were first described by H. Penna, of the yellow fever service.

In the last five months we have been able to investigate the disease clinically and epidemiologically, as well as to study the parasite in some of its morphological and biological aspects.

Clinically, the evolution of the disease is very similar to that of kala-azar: onset with fever of varying types, progressive emaciation, progressive anemia of a hypochromic type, leucopenia with relative monocitosis and rapid enlargement of liver and spleen. Hemorrhagic symptoms of mucosae are common. No skin lesions or skin color changes have been detected. Some cases have an acute evolution, with death occurring in from one to three months; others have a chronic course, with death occurring, in general, in from eight to fifteen months.

Parasites can be found rather easily by liver and spleen punctures and have also been detected in blood smears after white cell concentration. In human organisms parasites are always found in the form of leishmania, measuring from two to three micra, generally contained in the plasm of macrophages. Cultures have been obtained from spleen punctures in Noguchi and NNN mediums. Leptomonae grow and multiply abundantly in cultures, and their shape is identical with that of *Leishmania donovani*. Experiments are being carried on for identification of the species through comparison with all other known species of the genus *Leishmania*.

The formol-gel reaction has given fairly good results for diagnosis of clinical cases. Visceral lesions vary according to the chronic or acute evolution of the cases. In acute cases, hyperplasia of endothelial cells with monocitic infiltration, focal or generalized, is the principal sign, with a large number of parasites in mononuclear cells and extensive fatty degeneration. In chronic cases, fibrous lesions are dominant, with focal or generalized sclerosis, and a smaller number of parasites.

The disease has been found in almost all northern and eastern states of Brazil, and more recently, by Dr. Romaña, in the Argentine Chaco. No epidemic incidence of the infection has been found in any focus, but the disease exists endemically with scattered cases. No case of infection has been seen in towns, but investigation has shown the existence of jungle infection as a rule. Animal reservoirs of parasites are now being sought. Species of *Phlebotomus* have been found regularly in every focus.

The incidence of the disease, according to age, has been found to be as follows:

Under 6 years	53.1 per cent.
6 to 10 "	17.4 " "
Over 10 "	29.9 " "

Cases have been found in persons between the ages of 45 days and 56 years. Mortality investigation in some foci has shown a leishmaniasis death rate of 1.8 in the Amazon Valley, and one below 0.4 in the northeastern section of Brazil.

The treatment of clinical cases with antimonium derivatives—Neostibosan and Fuadine—has proved to be efficient.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETERMINATION OF THE INTERNAL GASES OF PLANT TISSUES

MAGNESS[1] has described a method of extracting the internal gases of plant tissues, uncontaminated by air, by submerging the material in mercury in a special tube to which is attached a mercury leveling burette that may be lowered to create a partial or almost complete vacuum in the tube containing the material. The gas escapes from the tissues, collects over the mercury and is then analyzed by means of the Bonnier-Mangin gas analysis apparatus.

The writers, in carrying out a large number of routine analyses, found the use of the Bonnier-Mangin apparatus rather tedious and time-consuming. It is frequently necessary to check the results several times to be certain of the accuracy of the values obtained. Consequently the Bonnier-Mangin apparatus was discarded and an Orsat gas analysis apparatus was used instead.

In order to make satisfactory determinations with the Orsat apparatus larger quantities of gas are necessary than is the case with the Bonnier-Mangin apparatus. To obtain an appropriate quantity of gas a larger extraction cylinder was provided. This was constructed in the laboratory by taking a piece of heavy-walled (2 or 3 mm) Pyrex glass tubing 35 mm in inside diameter, bending it at a right angle near one end and drawing it out to a nozzle to which a piece of heavy-walled rubber tubing was attached. A 1-liter aspirator bottle was attached to the other end of the rubber tubing, which served as a leveling bulb. The other or upper end of the glass tube was provided with a rubber stopper fitted with a capillary stopcock.

[1] J. R. Magness, *Bot. Gaz.*, 70: 308–316, no. 4. Illus. 1920.

A rather long rubber stopper was used and the small end was hollowed out into a short funnel with the end of the glass tube set flush with its bottom in order that the entire amount of gas could be driven from the tube by the mercury as the leveling bulb was raised. The glass tube was wrapped tightly at the upper end with adhesive tape to insure it against breakage as a result of the pressure exerted by the rubber stopper. The extraction cylinder is illustrated in Fig. 1. It was

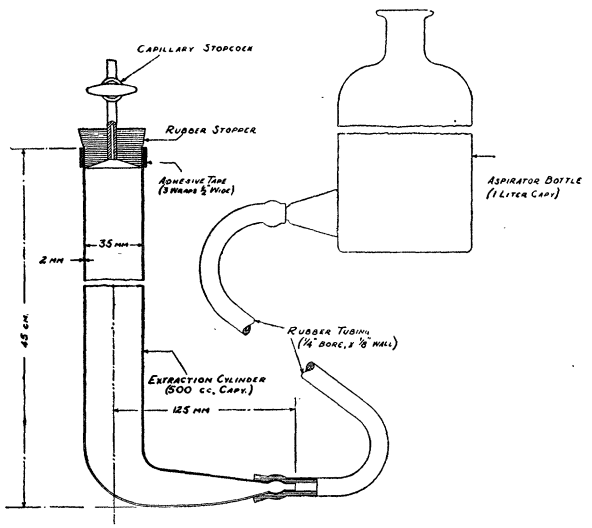

FIG. 1

45 cm. long and its capacity was approximately 500 cc and thus held sufficient material so that 10 to 15 cc or more of gas could be extracted at one operation. However, the cylinder may be made of any desired capacity. The danger of leakage appears to be no greater than with the Magness apparatus, and the connections can all be tested readily by repeated lowering and raising of the leveling bulb before the plant material is introduced. The extraction tube or cylinder may be made with a ground glass stopper as in the Magness apparatus if this modification is preferred.

After extraction the gas is transferred to the gas burette of the Orsat apparatus and its volume determined. It is then analyzed in the usual manner. In most of the tests the writers have used a 25 cc burette in place of the 100 cc burette regularly supplied with the Orsat apparatus, but a 10 cc burette may be used if only a small volume of gas is available. A 10 cc graduated pipette can be used for this purpose. If the material yields very small amounts of gas the gas obtained may be transferred to a Hempel-Winkler gas burette and stored until other extractions are made which may then be combined and analyzed. The absorption of the carbon dioxide and oxygen are carried out as in an ordinary gas analysis determination. Unless the capillary tube in the manifold of the gas analysis apparatus is very small, it may be advisable to correct for the volume contained in this capillary or manipulate the apparatus in such a manner that none of the extracted gas is in the capillary when a reading in the burette is made. In certain work where extreme accuracy is not required, acidulated water may be used in the measuring burette of the Orsat apparatus instead of mercury. This is somewhat more convenient.

The precautions to insure that the gas sample is not contaminated with the atmosphere are the same here as described in the Magness apparatus.

In working with pears about 250 grams of suitably shaped pieces were rapidly cut and dropped into the extraction cylinder. The leveling bottle containing mercury was then raised and the air in the cylinder driven out at the top through the capillary stopcock. The stopcock was then closed and the leveling bottle lowered about 10 inches for a few seconds and then raised. The gas thus collected was discarded, as some of it was at the surface of the tissue and may have been contaminated with outside air. The stopcock was then closed and the leveling bottle was lowered and held about 30 inches from the bottom of the extraction cylinder. The length of this extraction period was standardized at 5 minutes. It may prove desirable to construct a special cork borer of a size suitable for the material and apparatus and force the plugs of tissue out under the mercury in a manner similar to that suggested by Magness instead of filling the cylinder with pieces of tissue in the manner described.

The apparatus has been successfully used with eggplant fruits, pumpkins and Kieffer pears, with duplicate determinations checking very closely. The amount of gas in the eggplant fruit is 15 to 30 per cent. of its entire volume. The amount in the Kieffer pear is less than 1 per cent. of its volume. The successful use of this procedure with tissues of such extreme differences in their gas content indicates that the method is applicable to a wide range of tissues when a large quantity of the material is available. In the hands of the writers, at least, the proposed procedure has proved to be much less time-consuming and more reliable than the Bonnier-Mangin apparatus. It requires about 15 to 20 minutes to prepare the material and extract and analyze the gas by this method. It can not be used, however, when only very small amounts of material are at hand.

It may be noted that in the analysis of some fleshy fruits and vegetables the oxygen content of the gas in the tissues is much nearer the oxygen content of the air than might be expected. This is evidently due to the greater solubility of oxygen than nitrogen in the water contained in plant tissues. Thus the percentage of oxygen in air dissolved in water is around 34 to 35

per cent. at temperatures of 0° to 20° C.,[2] whereas the percentage of oxygen in normal atmosphere is around 21 per cent. In studying the gases obtained from fruit and vegetable tissues many investigators evidently consider that the gas obtained by subjecting the tissue to a partial or almost complete vacuum is obtained only from the intercellular spaces. According to Henry's law,[3] the concentration of the dissolved gas in solution is directly proportional to the concentration in the free space above the liquid. It is evident that in subjecting tissue to a partial vacuum considerable amounts of gas are extracted from the liquid contained in the cells as well as from the intercellular spaces. Magness[4] has mentioned this possibility. The difference in solubility of gases would also account, at least in part, for the very high CO_2 content in tissues after exposure to a much lower concentration of CO_2, as it is much more soluble in water than either O_2 or N_2. The writers have found that exposing Kieffer pears to an atmosphere containing 5.3 per cent. CO_2 at 60° F. resulted in the presence in the internal gases of 36 per cent. CO_2, whereas the gases from check lots in normal air had 18.6 per cent. Gerhardt and Ezell[5] obtained nearly 80 per cent. CO_2 from the gas of Bosc pears after a 24-hour exposure of the fruit to 35 per cent. CO_2 at 65° F. The gas extracted from Jonathan apples subjected to the same treatment contained nearly 50 per cent. CO_2. The higher CO_2 content in the gases obtained from pears was probably due to a higher proportion of the gas being extracted from the liquid contained in the fruit, as there is less intercellular space in pears than in apples.

It is possible that the gases dissolved in the solution within the cell are of more physiological significance than those contained in the intercellular spaces, as the dissolved gases are in more intimate contact with the protoplasm. For example, Heilbrunn[6] considers that the negative charge on the surface of protoplasm is due, at least in part, to the diffusion of carbonic acid from the interior of the cell.

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[2] C. D. Hodgman, "Handbook of Chemistry and Physics." Twentieth edition, pp. 890–891. Chemical Rubber Publishing Company, Cleveland, Ohio. 1935.

[3] F. H. Getman and F. Daniels, "Outlines of Theoretical Chemistry." Fifth edition, p. 141. John Wiley and Sons, Inc. New York. 1931.

[4] *Loc. cit.*

[5] F. Gerhardt and B. D. Ezell, SCIENCE, 80: 253–254, 1934.

[6] L. V. Heilbrunn, "The Colloid Chemistry of Protoplasm." Colloid Symposium Monograph. III: 135–151. 1925.

MANGE IN GUINEA PIGS

IN SCIENCE, for March 27, 1936, there appeared a short article on "Sulphocyanate Treatment of Mange in Guinea Pigs."[1] A safe and simple procedure has been used in our laboratories in treating mange in guinea pigs, rabbits and dogs. Raw linseed oil is applied to infected areas or the entire body, using a soft two-inch paint brush. The treatment is repeated at intervals of a few days. The animals "shampoo" themselves thoroughly; the oil absorbed is nutritive and mildly laxative. Hairs seemingly not infected will fall. We have had animals, completely denuded, make good recovery in body weight and return of good hair growth after treatment. Boiled linseed oil must not be used. The idea was borrowed from the custom of feeding flax seed to horses in the early spring in order to aid shedding of their winter coats of hair and from the fact that linseed oil is an excellent detergent for body surfaces soiled by heavy greases.

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PROTECTION OF EYEPIECES

THE upper lens of some makes of microscope eyepieces is set below the level of the surface of the casing for protection. Unfortunately, this also renders them very difficult to clean properly. Much of this cleaning can be avoided if a circular cover-slip 20 to 25 mm in diameter is placed on the upper surface. These cover-slips are easily removed and polished and will not interfere perceptibly with the use of the instrument. The rim of the eyepiece prevents them from sliding off when the microscope is tilted.

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[1] 83: 2152, 304, 1936.

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