Evidences of Parasitism," by Mr. Maurice C. Hall, Assistant Zoologist in the Zoological Division of this bureau, with the recommendation that it be published as a bulletin of this bureau.

Mr. Hall has devised a method of examining feces by means of which the presence or absence of parasites infesting the alimentary tract may be rapidly and accurately determined. Various methods of fecal examination are in vogue among medical and zoological investigators, and these are all critically compared with Mr. Hall's method, which is shown to yield superior results.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.

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A COMPARATIVE STUDY OF METHODS OF EXAMINING FECES FOR EVIDENCES OF PARASITISM.

INTRODUCTION.

Having occasion to make numerous examinations of feces for evidences of parasitic infection, the writer has devoted some time to developing a satisfactory method which would be capable of general application in routine examinations and which would give the surest results in the shortest time, one which would detect any existing infection, even though light, in any kind of feces, so far as the existing limitations of fecal examinations permit. The different methods which have been used and advocated, most of them being methods used in examining human feces, were tested on feces of various kinds. This paper deals with the results of these tests. The method which gave the most satisfactory results and which is hereafter referred to as the writer's method is a modification of previous methods.

Fecal examinations are of two sorts—first, examination with the naked eye for gross evidence of infection in the shape of entire parasites or fragments, and, second, microscopic examination for parasite eggs, embryos, etc. The first is very commonly, perhaps wrongly, neglected; the second is commonly used and will be considered first here.

METHODS OF MICROSCOPIC EXAMINATION.

SMEAR METHOD.

The microscopic method which is commonly used and advocated is what may be called the smear method. A small particle of feces is taken up with a toothpick or stirring rod, smeared on a glass slide in a drop of water or salt solution, covered with a cover glass, and examined under the microscope. This method is the oldest and the simplest, can be used under almost any conditions, and, in that it calls for no equipment beyond a toothpick, a slide and cover glass, and a microscope, is also the most inexpensive. It is usually considered satisfactory, providing a number of slide preparations, commonly 10, are examined. This method has been repeatedly commended by

Stiles (1902hh, 1903l, 1906a), is one commonly used by physicians (Jones, 1907, et al.), and was used by Garrison, Ransom, and Stevenson (1903a), and Stiles and Garrison (1906a) in Washington, and by Garrison (1908) in Manila.

SEDIMENTATION METHOD.

The simplest modification of this method consists in allowing the feces, if sufficiently fluid, to settle, and then examining the sediment; if the feces are too solid for this, large or small amounts are washed in a sufficient quantity of water, decanted as long as any matter will float, and the sediment finally examined. Stiles, in the papers just cited, Braun (Braun and Lühe, 1909), Garrison (1910), Letulle (1905), Jones (1907), and others have advocated this sedimentation method.

BURETTE METHOD.

I am informed by Dr. Ransom that one writer, in an article to which I have not a reference at present, has varied the preceding method by taking the sediment from the bottom, through a stopcock, a method designated in this paper as the burette method.

CENTRIFUGE METHODS.

Another variation of the sedimentation method is to use a centrifuge for the purpose of giving a more rapid and certain concentration of material. Pepper (1908) states that he has found repeated centrifuging, in which the sediment is shaken up each time with some fresh water, very useful. This is the process commonly known as washing. Bass (1909) uses the same device as part of his method. Braun (Braun and Lühe, 1909) notes that under certain circumstances the sediment may be centrifuged after sedimenting and decanting. He does not state under what circumstances this is to be done. Letulle (1905) admits the use of the centrifuge if needed, but objects to it on the ground that certain eggs suffer mechanical injury from its use. Stiles (1902hh, 1903l) states: "The centrifuge does not appear to be of any special value in fecal examinations."

SIEVE METHODS.

Another variation of technique consists in the use of one or more sieves or screens to take out coarse particles of undigested food and similar objects. Stiles (1902hh, 1903l) gives a qualified indorsement of this method in the following terms:

> In case an unusually large amount of large, coarse material is present in the feces, it is sometimes convenient to pour the entire mass through a sieve, re-

jecting the portion left in the sieve; or to wash the feces in a sieve, holding the latter under water. As a rule, however, the sieve is not very useful in fecal examinations.

On the other hand, the use of the sieve receives unqualified indorsement from Cobb (1904). In examining sheep feces for fluke eggs he boils a pellet or part of a pellet of sheep dung in water for a few minutes; puts it in a hemispherical brass sieve of 80 to 100 meshes to the inch, the sieve being in a watch glass full of water, and itself standing partly full of water; brushes the pellet through the sieve by means of a sable brush; sediments the fluid containing the fecal matter which passes through the sieve, and repeatedly removes the supernatant muddy fluid with a pipette until water poured on the sediment remains clear; and then brushes this sediment through a sieve of miller's silk bolting cloth of five meshes to the millimeter. The water which passes through the silk sieve is then examined for fluke eggs.

Bass (1909), Telemann (1908), and Garrison (1910) use a sieve of some sort in making examinations of feces.

GASTEIGER'S FILTER METHOD.

Gasteiger's (1904) method is an inversion of the methods where a centrifuge and sieve are used. Whereas with the latter the object is to screen out foreign particles larger than parasites and eggs and seek for the parasitic material at the bottom of the centrifuge tube, Gasteiger, in his search for eggs of *Ascaris* in the stalls of cattle, soaks the manure, straw, and mud in water; filters this water through some unspecified sort of filter, and examines the residue, not the filtrate, for eggs. This accomplishes to a lesser degree the same thing that is accomplished by sedimentation, sieving, and centrifuging—it gives a concentration of material.

BASS'S SALT SOLUTION METHOD.

Bass (1906) proposed a method which consisted in putting a quantity of feces in a vial three-fourths full of a nine-tenths saturated solution of sodium chlorid, shaking this well, allowing it to settle, and then examining a drop from the surface of the fluid. This method was proposed for the examination of human feces for hookworm eggs. According to Bass, all the eggs rise to the surface and large numbers may be found in one drop. Of course this method depends on the specific gravity of the parasite egg being less than that of the solution employed. Waddell (1910) states that if an examination of three slides, presumably smear preparations, did not disclose human hookworm eggs, this method of Bass's was used, and says: "By this method from 30 to 40 minutes could be saved per stool examined."

BASS'S CALCIUM CHLORID CENTRIFUGE METHOD.

Afterwards Bass (1909) modified the above method as follows: A quantity of feces is diluted with water, 1 in 10, and strained through gauze to get rid of coarse particles. What comes through is centrifuged, the fluid poured off, the centrifuge tube refilled and the fresh material and the old sediment centrifuged again, thereby constantly adding to the total sediment, until all the diluted feces have been used. The sediment is rewashed several times until all matter that can be washed out in this manner is removed. Then a calcium chlorid solution of a specific gravity of 1.050 is substituted for the water. This disposes of everything having a specific gravity below 1.050, and the sediment may be examined at this point. If much sediment remains, the heavier matter may be removed by centrifuging with a calcium chlorid solution having a specific gravity of 1.250. In this solution the eggs come to the top and a few drops from the surface may be removed and examined, or, better, some of the top fluid may be poured off, diluted with water sufficiently to bring the specific gravity below 1.050, and centrifuged. The sediment will now contain most of the eggs that were in the original amount of feces and may all be put on a slide and examined.

GARRISON'S CALCIUM CHLORID SEDIMENTATION METHOD.

Garrison (1910) has outlined a method which is essentially a modification of Bass's (1909) method. According to Garrison—

> The specific gravity of the ova is from 1.050 to 1.100 (old eggs sometimes higher, according to Bass). If the stool be liberally diluted with tap water the mixture has a considerably lower specific gravity, which varies, of course, with the character of the stool, but is usually about 1.005, so that the ova, together with the heavier sediment, sink to the bottom. [Allow to stand for an hour or more; decant and add fresh water repeatedly until soluble matter is washed out.] At any time during the sedimentation, but preferably after the specimen has been washed a few times, the coarser material may be removed by straining and washing the sediment through a fine wire gauze, using a small, strong jet of water. To completely wash a specimen until the supernatant water is clear may require quite a number of sedimentations, and it may be desirable to continue the process throughout one or more days. * * * Sometimes the feces contain heavy, gritty, solid material, which is particularly annoying in making slide preparations. The specific gravity of much of this material is sufficiently higher than that of the eggs to allow the use of a solution with a specific gravity between the two which will float the eggs to the surface and allow the heavy sediment to sink. This may be done by suspending the specimen, preferably after it has been well washed, in a solution of calcium chlorid containing 350 grams to the liter of water, which gives a specific gravity of about 1.200. (A saturated solution of the commercial salt has about the same specific gravity.) The top layer, containing eggs and the lighter débris, is decanted, leaving the heavy sediment behind.

WELLMAN'S SODIUM ACETATE CENTRIFUGE METHOD.

Wellman (1910) employs a method which appears to be a modification of Bass's (1909) method, but as it is not clear just what Wellman means by his statement of his method, the writer has not tested it. His method, which is for hookworm eggs, is given as follows:

> The principle involved was first employed independently by the writer and by Dr. Bass, of New Orleans, Dr. Bass being the first to publish his results. Our own method is to use two solutions of sodium acetate of specific gravities of 1.050 and 1.250, respectively. A portion of suspected feces is mixed with one of these solutions and centrifuged for about 10 seconds, the liquid decanted, then the other solution is poured on and mixed gently, the whole again centrifuged, and this process repeated until the eggs are all in one layer, the sand and other heavy ingredients of the feces remaining below and the light flocculent components lying above the zone in which the eggs remain. With this technique one slide contains as many ova as 50 or 100 slides by the ordinary method. Dr. Bass uses calcium chlorid for his solutions, but the writer has not been able to employ this salt satisfactorily on account of its hygroscopic properties.

It is unfortunate that Wellman does not state just why the hygroscopic properties of calcium chlorid are objectionable, as Bass employs this salt on account of these properties.

PEPPER'S ADHESION METHOD.

An interesting application of technique to a particular case is that of Pepper (1908). He takes advantage of the stickiness which he finds to be a property of human hookworm eggs in the following manner: Washed and sedimented feces are put on a slide for a few minutes and then immersed gently in water; on lifting it out the eggs are found adhering after everything else has been washed away. This process may be repeated and numerous eggs collected. Pepper does not find the same stickiness in the case of *Ascaris*, *Trichuris*, or *Tænia* eggs.

Stiles (1910) has given the smear method and the methods of Pepper (1908) and Bass (1909) for the examination of human feces for evidence of hookworm infection.

TELEMANN'S ETHER HYDROCHLORIC ACID METHOD.

Another modification of method consists in using chemical as well as physical means to secure a concentration of parasitic material. Telemann (1908), who appears to have been the first to use chemical methods in examining feces for parasite eggs, uses the following technique: Small particles are taken from a number of places in the feces under investigation and shaken up in a mixture of equal parts of ether and pure hydrochloric acid in a reagent glass. (I believe that I have had slightly better results from adding ether first, stirring well, and then adding the hydrochloric acid.) The ether dissolves

the neutral fats and free fatty acids, while the hydrochloric acid dissolves the albuminous matter, as casein, etc., soaps, mucin, phosphates, and various calcium salts. This mixture is then filtered through a fine hair sieve to eliminate large particles, and the liquid is then centrifuged about a minute. In the centrifuge tube will be found three layers, an upper one of fats dissolved in ether, a middle one of bacteria and small detritus in the acid, and finally a sediment of small food particles, mostly cellulose and muscle fibers, and parasite eggs. The parasite eggs, being relatively more concentrated in the sediment than in the original feces, can readily be found in the usual manner by microscopic examination of a slide. This method has been tested and commended by Quadflieg (1909), who notes, however, that it should be supplemented by the smear method, as the latter seems to be superior in the case of certain parasites. Pfister (1909) commends the method as being satisfactory in searching for *Bilharzia* eggs.

CULTURE METHODS.

A final modification of technique is to use culture methods, the feces being kept moist and warm, and after a proper interval being examined for embryos which have developed and escaped from the eggs. Under certain conditions this method gives a concentration of material on the glass receptacle or in water contained in depressions on the surface of the feces.

PURPOSE OF METHODS.

Inasmuch as the writer's method is a variation of some existing methods already outlined, it may be in order at this point to find a basis for the selection of a method by considering what the purpose of the microscopic examination and the reason for any method may be.

Obviously, the purpose of the examination is to detect parasites and their eggs in the feces if they are present. The evident way to accomplish this is to examine the feces on a microscopic slide preparation after they are so thinned as to permit of examination. Every method, no matter how complicated, includes these essential steps. The smear method accomplishes this in the minimum number of steps: fluid feces are examined at first hand; solid feces are rendered fluid and then examined or are mounted in a drop of water.

ADVANTAGES OF CONCENTRATION.

The purpose of all modifications of this simple proceeding, as the preceding sketch shows, is to attain one object—the concentration of parasites and their eggs in order to facilitate the detection of infection. This concentration is accomplished by increasing the time spent on the treatment of the feces previous to their microscopic ex-

amination. At the same time it increases the likelihood of finding parasites and their eggs when these are present.

The justification for thus shifting the greater amount of time from that of microscopic examination to that of preliminary treatment is readily found. Microscopic examination of feces for parasites and their eggs is one which calls for some degree of mental concentration. The judgment is constantly exercised in passing upon various objects which catch the eye and by a superficial resemblance in size, shape, color or refraction to parasites and eggs call for a determination of their spurious character. The determination of some of these forms requires the use of the higher powers of the microscope and some little time in examination. In addition to the fact that microscopic examination makes considerable mental demands, is the fact that microscopic work is eyestrain—a matter of considerable importance to anyone who has much work of the kind to do—and the saving of eyestrain by eliminating part of the objects which must be picked up by the eye, only to be rejected as not being the thing sought for, is greatly to be desired.

On the other hand, the treatment of feces preliminary to microscopic examination calls for little mental concentration and no eyestrain, and so leaves the mind free for other things and prolongs the period of usefulness of the eyes. Moreover, the fact that this preliminary treatment can largely be left to the operation of natural laws and to power machinery also saves to the operator the time when things can be left unattended.

PROPERTIES USED IN CONCENTRATION.

Concentration, the object of all improvements on the smear method, is obtained by eliminating as much as possible of the nonparasitic material by taking advantage of differences in physical, chemical, and biological properties between such material and the parasites. These differences are in most cases those of specific gravity, of size, of physical and chemical solubility, of adhesiveness, and of capacity for growth and development.

PHYSICAL PROPERTIES.

The elimination of matter having a specific gravity less than that of the parasites is accomplished by sedimenting in water and decanting material which floats or remains suspended, and by centrifuging in water, and is based on the general truth that parasites are heavier than water and will settle to the bottom. Dock and Bass (1910) lay emphasis on the fact that—

> Most * * * food particles are irregular in outline and shape, making their surface per given weight greater than that of the round, oval eggs.

Owing to the greater resistance offered by such surfaces, these particles are slower in reaching the bottom.

In Bass's (1909) second method additional matter of specific gravity less than that of the parasite is eliminated by the use of a calcium-chlorid solution having a specific gravity of 1.050. In this method and in Bass's (1906) first method matter having a specific gravity greater than that of the parasites is also eliminated, in the first method by the use of a nine-tenths saturated solution of sodium chlorid and in the second method by the use of a calcium-chlorid solution having a specific gravity of 1.250. Garrison (1910) varies this method by using only one solution, of specific gravity 1.200, and Wellman substitutes sodium acetate for calcium chlorid.

These methods and the sedimentation and filtration methods likewise eliminate matter which is soluble in water. Washing not only assists in concentrating material by removing fine suspended and colored soluble matter, but in so doing it gives a clearer background for microscopic work.

The elimination of matter larger than the parasites is accomplished by the use of sieves with a mesh aperture of such size that it will permit the passage of parasitic material, but not of coarse fecal particles.

The elimination of matter having different adhesive properties, as advocated by Pepper (1908), has only a limited application and needs no discussion in a consideration of general methods. The writer has tested this method and finds it very satisfactory for the purpose for which it was proposed. Dock and Bass (1910) commend Pepper's method, and state in comment:

> The best results are obtained if a part of the fecal material is removed by use of the centrifugal. Otherwise the method is often disappointing.

Other methods of differentiation, as staining reaction, etc., might be devised. In fact, as later indicated, the writer has attempted something of the sort without obtaining satisfactory results. Nevertheless satisfactory methods might be devised.

CHEMICAL PROPERTIES.

The elimination of matter having a different chemical solubility is accomplished by the addition of chemicals which will dissolve part of the nonparasitic substances with the formation of new and soluble substances. Such chemicals will also dissolve a number of things forming a mere physical solution.

BIOLOGICAL PROPERTIES.

The elimination of the nonparasitic matter lacking the biological properties of growth and development is accomplished by the use of culture methods. These methods, which are indispensable in bacteriology and of increasing importance in protozoology, in both of

which the reproductive faculty may be taken advantage of, are of limited use in the examination of feces for worm parasites, as multiplication of the parasites under these circumstances is only possible in such exceptional cases as *Strongyloides*.

THE THEORETICAL IDEAL.

From the foregoing it would appear that the best technique should eliminate matter of specific gravity different from that of the parasites by sedimentation, or, since it is more rapid, by centrifuging, in solutions of such strengths as to get rid of the lighter nonparasitic material at one time and the heavier at another; such technique should eliminate matter of different size by sieving; finally it should, in the course of the above processes or the filtration method, eliminate matter of physical or chemical solubility different from that of the parasites by the use of suitable reagents. Such a method would be expected to give a slide with the maximum amount of parasitic material and the minimum amount of other material. Incidentally, this treatment would comminute the resultant sediment so that it would give a clear, uniform microscopic field, superior to that obtained by the smear method. An ordinary smear of feces, subjected to no preliminary treatment, makes a very trashy and indifferent microscopic preparation, especially in the herbivora, where large plant particles are easily capable of concealing the evidences of parasitic infection, and the same objection holds good to some extent of smear preparations of any sort of feces.

PRACTICAL OBJECTIONS.

In actual practice, experience modifies the view that we should do all the above things and shows that there are other things that must be considered. Among these are (1) the possible injury to parasites or eggs by certain forms of technique, and (2) the possibility that in general work certain methods may prove to have only a limited application, or the concentration resulting from the application of certain methods may be too insignificant to warrant the time and effort expended. A discussion of these points can best be given after consideration of the writer's method.

GROSS EXAMINATION.

The naked-eye examination of feces for gross evidences of parasites, as stated at the beginning of this paper, receives too little attention, and it may be fairly assumed from the lack of statements showing a definite technique that it usually consists of perhaps a hasty glance at bottled material or possibly some picking over of the feces with a dissecting needle or a stirring rod at the most.

The likelihood of finding easily identified mature worms or tapeworm segments warrants a more careful examination. Garrison (1910) has recognized this fact and given a method of collection as follows:

> In the case of the larger worms, such as *Ascaris* and the large tapeworms, which are easily picked out, about the only precautions needed are to clean the worms of the fecal débris and to keep them as fresh as possible until they are killed. This is best done by transferring them promptly from the fresh stool to a dish of warm physiological salt solution. * * *
>
> Smaller worms, like hookworms, pinworms, whipworms, and the "dwarf tapeworm," require careful searching, and this is best done by spreading portions of the fluid stool in a thin translucent layer in a glass dish or on a glass plate (table top), the glass being placed over a black background.
>
> In picking out the still smaller forms, such as the adult trichinæ, the minute intestinal fluke, *Heterophyes*, and the detached heads of tapeworms, a hand lens is almost necessary, and it is advisable to make a routine practice of running over the material with a magnifier of low power in every case.
>
> Should the stool not be sufficiently fluid to admit of the above manipulation, it must be thoroughly mixed and diluted with water, and this brings up the method of sedimentation, which, while of special use in collecting ova, is frequently a valuable help in finding the smaller worms.

THE WRITER'S METHOD.

The writer's method includes both macroscopic and microscopic examination. Concentration is attained by careful comminution of feces, the use of sieves, sedimentation and centrifuging, and washing in water. The result is checked by centrifuging one tube of material in a calcium chlorid solution with a specific gravity of 1.250.

The illustration (fig. 1) is intended to show at a glance the apparatus needed (except for pipettes and brushes) and the method and order of its use. It is, of course, evident that the reader will be familiar with the individual pieces of apparatus.

COMMINUTION OF FECES.

In any method which aims at a concentration of the parasitic material present it is first necessary to break up the fecal masses in order to release the embedded and adherent parasites and to put the feces in such shape that they can be treated by any of the processes outlined in the preceding part of the paper. This is a feature to which it seems that too little attention is paid. Of the methods of comminuting such fecal masses, boiling in water, as advocated by Cobb (1904) for sheep feces, is fairly good, but not so effective for sheep feces as the general method used by the writer, and has obvious objections in the cases of human feces or those of the carnivora. Stiles (1902hh, 1903l) states that the feces should be shaken or stirred thoroughly, and Telemann (1908) notes that the feces

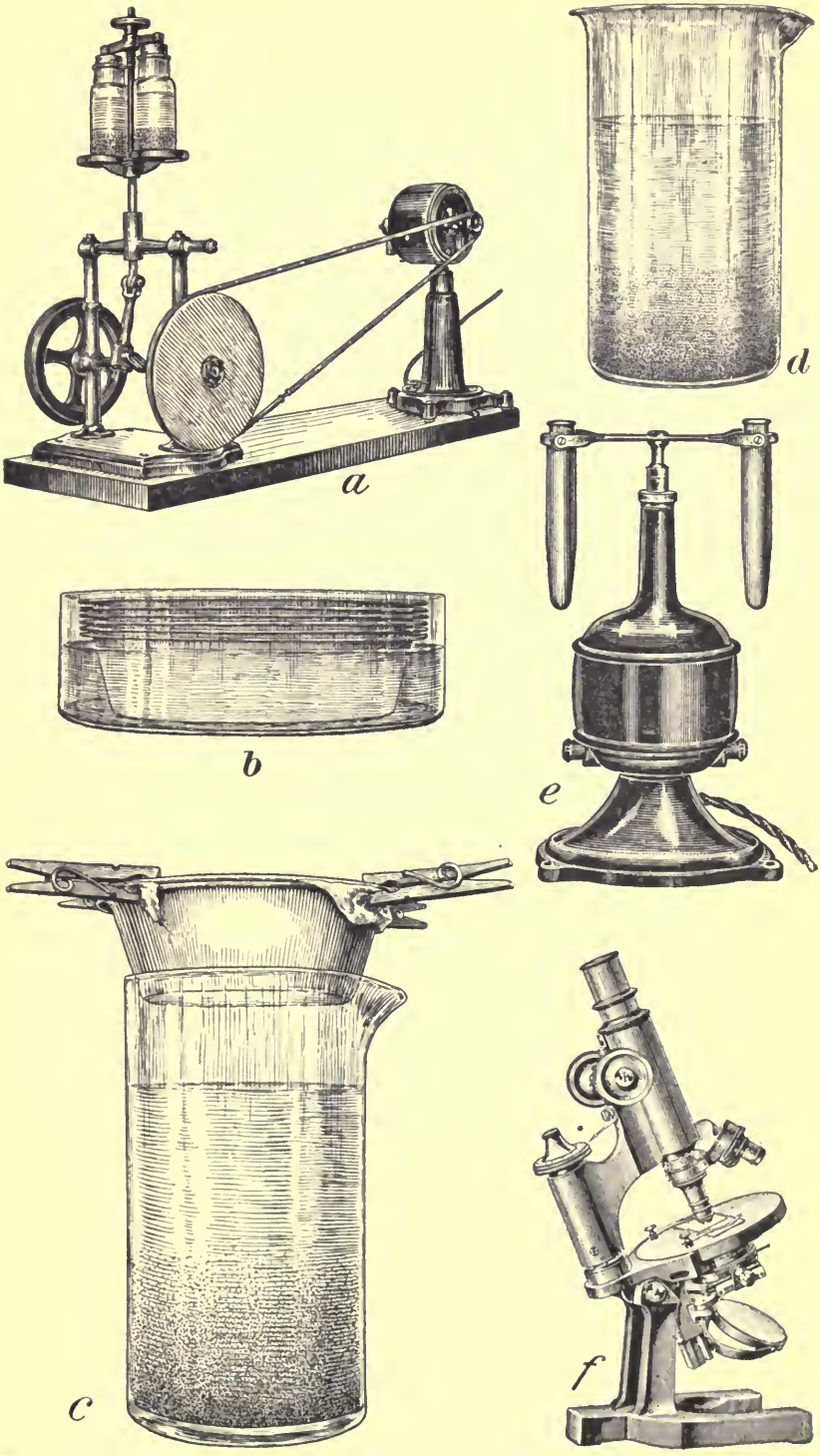

FIG. 1.—Apparatus used in the writer's method of examining feces: *a*, Shaker; *b*, brass sieves and dish; *c*, silk sieve and jar; *d*, beaker; *e*, centrifuge; *f*, microscope.

should be shaken in the mixture of ether and hydrochloric acid. Garrison (1910) advises the use of large quantities of water, 5 to 10 liters, for the first sedimentation of large solid stools, the water to be run in violently and the mixture well stirred.

The writer takes particular care to break up the feces thoroughly. The method consists in shaking the feces in a rubber-stoppered, wide-mouthed glass bottle about three-fourths full of water. The entire fecal sample, up to 4 or 5 ounces, is used. It is sometimes desirable to break or crush with a stirring rod such hard fecal masses as sheep feces. It is also sometimes desirable to add shot to hard fecal masses. In such cases the most satisfactory results were obtained with about 100 lead shot having a diameter of 3.8 millimeters; shot with a diameter of 8 millimeters was not as effective in breaking up feces, and had the additional disadvantage that it blackened the glass. The use of shot is to be avoided as a rule, for the reason that gross parasitic material is apt to be damaged by it. At first the bottle containing the feces and water and, if necessary, the shot, was shaken rapidly by hand, but as the amount of fecal examination in this laboratory warranted the use of a machine for this work, a shaker of the kind used in mixing "milk shakes" was installed and connected by belting with a pulley wheel fitted on an electric-fan motor. This apparatus (fig. 1, *a*) is very rapid and effective in its work. The bottles are lifted a distance of 5 centimeters and dropped back again at a rate of about 500 times per minute. The same machine operated by hand would doubtless be very good.

SIEVING.

After having been broken up in this manner the feces are poured through a set of six brass sieves. The sieves have a mesh aperture ranging from 3 millimeters in the largest to about one-fourth of a millimeter in the smallest. They are made by taking tin pans with a bottom diameter of about 6½ inches and sides 2 inches high, cutting out the bottom, leaving a flange near the sides, and soldering onto the flange brass screening with meshes of various sizes. These sieves are copied from a set used by Dr. Cobb in collecting free-living nematodes. The pans, of course, tend to rust, as it is not always convenient to dry them after use. Dr. Cobb tells me that he avoids this by the use of oil or grease warmed on the pans and then carefully wiped off. As this practice seems inconvenient also, the pans used by the writer have been enameled to prevent rusting. This makes the pans bind a little when nested. A set coated with shellac is being tried. Galvanized-iron, brass, or aluminum pans would presumably be better, though the two latter would be more expensive. The brass sieves which can be purchased already made are not beveled

and hence can not be nested. They can be superimposed, one on another, but the result is a stack which is so high that it does not permit of six of them being set in a shallow dish of water with the water standing above the screen in the upper sieve.

The sieves are nested in the order of mesh aperture, with the coarsest on top, and placed in a large porcelain evaporating dish, or a large glass crystallization dish (fig. 1, *b*). The feces are poured into the top screen and pass through the screens to the evaporating dish, particles of different sizes being held by the different screens. The use of fewer screens would not be a gain, as too coarse material poured on a screen clogs it. Tap water or normal salt solution is poured in the upper sieve until the water stands in the evaporating or crystallization dish at a level above that of the bottom of the upper sieve. This sieve is lifted and shaken a little until the fine matter has passed through. It is then lifted out and put in a large crystallization dish half full of water or salt solution and the matter it contained examined on the screen or washed into the dish. Gross parasitic material is picked out, the screen rinsed, the dish emptied and refilled if the amount of discoloration or trash present warrants it, and the process is repeated with the remaining sieves. The gross material left on the screens is thoroughly cleaned, and the likelihood of wasting time examining citrus pulp vesicles, vegetable fibers, etc., as possible parasites is reduced to a minimum.

The sediment left in the evaporating dish after removing the finest sieve is poured onto a screen of miller's silk bolting cloth with a mesh aperture of 0.117 to 0.134 millimeter and the finer particles washed through into a tall jar (fig. 1, *c*). The mesh aperture of this sieve diminishes as the cloth shrinks with use, and in a cloth which has been in use for several months it has diminished to a size of 0.070 to 0.080 millimeter. Such a mesh is too fine to permit the passage of the eggs of such important species as *Fasciola hepatica*. Some No. 120 mesh brass screen has recently been obtained which has a mesh aperture rated at 0.117 millimeter, but varying from 0.103 to 0.120, according to the writer's measurements. This promises to be a very satisfactory substitute for the bolting cloth. Presumably it will retain its mesh aperture, and will be more durable.

When the shot are used, they are poured with the feces into the coarsest of the brass sieves. They are subsequently poured from the sieve into a petri dish with whatever coarse fecal matter may accompany them and the fecal matter easily removed by a stream of tap water. It might be supposed that fecal matter of some sort would stick to the shot, but it does not do so. Parasitic material that might adhere can be destroyed by dry heat. Lead shot have the advantage over steel shot that they may be kept in a formol solution without rusting, while steel shot would need careful drying, or else keeping in oil.

Where small amounts of feces are used, two pieces of brass tubing, 2 inches in diameter and 1¼ inches high, beveled to fit one in the other, and the lower one furnished with three projections to hold it on glassware of not over 3 inches in diameter, are used to hold the bolting cloth of the silk sieve, the cloth being caught and held taut by the beveled surfaces. Where large amounts of feces are used, and large amounts—not to exceed 4 or 5 ounces—should be used whenever obtainable, two enameled tin pans, with a bottom diameter of 4½ inches and with the bottom cut out so as to leave a narrow flange, are used in place of the brass tubing. The cloth is held between the upper flanges of the two tins, and the cloth and flanges held together by four small clothespins of the sort provided with a wire spring to hold the jaws together. This device I also owe to Dr. Cobb. The sieve formed of the tins and the bolting cloth is the right size for use with a jar 10 inches high and 5 inches in diameter (see fig. 1, *c*). When necessary a soft brush is used to brush the feces through the fine brass sieves or the silk cloth. Cobb (1904), as previously noted, uses a brush for the same purpose. These sieves will work better if water is poured through them or if they are dipped in water before the fecal matter is poured on.

The sieves, as well as all other apparatus coming in contact with the fecal material, are washed promptly with boiling water, the sieves being also scrubbed with a stiff brush. This prevents any parasitic material from remaining to contaminate a subsequent fecal specimen, and thereby giving inaccurate findings. A microscopic examination of the silk sieve shows that it washes clean very readily, and when rinsed retains very little of the material poured on it. Experiment shows that eggs pass through this cloth very readily, less than half of 1 per cent of even such large eggs as *Toxocara* remaining when the fecal matter is first brushed through. A smear made from the residue on the bolting-cloth sieve showed 4 eggs in one case where the slide preparation from the centrifuged sediment showed 860 eggs; another smear from the residue on the bolting cloth showed 1 egg where the slide from the sediment showed 475 eggs. In the latter case, a fair estimate of the amount of material on the cloth and the amount from which the smear was made indicates that there were probably not more than 10 eggs left on the cloth, while thousands had passed through it. Those that are left are held by the jelly-like residue obtained at this point, and not because the screen mesh is defective or too small. The writer is not aware of any parasite eggs which are too large in their smaller diameter to pass through a mesh with a diameter of 0.117 to 0.134 millimeter. The number remaining after clean water has been poured through the cloth into the jar is entirely negligible.

In working with human feces, or where dangerous infection may be present, the silk cloth may be kept in a jar of formol solution

when not in use. In the course of a large number of experiments nothing has yet indicated that parasitic material from one examination has remained to subsequently contaminate other fecal specimens. Parasites that might be suspected of remaining after the cloth had been washed in boiling water might be destroyed by prolonged boiling or subjection to dry heat—experiment shows that eggs so treated are distorted or characterized by the formation of air spaces or oily areas—or fresh pieces of cloth could be used each time. This last, however, would be somewhat expensive, as this cloth retails in Washington in half-yard widths at about $5 a yard. It would be cheaper to use the No. 120 mesh brass screen. This costs $1.85 a square foot, but would be permanent.

SEDIMENTING AND CENTRIFUGING.

The feces which pass through the silk sieve are sedimented with plenty of water in the jar. After decanting, the sediment is transferred to a beaker (fig. 1, *d*) and may now be washed if desired. The entire sediment, or as much as seems desirable, is then centrifuged (see fig. 1, *e*), repeated centrifuging with the addition of fresh material adding to the total centrifuge sediment, and may be washed at this point also, as advised by Pepper (1908) and Bass (1909). The writer sometimes washes the material at both points, the second supplementing and completing the first. It is usually sufficient to wash the sediment in the centrifuge. Bass has called attention to the important fact that a centrifuge should only be run the minimum time necessary to bring down the eggs. This time will vary with different centrifuges. With a centrifuge running 3,500 revolutions per minute Bass allows 4 to 10 seconds. I find this enough time with a centrifuge running 1,230 revolutions per minutes. After the material in the two centrifuge tubes is washed in water, one tube is left alone; the water is poured off the other and calcium chlorid solution, with a specific gravity of 1.250, is added to the sediment. After centrifuging, a slide preparation is sometimes made from this tube direct. In most cases the top cubic centimeter is pipetted off, shaken up with 14 cubic centimeters of water, and centrifuged. This is the more satisfactory and certain method.

PREPARATION OF SLIDES.

By means of a long pipette, a drop of sediment is drawn up from the bottom of the tube in which water alone is used, placed on a slide under a cover glass, and examined with a microscope (fig. 1, *f*). A second slide is made from the other tube. This second slide is either made directly from a drop taken from the surface of the calcium chlorid solution, or from the bottom in case the top cubic centimeter

has been added to water and centrifuged. The second slide is used as a check on the first. It sometimes has fewer eggs, especially when pipetted direct from the top, but it is a cleaner preparation, is easily examined, will sometimes have more eggs, especially if made from the sediment where the top cubic centimeter of the 1.250 solution has been centrifuged with the addition of water, and occasionally throws additional light on the material under examination. The pipettes are rinsed thoroughly, and when dried are heated in a Bunsen flame for a short time to destroy any eggs that might adhere, thus preventing contamination in subsequent examinations.

CONCENTRATION OBTAINED BY THE USE OF SIEVES.

In examining the feces of 35 sheep, the entire amount of feces passing through the sieve was centrifuged in order to give a uniform comparative study and to determine the amount of concentration attained by the use of sieves, and due to them alone. To eliminate other factors, the sediment was not washed and the centrifuge was run for long periods till everything had come down. Centrifuging the entire amount of feces necessitated the repeated filling of the tubes of a two or four arm centrifuge. A comparative examination of slides made from the sediment obtained by centrifuging a single tube full of the material, with slides made from the total sediment, showed that the concentration was the same in both cases, a result which would be expected from a theoretical standpoint. While the concentration is the same, the total amount of parasitic material present is, of course, much less in the single tube.

Using moist fecal pellets, the concentration obtained was 4:1. The concentration varies with animals of other species, with food habits, and with the condition of the particular fecal specimen examined. At the same time, the concentration is always sufficient to warrant the use of the sieves. The microscopic field obtained after treatment of feces in this way is very much more satisfactory than the field obtained in the smear method, and where the same number of slides are examined the likelihood of finding evidences of existing parasitism is certainly more than four times greater in cases where the feces have been subjected to thorough sieving.

SUMMARY OF METHOD.

The writer's method is, then, merely a modification of existing methods, and might be termed a comminution-sieving-sedimentation-centrifuge method in which water alone is depended on as a medium for these operations, a slide made after centrifuging in a calcium chlorid solution with a specific gravity of 1.250 being regarded principally as a check on the method as given.

COLLECTION INDEX.

The writer has had no opportunity to work out an index showing the relation of the count obtained by the writer's method to the number of worms present. In few cases where the feces of animals are examined in this laboratory are the animals examined post-mortem within a short time. In one instance, 3 grams of feces were taken from the rectum of a sheep during a post-mortem examination for parasites, and after treatment by the writer's method, noting the time element and the amount of water used in each case—which data need not be given here—a slide was made of one-tenth of a cubic centimeter of sediment. This slide showed 3,325 nematode eggs and embryos. The intestines and fourth stomach showed a correspondingly high degree of infection with numerous species of worms, and it was impossible to determine the relation between the number of female worms of any species and the number of eggs of that species under the circumstances.

In spite of the relation found by Cobb (1904) as regards flukes, by Leichtenstern and by Grassi and Parona, according to Dock and Bass (1910), as regards hookworms, between the number of parasites present and the number of eggs in a given fecal sample, the examinations made by the writer do not indicate that anything of the sort is apt to prove of use in routine examinations for parasites in general. Dock and Bass (1910) have come to similar conclusions and cite the work of Ashford and King to the same effect. Not only is there a certain element of chance in the detection of eggs when they are present in light infections, but there are certain conditions which permit of the existence of parasitism without the presence of eggs in the feces to indicate it. Some of these conditions are as follows:

(1) Infection with male nematodes will not be indicated by eggs in the feces. Occasionally the only nematodes of a given species which are present in an animal will be males. Such infections are usually light.

(2) The above condition will not be true of the flukes and tapeworms, as they are hermaphroditic; but in the case of these worms, and the nematodes also, infections which are so recent that the infecting larvæ have not yet reached the egg-producing stage will not be indicated by eggs in the feces. Such infections may be heavy.

(3) Egg production in female or hermaphroditic animals may be interrupted in various ways. Thus tapeworms may be broken, perhaps by intestinal peristalsis, at points anywhere from just back of the head to just in front of the gravid proglottids, and the feces will show no more eggs or proglottids until new gravid proglottids are formed. Dock and Bass (1910) report that in an examination of 247 female *Necator americanus* by Bass, 7 per cent were found to

contain no ova. Bass suggested the possibility of old age as an explanation of this. Dock and Bass (1910) state that they found 9.25 per cent of 397 female *N. americanus* barren in cases where the infection was of long standing, while only one-third of 1 per cent of 120 female hookworms from the dog were without eggs. They state, however, that Dr. Stiles does not think that hookworms live long after old age induces cessation of ovulation.

(4) A thorough purging of the intestinal tract may remove an accumulation of parasite eggs, with the result that numbers of eggs will be found at this time and none a day or two later. Dock and Bass (1910) comment favorably on Ashford and King's statement that hookworms cause an increase of mucus at the site of their feeding ground, and as this mucus often comes off en masse and contains most of the eggs the actual feces would contain relatively few. They also note that Ashford and King say that eggs are more difficult to find in diarrheal stools, and that they saw cases of heavy infection that at times had no ova in the feces. Dock and Bass (1910) call attention to the fact that the amount of feces varies with different persons and from time to time in the same person. They also state that it is a common experience to find no eggs in the feces within a few days after a course of thymol and subsequently to find many eggs. They say that Dieminger found that the number of eggs was very much diminished when patients were drinking hard.

It follows from the above that little weight can be laid on a collection index. In the writer's experience, consecutive and careful examinations of the feces of a given animal show days when eggs of various kinds are abundant and days when they are scarce or missing, and the collection index for the various days would present a striking disagreement. This would be particularly true of cestodes.

Negative examinations must be considered doubtful and must be checked at intervals if infection is suspected.

TESTS OF UNPUBLISHED METHODS.

Numerous tests of unpublished modifications of technique were made in connection with the development of the writer's method. Among these was an attempt to increase the specific gravity of parasites and their eggs by adding mercuric chlorid before centrifuging. Comparative tests did not indicate that this method was of value, and any adaptation of technique which would make it so would probably not repay in results what it required in time. Tentative attempts to use a differential stain were unsatisfactory. In one of these, sulphuric acid and iodin were added to slide preparations to distinguish cellulose substances which might otherwise have to be examined as

possibly parasitic. By this method cellulose was colored violet or black, and nematode eggs brown, or at times black, with a light areole where the shell showed at the periphery. As such a method lessened the refractive index which makes most nematode eggs so conspicuous, the use of these reagents was discontinued as giving no improvement in the resulting preparations. Stubbendorff (1893a) has noted this test and a number of others in a note on the differential diagnosis of parasite eggs and plant spores.

COMPARATIVE TESTS OF THE WRITER'S METHOD AND OTHER METHODS.

For about a year comparative tests of the writer's method and other methods were made in the examination of feces of various kinds. So far as possible equal amounts of feces were examined and the slides made from equal amounts of material. The parasite eggs and embryos were carefully counted with the aid of a mechanical stage, and the results compared.

SMEAR METHOD.

Comparative tests of the smear method and the writer's method, some results of which are given in connection with other tests, indicate that the smear method is much less certain and effective. A diagnosis made by the writer's method would be much more complete and adequate than one based on the usual ten smear preparations.

SEDIMENTATION METHOD.

The simple sedimentation method is so obviously inferior to methods involving sieving and centrifuging, and improved modifications of the sedimentation method were found so inferior to the writer's method in actual test, that the simple method was not even given a comparative test.

BURETTE METHOD.

A test of the sedimentation method in which the sediment was taken from the bottom by means of a stopcock did not give as good results as the centrifuge method. In this test a burette of 25 cubic centimeters capacity was used. Ten cubic centimeters of water were first put in it, with the idea of washing the feces at the bottom of the burette instead of at the top, as is commonly done where water is poured over a sediment and the sediment shaken up in the water, allowed to settle, and the fluid then decanted. It appeared that in this way the sediment could be taken out promptly and already washed. In testing, the sediment obtained after screening and de-

canting was shaken vigorously and divided into two equal parts, one being centrifuged and washed, the other being poured onto the 10 cubic centimeters of water in the burette. As the sediment settled it could be traced by the discoloration of the water, and when the discoloration reached the bottom one preparation was made by opening the stopcock and taking a drop of water on the slide. Other slides were made at intervals up to an hour. In one specimen of dog feces where there was an infection with *Dipylidium*, *Ascaris*, *Trichuris*, and *Ancylostoma*, the best of six slides made in this way showed a total of 27 eggs, while a slide made after centrifuging with calcium chlorid of 1.250 specific gravity showed 77 eggs and one centrifuged in water alone showed 181 eggs. In the last case there were more eggs of any one of the four species of parasites present than there were of all together in the best of the slides made by the burette method.

In another test, feces from a dog infected with *Toxocara* and *Tænia* were tested by the burette method without the clean water at the bottom, the sediment obtained after screening being poured into the empty burette. The best of two slides made by this method showed 58 eggs, a slide made by the smear method showed 46 eggs, a slide made by centrifuging in the solution of 1.250 specific gravity showed 117 eggs, and a slide made after centrifuging in water alone showed 215 eggs in one-fourth of the cover-glass area. The slide started to dry at this point and the count was discontinued. The estimated total for the slide was of course four times 215, or 860 eggs. A smear preparation made from the residue on the bolting-cloth sieve showed four *Toxocara* and no *Tænia* eggs.

GASTEIGER'S FILTER METHOD.

A test of the filter paper and funnel as a substitute for the centrifuge indicated at once that nothing was gained by scattering a sediment over a filter paper instead of concentrating it in the bottom of a tube. The method was also slow and offered the usual chance of a break in the filter paper, necessitating a second filtration. I can not imagine any use of the filter in fecal examinations for parasites that would not be better subserved by the use of the sieve followed by centrifuging.

BASS'S CALCIUM CHLORID CENTRIFUGE METHOD.

Inasmuch as Bass's salt solution method is held by its author to be inferior to the calcium chlorid method, it need only be noted here that in the few tests of the first-named method made by the writer

its inferiority to the calcium chlorid method and to the writer's method was quite apparent.

In comparative tests of Bass's calcium chlorid method, extending over a number of months and involving the examination of the feces of man, the dog, sheep, eland, hartbeest, and chicken, it was found that this method secures a high degree of concentration of material, but in eliminating nonparasitic material it also eliminates some parasites. Part of these seem to be left in the rejected sediment at the bottom of the tube containing the solution with a specific gravity of 1.250, as repeated examinations of this rejected sediment showed numbers of parasite eggs and embryos in nearly all cases, indicating that endosmosis brings the specific gravity of the parasites up to that of the surrounding fluid. Some parasites seem to be lost also in the repeated handling due to the use of the solution with a specific gravity of 1.050, as more parasites were recovered where the use of this solution was omitted and the 1.250 solution alone used than where both were used, as Bass directs. Where slide preparations were made from equal amounts of sediment secured by Bass's method and the writer's method, the amount of parasitic material to a slide was usually greater when prepared by the writer's method. There is, furthermore, a distortion of parasite eggs and embryos due to osmosis. This distortion and destruction of parasites constitutes at once a limitation and a defect of the method. It renders it unsuitable for the collection of live material and makes it more difficult to recognize and identify some parasites, such as nematode embryos.

In an examination of some human feces from a case of hookworm infection, which material was obtained through the courtesy of Dr. Stiles and his assistant, Mr. Crane, a smear preparation of the feces showed 2 eggs, a preparation made by Bass's method showed 84 eggs, and a preparation made by the writer's method showed 348 eggs. An explanation of the failure of Bass's method to give as good results was found in an examination of the sediment in the tube containing the strong calcium chlorid solution. A preparation made from this sediment showed 122 eggs.

In an examination of the feces of a number of dogs, the feces were treated according to the method given by the writer, according to that of Bass, and according to Bass's method without the use of his solution of 1.050 specific gravity. The method involving the use of the 1.250 solution, but not the 1.050 solution, is given in the table below as Bass's modified method. The slides were made by drawing up definite equal amounts of sediment in a graduated pipette from the bottom of the centrifuge tube. The results of the tests are given in the following table. Generic names refer to eggs found.

Comparative tests of three methods in examination of dog feces.

Feces.	Method.		
	Writer's.	Bass's.	Bass's modified.
	Eggs.	*Eggs.*	*Eggs.*
Dog No. 1	1 Nematode embryo... 8 *Ancylostoma*	Negative	Negative.
Dog No. 2	28 *Ancylostoma* 28 *Trichuris*	5 *Ancylostoma* 18 *Trichuris*	23 *Ancylostoma*. 3 *Trichuris*.
Dog No. 3	Negative	4 *Toxocara*	Negative.
Dog No. 4	13 *Ancylostoma*	10 *Ancylostoma* 1 *Toxocara*	16 *Ancylostoma*. 1 *Toxocara*.
Dog No. 5	20 *Ancylostoma*	3 *Tænia* 10 *Ancylostoma*	36 *Ancylostoma*.
Dog No. 6	4 *Toxocara*	1 *Toxocara*	2 *Toxocara*.
Dog No. 7	258 *Tænia*	209 *Tænia*	299 *Tænia*.

An examination of the above table shows that the results obtained by the use of the writer's method and of Bass's method without the step involving the use of the solution of 1.050 specific gravity, are on the whole superior to those obtained by the use of Bass's method. Dock and Bass (1910) state of Bass's method:

> Gage and Bass conclude, after this extensive experience [the examination of the feces of 315 students by the smear method, by sedimentation and centrifuging, and by Bass's method], that for all practical purposes washing with water alone is all that is necessary and that the washing with calcium-chlorid solution is unnecessary except for special purposes.

The fact that the use of the strong calcium chlorid solution gives at times better results than the use of water alone is the writer's warrant for making use of it in one centrifuge tube to check the findings from the other tube.

GARRISON'S CALCIUM CHLORID SEDIMENTATION METHOD.

Garrison's method of using calcium chlorid and sedimenting instead of centrifuging has come to the writer's attention too recently to permit of adequate tests. A test made by sedimenting in a centrifuge tube instead of centrifuging did not give as good results as centrifuging. Sedimenting has the disadvantage of being slower than centrifuging.

TELEMANN'S ETHER HYDROCHLORIC ACID METHOD.

A number of tests were made to determine the applicability of Telemann's (1908) chemical methods to the examination of feces. Telemann himself states that he has found his method satisfactory in a large series of human and animal feces. He does not specify what animals are included in the series, but his statement may be believed in any case. At the same time, the tests made in this laboratory indicate that his success was due more to the use of the hair sieve than to the use of chemicals. The latter probably did

more in mechanically breaking up the feces by a slight amount of chemical action than in actually concentrating parasitic material by eliminating nonparasitic matter soluble in ether and hydrochloric acid. As previously stated, the tests of the writer's method on sheep feces gave a constant theoretical concentration of 4:1. The addition of ether and hydrochloric acid to a given 0.5 cubic centimeter of sediment so obtained gave a reduction to 0.4 cubic centimeter, or an additional theoretical concentration of only 5:4. When the ether and hydrochloric acid were added to the fresh feces a somewhat smaller sediment was obtained. This does not indicate a greater concentration, as it appears to, but a lesser breaking up of feces by these reagents than by water, so that more agglomerations of material that should be broken up to allow the finer matter to pass the sieve are left unbroken, and the small as well as the large particles are held by the sieve. This is evident from the result obtained by adding these reagents last to determine the action due to them alone.

Another result sometimes obtained by using Telemann's method on sheep feces was that a plug of plant material formed at the top of the centrifuge tube and held in its mesh almost all the fecal material. The feces of herbivora are composed largely of plant matter and hence largely of cellulose. This is removable to a great extent by sieving but is not at all soluble in Telemann's reagents. This is also true of the feces of such birds as chickens and pigeons, as tests with such feces indicate.

On the other hand, the feces of man and of the carnivora have less plant matter of the sort and more matter that is soluble in ether and hydrochloric acid. Nevertheless, comparative tests indicate that the writer's method is practically as good as Telemann's for these feces also. In tests with human feces where the writer's method gave a theoretical concentration of 4:1, Telemann's method gave the same result. In one case the sediment obtained by the writer's method was treated by Telemann's method, and after shaking up and stirring was again centrifuged. The resultant sediment showed no reduction in volume as a result of the action of the chemicals. In another case where the writer's method gave a sediment of 0.85 cubic centimeter, an application of Telemann's method to this sediment reduced it to 0.65 cubic centimeter. Even more surprising results were obtained with dog feces, used as representative of feces of the carnivora. In test cases sieving in water gave a concentration of 5:1. In one case the application of Telemann's method to the sediment so obtained reduced it to half of its volume, thereby doubling the concentration. Although the concentration was doubled, the fact that the writer's method had reduced the original 1 cubic centimeter to 0.2 cubic centimeter, thereby eliminating 0.8 cubic centimeter, while the chemicals had only reduced it by 0.1 cubic centimeter more, indicates that the

additional solution of material accomplished by the use of these chemicals was but slightly more than the solution accomplished by water alone. In two other cases where dog feces had been allowed to stand a long time in water and had been shaken up for four or five minutes before being sieved and centrifuged, the resultant sediment of 1.4 cubic centimeters was actually increased after the application of Telemann's reagents followed by centrifuging to a flocculent sediment of 1.9 cubic centimeters.

The results of comparative tests of Telemann's and the writer's method were quite unexpected, as the writer had used Telemann's method for a year and only abandoned it when its inapplicability in the case of sheep feces became too evident to overlook. As has been stated previously in this paper, the success of Telemann's method appears to be due more to the efficiency of a slight solvent action in mechanically breaking up feces and putting them in condition to sieve, than to an extensive solvent action. Much that might be dissolved in the chemicals is soluble in water, much that might be dissolved is perhaps taken out by the screen where water alone is used, and the fact that the slight solvent action breaks up the feces and makes them easy to sieve seems to be the feature to which the results obtained by this method are to be attributed.

The feces of herbivora have little material that is soluble in Telemann's reagents, and the breaking up secured by them is inferior to that secured by the use of the writer's method. Human feces break up rapidly in Telemann's reagents, but the method advocated by the writer takes about the same time and gives as good results. Of Telemann's method, Dock and Bass (1910) say:

> We have found the method admirable with some stools, but in others not enough solution occurs to permit a concentrated layer of eggs to be thrown down.

Telemann's method has one disadvantage which neither Telemann (1908), Pfister (1909), nor Quadflieg (1909) mentions in connection with it—it injures the microscope. The fumes of pure hydrochloric acid attack the lens mountings and also the stage and the lenses themselves. The use of vaseline to seal the edges of the cover glass affords an uncertain protection, and the use of high powers under such circumstances is decidedly unsafe. In the writer's opinion the injury to the microscope would of itself be sufficient reason for discarding this method.

CULTURE METHODS.

Quadflieg (1909) has recommended using culture methods as an aid in detecting infection. Tests of this method did not indicate that it added to the certainty of detecting infection, though live embryo nematodes could be detected by the use of a dissecting microscope

with low-power lenses. As the determination of nematode genera and species is in general more difficult from the embryos than from the eggs, the usefulness of the culture method is limited.

Emerson (1910) uses the following method in searching for *Strongyloides intestinalis:*

> The stool should be placed in a dish, a small depression made on its surface, this filled with water, and the stool left in a thermostat overnight. If embryos are present, they are easily found * * * actively swimming in this water.

Culture methods have been used by Ransom (1906i) in studying *Hæmonchus contortus* in sheep feces. Dr. Garrison tells me that he has seen the culture method used successfully in Army work in examining a company for hookworm infection.

It is evident that such methods are suitable only for special purposes and not for general work.

OBJECTIONS TO CERTAIN METHODS.

The reason for rejecting some of the methods outlined in the hypothetical ideal treatment previously mentioned is, as was stated, that in actual practice objections of two general kinds may be urged against them. These objections are: (1) The injury to parasites or eggs; (2) the unsuitability of the methods for certain kinds of feces or the fact that the concentration resulting from the application of the method may be too insignificant to warrant the time and effort expended. It will be fairly evident from the discussion of methods already given just how those methods fail in these two respects, but a brief summary will indicate some general truths.

INJURY TO PARASITES.

In regard to the first point, it is evident that if parasite eggs or embryos are to be used in feeding experiments to produce infection, or are desired alive for study, or if the movement of embryos is depended upon to indicate viability or to aid the eye in detecting or locating the parasites, certain methods, such as boiling (Cobb, 1904) or the use of chemicals which will kill by poisoning or by rapid osmosis (Telemann, 1908; Bass, 1909), can not be used. Bass's (1909) method has the further objection that it not only kills, but distorts. Letulle (1905) urges that certain eggs, such as those of *Bilharzia* and *Uncinaria*, are mechanically injured by the use of the centrifuge. I have not found this true of the eggs of the human, sheep, or dog hookworm, or any other parasites encountered, but there might be cases where the objection would hold good. The writer's use of shot would work occasional injury to parasite material and it should be avoided when possible. In almost all cases, the feces can be broken up in a little longer time by shaking in water alone, as experiments show.

LIMITED APPLICATION OR INCOMMENSURATE RESULTS.

In regard to the second point, experience shows that in using specific gravity as an aid in concentration it is safe to assume that the specific gravity of a parasite is greater than water, though all eggs will not go to the bottom, as some are held up by lighter material, bubbles, etc., but it is not safe to rely on a parasite floating on a solution of specific gravity greater than itself for any length of time, owing to the action of endosmosis. An additional objection to this method advocated by Bass (1909) is that experience shows that all the steps are not warranted by the results. In most cases the simpler methods used by the writer give as good or better results. The use of the 1.050 solution appears to be a defect of the method. The concentration attained by it seems trifling in any case and at times it apparently results in actual loss of parasite material. Finally, under the same class of objections, experience shows that the concentration obtained by the use of chemicals instead of water (Telemann, 1908) is no greater than that obtained by the use of water alone, and hence there appears to be no reason for using the more expensive chemicals, especially in view of the injury to the microscope which one of them occasions. It might also be urged that the odor of these chemicals is such that one would prefer to avoid them, though it is also true that they deodorize the feces after a fashion, and that while they kill the parasites they also disinfect the feces in cases where the killing is immaterial.

DISINFECTION OF FECES.

Disinfection and deodorization of feces can be more easily accomplished, if desired, by the use of formol solution instead of water, as suggested by Letulle (1905), Jones (1907), and Garrison (1910). In a discussion of this point before the Helminthological Society of Washington, December 1, 1910, Dr. Stiles advocated the use of coal-tar disinfectants on the ground that protracted work with formol material would cause headaches. The writer has never experienced any inconvenience from the use of formol. Dr. Stiles also pointed out the great desirability of using some disinfectant, for the reason that the greater number of fecal examinations now being made in this country are for evidence of hookworm infection, and the localities with the greatest amount of hookworm infection have a high typhoid index. In using a disinfectant, the writer's method of comminution has the advantage of bringing the disinfectant into intimate contact with all parts of the fecal mass. The thorough breaking up of the feces and the use of large amounts of water reduces the odor to a point where it is almost imperceptible in most cases.

SUMMARY.

After testing the various methods as above indicated, the writer finds that the best results in routine examinations of feces of all kinds are obtained from the simple method already given. Briefly, the method consists in breaking up the feces very thoroughly by shaking in water, adding a quantity of small shot if necessary or desirable; sieving through a set of brass sieves and then through a silk bolting-cloth sieve or a sieve made with a jeweler's fine-meshed brass screen, examining the material left on the sieve for parasites; sedimenting (and washing); centrifuging (and washing)—one tube being filled with calcium chlorid solution of 1.250 specific gravity, centrifuged, and if desired the top cubic centimeter removed with a pipette, shaken up in a tube with 14 cubic centimeters of water and centrifuged—and then making a microscopic examination of a drop of sediment from the bottom of the tube centrifuged with water, and one from the top when the calcium chlorid solution alone was used or from the bottom in case water was added to the top cubic centimeter. The material is washed at either or both of the points indicated.

ADAPTABILITY OF METHOD.

The writer does not claim that the method advocated here is the best possible method. It is, however, the method which his experience shows to be the best for routine examination of various kinds of feces after comparative tests with other methods. It serves very well for the feces of man, and of the carnivora, herbivora, and birds, so far as fecal examinations for representatives of the last three groups indicate. It is not only of service in examining feces for worm parasites, but also for coccidia. It has not been tested for other protozoa. Presumably the writer's comminution method would damage flagellates, ciliates, or amebæ. It is often useful in detecting parasitic infection of stomach and intestinal contents. It has the advantage of speed and certainty over the smear method or sedimentation methods. It takes longer to make the microscopic preparation than in the smear method, but the resulting concentration justifies it. Nor is it a long or difficult process. The time required for each step is slight—a minute to shake up the feces, another to sieve them, and another to centrifuge them, leaving out the sedimentation after sieving, which needs no attention, and the time spent in examining material on the screens, which examination is incidental to the technique, not part of it. As everyone who has used laboratory methods knows, the total time necessary to perform three one-minute operations is not just three minutes or, as a rule, even six minutes, as preparation, intermediate steps, cleaning up, etc., add considerably to the time necessary. At the same time, the method outlined here is reasonably rapid and takes less time than is required to examine the additional slides

which the use of the smear method would demand. I find that the careful examination of a slide not uncommonly takes 9 or 10 minutes. Garrison, Ransom, and Stevenson (1903a) state that the examination of 100 preparations is an average day's work for one person. This is a little over four minutes for each preparation. Stiles (1909) states that a thorough examination of 10 slides will take 40 to 60 minutes, or 4 to 6 minutes for each preparation. Dock and Bass (1910) claim to look over an ordinary slide thoroughly in 2 to 4 minutes. If one slide prepared by the writer's method be considered as equivalent to 4 smear preparations, and experience shows that it is much better than this, then it will save time to spend 8 minutes in obtaining a sediment and 8 more in examining 2 preparations made from this sediment, rather than spend 32 minutes examining 8 smear preparations. Where positive information as to parasitic infection is desired, the best methods and the time necessary for these methods are abundantly warranted. Dock and Bass (1910) state that Gage and Bass, in the examination of the feces of 315 students, found only 47 per cent of the cases of intestinal parasitism by the smear method, the remaining 53 per cent being found by the use of centrifuge methods. They note cases where the examination of 25 smear preparations failed to show infection, although the existence of infection in these cases was demonstrated by preparations made after sedimenting and centrifuging.

Only in those cases where the necessary apparatus for better methods is not available, or where evidence of heavy infection is sought for with a view to immediate medical treatment, would the writer consider the routine use of the crude smear method warranted. In exceptional cases its use might be warranted in examining one or two slides for specific infection where there is likelihood of the infection being heavy enough to be promptly discovered by this method.

ECONOMY OF METHOD.

If the time of a physician, veterinarian, or scientist is of any value, then the smear method is not even more economical than the writer's method. The centrifuge is a thing which the workers just mentioned should have for purposes other than fecal examinations, and the only additional pieces of apparatus required—the screens—are inexpensive and durable.

CONCLUSION.

The method of examining feces for evidences of parasitism which consists in putting the feces through a process of thorough shaking, sieving, sedimenting, and centrifuging appears, from a theoretical standpoint and in actual experience, to give the best results in the shortest time and with a minimum financial expenditure when the value of time saved is considered. It is therefore advocated as a practical routine method of examining feces of all sorts.

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