HEALTH & DISEASES

This column is taken care of by the "Studygroup for Diseases and the Optimum Keeping and Breeding of Terrarium Animals" of the Belgian Society "Terra". If there is a question concerning health or diseases, feel free to contact the president of the Studygroup: Mr. Hugo Claessen, Arthur Sterckstraat 18, B-2600 Berchem, Belgium. He will try to answer your question in this column to the benefit of all members.

MICROSCOPY, PART I.

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Contents: Introduction - Microscopic domains - Microscopic techniques - Microscopical equipment - Prescriptions - Glass work.

INTRODUCTION

As mentioned in previous articles, microscopy is a very good method to discover various infections, long before the break out of symptoms. In view of the interest and the questions asked, we conclude that many terrarium keepers make a regular examination of the faeces of their animals. Examination of sick or dead animals has to be encouraged, as one has to try to get as much information as possible. This information about research, treatment and progress of the infections has to be published, so that everyone is able to follow the

development in treating sick animals. Until recently there was not enough published but now regular articles appear in magazines or/and books about diseases and their treatment. This is partly to blame on the fact that in some countries, certain languages will not or cannot be read. German amateurs can not get information that is published in Dutch, etc. That is why it is recommendable to publish articles in an universal language or to add a resumé in another language.

MICROSCOPIC DOMAINS

The following microscopic domains are very important for medical and veterinarian purposes: bacteriology, parasitology, and histology.

A. Bacteriology.
In bacteriology one tries to study bacteria, fungi and yeasts by making universal or specific stainings. By microscopic research you can determine which group of bacteria the species belongs. Fungi and yeasts are easily recognized and traced. By making different stainings with bacteria, one can distinguish round, stick-formed, Gram-positive (universal bacteria), Gram-negative (ill-making bacteria), etc. This can be done simply, if you have access to a number of chemicals and if you know the working method. You can research fairly quickly mucus, pus, blood and faeces for these organismes. It is a good experience and easily learned by amateurs.

B. Parasitology.
Parasitology is almost the same as bacteriology. The only differences are the methods of staining and the treatment of the material. The preparation-techniques to discover the parasites in small amounts differ substantially. Most of these techniques can be easily performed by amateurs. You

do not need extra equipment, though it is better and more comfortable to work with dedicated apparature.

C. Histology.
Histology is the theory of the tissue. After cutting and staining pieces of tissue, for example from the liver or the kidney, into very thin slices (normally in a mono-cellular layer of 0.001-0.008 mm) you can look at them under a microscope. The different structures become visible. In this way you can clearly notice the changes which occur in infected tissue. You can practically always find the cause of death, even if there weren't any external (macroscopic) changes visible. This is the best way to ascertain the cause of death. Unfortunately histological research is time-consuming, and expensive due to the use of many chemicals. You have got to have experience and need special equipment.

MICROSCOPIC TECHNIQUES

The different microscopic techniques which are used, are for every domain approximately the same. They have been developed to solve particular problems in the process. Every technique will be listed separately. The compositions of the various solutions can be found in the appendix.

Universal actions.
The underlisted, systematically described techniques must be performed in a particular order to obtain correct result.

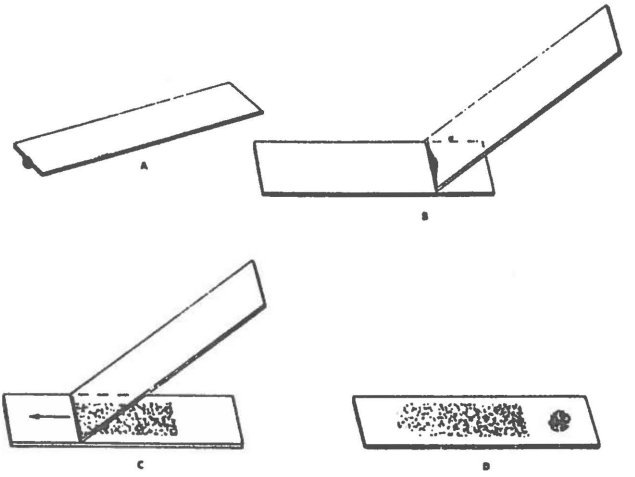
A. Sampling
How you sample depends on what purpose the sample is for. You have to decide if you want to research the parasites alive or dead. In bacteriological research you have to use ste-

rile material. When blood coagulates, you can not use it, so you have to prevent this. You must keep the sample in its original state as making changes in the sample can lead to false conclusions.

- Feaces
  In a clean glass pot (10-20 ml) you place, using a clean spoon or spatula, a small sample of faeces (about the size of a pea), preferably from the middle of the faeces, as this is usually still moist. Add 2-4 ml physiological salt-solution. The bottle is closed and strongly shaken. Treated this way, it can be kept for a couple of days in the refrigerator. It can be examined for coccidia, flagellates, and worm-eggs. For amoebes the sample should be examined within one hour.

- Pus
  As pus is practically always researched after staining you have to place it directly onto a slide. The wound or inflammation has to be disinfected carefully with disinfection alcohol. After disinfection you squeeze the wound carefully and the pus which comes out is put on one or more slides. The pus on the slide is spread thin (about 1 cm^2 surface). When the pus is too thick you can mix it with a drop of physiological salt-solution. Now you have to dry the slides as quickly as possible, for instance by heating them (40-50^{0}C). The slides can be preserved for a long time and are useful for bacteriological research.

- Blood
  The best way to research blood is after staining. You can place it directly on a slide as follows: put one drop of blood on the slide and spread it with another slide as thin as possible (see fig.). The slide has to be dried in



the air, without heating. If you want more blood, you have to suck it up with a hypodermic syringe from a bleeding wound or from a vein (in snakes the best place to do this is in the vena palatina in the upper jaw). Before you do so, you have to take up a bit of bloodpreservation-expedient. After the blood has been removed, place the mixture in a sterile glass bottle.

- Organ preparation
  Sometimes, pending a dissection, you want to look for parasites in a particular organ. Take a small glass bottle and fill with 2-3 ml physiological salt-solution. Then cut a piece (about 1 cm^3) of the organ to be examined. Place this in the bottle and mash it with a glass stick. Now you have got a liquid which you can study directly or stain, then study.

B. Fixation

The samples mentioned above are only suitable for direct research or for research on living parasites. If you want to preserve the samples

you have to fix them. This means treating them in such way that they stay exactly the way they are for a long time (sometimes for years). There are three different techniques:

- Watery suspensions
  The original watery suspensions are fixed after research, if you want to preserve the sample. This causes death of everything in the sample. You simply add double amount of fixing agent to the already extant liquid. The most-used solutions are: neutralized formalin or Schaudinn's Fluid. The samples can be sent off for further research or preserved for years.

- Slides
  The dried slides are put in methanol (free from water) for 5 minutes. After this you let them dry. They can still be stained even after years.

- Histological preparations
  If you want to preserve a piece of tissue for histological research, you simply put it in a Bouin's Fluid or in 4% formalin. Fill a small bottle with 10-20 ml liquid and put one or more pieces of tissue (about 1 cm^3) in it. This can be kept for years.
  It is also possible to fix a whole animal. You need a glass pot half filled with 4% formalin. With a hypodermic syringe carefully inject 4% formalin into the animal, especially in the abdominal cavity. This must be done until the animal has clearly swollen. Place the animal into the pot and add more formalin until the animal is completely covered.
  On all the bottles and pots stick labels with clear and complete data.

C. The making of a preparation
The method sometimes applied in faeces research, for making a preparation, is based upon the

difference in specific gravity between wormeggs and the remaining waste products in the faeces. For this take a synthetic or glass bottle of 20 ml. The opening must be a little bit smaller than a slide. You have to filter 2-3 ml of the faeces through sterilized gauze into the bottle. Then you fill the bottle with separation fluid. The fluid has to come just above the edge of the bottle. Now you carefully lay a slide on top of the bottle and let it stand. The heavy fragments (sand, etc.) will slowly sink. The wormeggs will slowly rise as they have a lesser specific gravity, and stick to the slide. After 15-20 minutes you can take the slide off the bottle and lay it on another slide. Now you can observe it for wormeggs, flagellates, cysts, and coccidia.
In a well equipped laboratory centrafugal force can be used to separate the sample, and then you can take fluid off the surface and examine. You can also make a spreading, fix it, and preserve the wormeggs.
During a dissection you sometimes find living worms in the alimentary canal. These can washed with water and preserved in a physiological salt-solution. After 24-48 hours put them in 4% formalin. In the physiological salt-solution you will find a sediment containing almost exclusively, wormeggs. From this you can make a spreading, dry it and fix it. Now you have a preparation of the eggs and the corresponding worms in formalin.

D. Staining/differentiation
After slides are fixed, you can stain them. The staining is done to make particular structures visible (bacteria, etc.), or to make a distinction by staining in such a way that particular cells stain and others do not. The staining is founded on the difference in acid-

degree or chemical reaction of the different elements. When a slide is stained with a basic stain, all the acid components will react with the basic stain, while the basic components do this less or not at all. Usually you work as follows:

- To colour with a stain, you dip the slide for some minutes in the stain or pour the stain over the slide.
- Wash away excess stain with water or a solvent.
- Now you must differentiate with a solvent, to dissolve the weakly bound stain. The strongly bound stain remains.
- Wash away the differentiation fluid.
- Now you have to stain it again, with a contrasting stain, that colours all non-stained parts.

For example a normal Gram-staining for bacteria: You first stain with a blue stain, differentiate and stain again, but with a red stain. The bacteria which are blue-coloured you call Gram-positive bacteria and the red bacteria are Gram-negative.

E. Dehydration/mounting

The original slides have to be covered with a coverslip if you want to preserve them as a preparation. This is done by putting one drop of mounting medium on a dried and stained slide. With a pair of tweezers lay a clean coverslip onto the drop. Take care that there are no air bubbles between the slide and the coverslip. The mounting medium will equally divide when you push softly on the coverslip.
Many mounting mediums are incompatible with water. If you want to prepare larger parts, for example worms, fungi or insects, you have to dehydrate them. For this you have to replace the body water with a solvent that is compatible with the mounting medium. If you do not do this,

and you let everything dry first, the enclosed objects will shrivel and become useless.
To replace the water you successively put the slides into 50% alcohol, 75% alcohol, 95% alcohol and then in 100% isopropylalcohol. After this you put the slides twice, for some minutes, in toluene, xylene or chloroform. Now you can mount it.

MICROSCOPICAL EQUIPMENT

Having discussed the most common methods, we now turn our attention to the prescription of the different solutions and stains. There are a lot of different stains and compositions, but we only present a couple of universal prescriptions and their specific use and should present no problems for the amateur.
After the prescriptions follows a list of necessary laboratory materials and a few that we recommend.

PRESCRIPTIONS

- Physiological salt-solution: 6 g Sodiumchloride (NaCl) in 1 litre water.
- Alcohol 95%: Denaturised disinfection alcohol (Ethanol).
- Alcohol 75%: 100 ml Alcohol 95% + 20 ml water.
- Alcohol 50%: 65 ml Alcohol 95% + 45 ml water.
- Methanol 100%: pure methanol alcohol (poisonous).
- Isopropanol 100%: pure isopropyl alcohol.
- Toluene: poisonous, can change the blood when inhaled for a long time.
- Mounting media that are incompetible with water

and alcohol: buy Caedex. Dries very slowly (2-3 months).

- Mounting media holding acohol, incompatible with water: buy Euparal. Dries slowly (1 week).
- Mounting media holding water: Polyvinyl lakto-phenol, Glycerin, Gelatine.
- Formalin 4% neutralised:

Formalin 37%	100 ml
Sodiumdihydrogenphosphate ($NaH_2PO_3H_2O$)	4 g
Disodiumphosphate (Na_2HPO_3)	6.5 g
Water	900 ml

  After dissolving everything it must be filtered.
- Bouin's Fluid:

Alcohol 95%	50 ml
Formalin 37%	30 ml
Acetic acid 100%	7.5 ml
Saturated solution of picric acid in water	12.5 ml
Distilled water	13 ml
Copper acetate ($Cu(CH_3COO)_2$)	1 g

  The acetic acid is only added when fixer is actually needed.
- Schaudinn's Fluid:

Saturated solution of mercury-chloride ($HgCl_2$) in water	66 ml
Alcohol 95%	35 ml
Acetic acid 100%	1 ml
Polyvinylalcohol	5 g

  Solutions of mercury-salts are very poisonous! Acetic acid must only be added at the time of need.
- Lacquer:
  Melt 50 g pure shellac resin together with 50 g beeswax.

- Separation fluid:
  Saturated solution of Sodiumchloride (NaCl) in water. 360 g NaCl in 1 litre H_2O: Trematode eggs stay under.
  Zinc sulfate ($ZnSO_4$) solution: 330 g $ZnSO_4$ dissolved in 660 ml water: Trematode eggs float.
- Blood preservation expedient:
  3.8 g trisodiumcitrate ($HO - C_3H_4(COONa)_3$) in 100 ml water.

GLASSWORK

The material needed depends on what you want to do. You can collect most things yourself only buying a few. Ask friends to keep their medecine bottles. All sorts of glass pots and bottles are useful.
- Little glass pots: use penicillin bottles with a rubber stopper. First you must clean and sterilize them in a pressure-cooker (above the water). Let it boil for 20 minutes.
- Little bottles and pots for stains and fluids.
- Slides and coverslips for the microscope you can buy at an optician's.
- Sterile needle: a darning needle assembled on a wooden handle.
- Hypodermic syringe: plastic throw-away syringes of all sizes.
- Glass sticks: 15 cm long and 4-6 mm thick.
- Scalping knife: a razorblade assembled onto a wooden handle
- Sterilized gauze.
- Small plastic or glass funnel.

You can buy a lot of laboratory equipment not strictly necessary:
- a slide carrier, in which you can stain various slides at the same time.
- tweezers: pointed and round
- graduated cylinders: 100 ml - 50 ml - 10 ml

- beakers and erlenmeyers: 500 ml - 250 ml. To make the solutions.
- glass funnels and filter papers.
- pipettes to measure small amounts of liquid.