indicates that in the herbaceous forms an increase of moisture content in the leaves causes a marked decrease in osmotic pressure, whereas for the ligneous forms the decrease, if it occurs, is relatively slight.

For the water content of the leaves and the total solids in the sap we have again a negative correlation, both for the ligneous and for the herbaceous forms, as well as for the 2 forms combined. Here again, the ligneous forms show a much lower correlation than do the herbaceous forms, indicating that physiologically the 2 forms differ to a very marked extent.

It will be noted that in none of the series in which bound water is a factor do we find high coefficients of correlation. This justifies the labor necessary for making these calculations, inasmuch as it demonstrates that the measurement of bound water in a plant sap is the measurement of a factor which is essentially independent of the properties which have previously been measured, and that the bound-water values cannot be obtained from any of the ordinary measurements. Consequently these data can be taken as indicating that measurements of bound water may prove helpful in studying the properties of plant saps as related to geographical distribution.

Another point brought out by the table has already been referred to, namely, the marked differences which are shown in certain of the relationships, when one compares ligneous with herbaceous plant forms. It should be obvious that there is a marked difference in the physico-chemical properties of the plant saps of these two forms, as has already been pointed out.[13, 14, 15]

3984

Improved Method for the Extraction of Melanin from Human Urine.

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The futility in expecting to obtain an unaltered metabolic product of a complex character like human or animal melanin after prolonged boiling with caustic or concentrated mineral acids, was pointed out by Gortner[1]. The method of isolation of urinary melanin by direct precipitation of the urine either with basic lead acetate or with barium hydroxide suffers from the disadvantage

[1] Gortner, R. A., *J. Biol. Chem.*, 1910, viii, 341.

that a great variety of compounds besides the melanin is taken down by either method; which fact does not facilitate the purification.

Due to the great scarcity of melanuria in cases of melanosarcoma in man, the problem of a simple and at the same time mild method for the isolation of melanin from human urine is only seldom attacked. The fact that we are engaged in the isolation of another cyclic urinary compound[2] made it possible for us to use the occasion of obtaining melanotic urine from a middle aged man with multiple metastases from a melanosarcoma of the skin. The abundance of melanin produced and circulated in the organism in this patient was demonstrated not only by the occurrence of melanin in his urine but also by a unique diffuse melanotic discoloration of the skin giving a slate colored character particularly to face and hands.

For the isolation of the melanin the following procedure has been employed: The urine is evaporated on a water bath in a current of air and the residue is extracted with absolute methyl alcohol. The extraction is carried out in a tall cylinder or beaker and the salts allowed to settle slowly. This process is repeated several times with fresh washings of methyl alcohol until the final washing is straw colored or colorless. This extracts a variety of substances leaving the bulk of the salts as well as all the melanin. The same procedure is then carried out with methyl alcohol acidified slightly with HCl, about 2 drops of concentrated HCl per 100 cc. of absolute methyl alcohol. This extracts the melanin. The acidified methyl alcohol from repeated washings is united, the melanin precipitated by the addition of ether, the solutions centrifuged and the supernatant liquid decanted. The precipitate, which consists of almost pure melanin, is redissolved in acidified methyl alcohol and reprecipitated with ether. The same process is repeated a second time. The precipitate is now washed several times with water, then with ethyl alcohol, finally with ether and dried in a current of dry air.

The product obtained is a dark brown or black powder, having the properties ascribed to melanin. It is insoluble in water, in neutral methyl alcohol, neutral ethyl alcohol and ether. It is slightly soluble in dilute acids, completely soluble in concentrated mineral acids and in alkali. It is somewhat soluble in alkaline methyl alcohol and highly soluble in acidified methyl alcohol. It does not reduce phosphomolybdic acid. It may be precipitated from the urine by dialysis, or carried down by saturating the urine with ammonium sulfate.

[2] Medes, Grace, Berglund, Hilding, and Lohmann, Anne, PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 210.

It does not give the biuret reaction or the Hopkins-Cole reaction. Neither does it give a positive Millon's reaction. This corresponds to the observation of Gortner's (personal communication) that the melanin obtained from black wool of sheep without application of strong reagents gives a negative Millon's before hydrolysis.

The yield of melanin in our patient amounts to about 500 mg. per 24 hours.

3985

Experimental Tularemia in Birds.

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It has previously been reported that grouse are susceptible to experimental tularemia.[1, 2] Inoculation of an abrasion through the skin of a ruffed grouse results in a fatal septicemia.

Preliminary experiments which have been carried out on the susceptibility of the Hungarian partridge to experimental tularemia, indicate that this bird is highly susceptible to the disease, a fatal infection resulting from an open inoculation of a skin abrasion.

The pigeon appears to be relatively more resistant. In this bird an intramuscular injection causes the formation of a local lesion, and in some cases this is followed by a generalized invasion by the organism. Even with the production of a septicemia, no clinical symptoms have been observed in the pigeons in our series and those not killed have recovered.

The ring-necked pheasant also appears to be relatively resistant to experimental tularemia.

The domestic chicken appears to be absolutely resistant to the disease. Organisms injected intramuscularly do not appear to invade and produce a general infection.

[1] Parker, R. R., and Spencer, R. R., Sixth Biennial Report, Montana State Bd. of Entomology, 1925-1926, p. 30.

[2] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., in press.