PLANT CHEMISTRY LABORATORY.

Director: Dr. J. Melville.

The Plant Chemistry Laboratory has continued its investigations along the lines which were indicated in its initial annual report last year. The staff has been engaged largely on what can be termed the initiatory stages of a long-term investigation, This has involved the application of the most approved methods of analysis for single substances or groups of closely related substances, and the working-out of new methods for materials whose presence has been known or suspected but for which analytical methods have not been available.

The object of the investigation is, in general terms, a much fuller knowledge of the chemical substances which are present in aqueous plant extracts—*i.e.*, of those metabolites which can be translocated and which are assumed therefore to be of prime importance in the synthesis or breakdown of protein. The need for such an investigation is becoming increasingly obvious. Before it is possible to correlate stock health with the pasture on which the animals are grazing, a knowledge of the materials present in the ingested food, together, with an appreciation of their pharmacological as well as their nutritional properties, is essential; while, almost equally desirable, are analytical methods whereby the concentrations of such materials can be quantitatively determined.

The Cyanogenetic Glucoside of White Clover.

The ability of certain strains of white clover to produce hydrocyanic acid (HCN) has been of great interest to pasture and animal investigators. The relative amount present in various lines is being used in New Zealand as a means of seed certification, while it is obvious that the presence of so toxic a substance as HCN in this universal pasture plant is a matter of concern to the veterinarian. Although no case of HCN poisoning on clover has ever been recorded, it is obviously desirable to have further information on the glucoside which produces HCN, and on its breakdown to give free HCN under the action of the corresponding enzyme.

The isolation of the glucoside, lotaustralin, has been accomplished by Australian workers, but the yield obtained from white clover by their method was so small that it gave little promise of being suitable for building up a reasonable quantity of the material. By working with fresh material, and extraction of a clarified and concentrated extract with ethyl acetate, the yield has been much increased, and it is hoped that sufficient can be prepared for toxicological studies.

In a study of the possibly toxic effects of clover containing high amounts of the glucoside it is important that due weight be given to the way in which the glucoside is broken down by the corresponding enzyme It must be stressed that in the absence of a hydrolytic enzyme, lotaustralin would be quite harmless even if fed in comparatively large quantities. Only in the presence of the enzyme is the glucoside dangerous, and then only if conditions in the digestive tract are such that HCN is rapidly liberated.

That an enzyme capable of causing the liberation of HCN from the glucoside is present in clover is evident from the breakdown which occurs when the leaves are cytolysed or allowed to autolyse; but, due to the large range of concentrations of the enzyme in various lines of clover, and to the difficulty of obtaining highly active preparations, the preliminary work has been carried out on linseed. This easily available raw material is an excellent source of the enzyme, and by the use of methods elaborated for the purification of other similar materials a product of high activity has been obtained. The most active preparation hydrolyses one hundred and twenty times its weight of glucoside in two hours, representing an increase in activity over the original linseed of two hundred times. It is believed that even this material is far from pure, but it was sufficiently free from extraneous materials to make possible a precise study of its reaction with the purified glucoside.

The purification of the enzyme from white clover is a much more difficult operation, but it has been possible to show that its properties are practically identical with that from linseed. The conditions of pH and temperature under which the enzyme hydrolyses the glucoside have been accurately defined. It should be possible, therefore, to supply both glucoside and an active preparation of enzyme for toxicological work, while the information as to optimum conditions for hydrolysis will be of value. The preparation of the enzyme from linseed has been very useful in determining the quantity of glucoside in fresh clover, since individual variations in the quantity of enzyme present have no effect, and it is necessary only to allow the reaction to proceed overnight to get maximum amounts of HCN. The value of this in breeding and selection work has already been demonstrated in a study which is being carried out on white clover by the Grasslands Division.

One further observation has been made as a result of these investigations—viz., the extraordinary rapidity with which a certain amount of the glucoside is broken down after the clover is cut. It has been established beyond doubt that there exists no free HCN in the leaves on the plant; yet in certain cases it has been found that ten minutes after cutting no less than 25 per cent. of the total glucoside has been broken down. Further breakdown does not occur until the leaves begin to autolyse, which generally requires more than forty-eight hours. What initiates this almost explosive reaction is unknown, but it illustrates how carefully interpretations of analyses of cut herbage must be made.