experimental conditions have been examined, but in no case has excretion been detected either by benefit to non-legume in association or through analysis of the sand.

At the invitation of Prof. Virtanen and with the aid of Dr. S. v. Hausen, I conducted experiments at the Biochemical Institute in Helsingfors during the fall of 1936. In spite of unfavourable weather, which restricted nitrogen fixation, definite evidence of excretion was obtained with peas grown in sterile containers. The experiments were repeated this spring at Madison under identical conditions with the exception of the sand used—and of necessity with different environmental conditions, for example, type of greenhouse and weather conditions. In these experiments, excretion has been detected in *part*, but not *all*, the cultures independent of the quantity of nitrogen fixed. The cause of the discrepancies is not readily apparent, but the chief factor in associated cultures appears to be the manner of development of the plants. Our experiments indicate that excretion may be profoundly affected both qualitatively and quantitatively by the species of plant, nature of substrate and in associated cultures by the relative rate of growth of the different species. An environment that delays the reproductive stage appears to favour the excretion process.

Without minimizing the importance of Prof. Virtanen's studies, especially as they relate to the chemical nature of the excreted compounds, it is emphasized that excretion of nitrogen by leguminous plants is not universally obtained, even under experimental conditions which are apparently identical. For this reason the questions as to whether the phenomenon actually occurs in the field and to what extent it is responsible for the beneficial effects of associated culture of legumes and non-legumes remain unanswered. Until the factors which control the process are defined and the origin of the discrepancies known, it should be recognized that application to practical agriculture is only an attractive possibility and not an established fact.

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Succession of Broods of *Lebistes*

THERE seems to be a point in the natural history of *Lebistes* which is not generally known among those using this fish as a 'laboratory animal' in Great Britain. As it affects certain types of research very markedly, the following information will be of interest to those using this fish.

As a result of one mating, a female *Lebistes* may produce a succession of broods over a period of from six to eight months. From personal experience, I cannot vouch for the length of time mentioned (my authorities are Mellen and Lanier) as I am only now in the process of testing it, but that spermatozoa do remain alive within the body of the female and fertilize one brood after another is definitely established. The exact details of my data may be of interest. Mr. Gillespie sent me up some specimens from the Zoological Society of Scotland's Aquarium on February 3 last. As a result of the rigours of the journey, all were dead by the morning of February 5 except two females. Hoping for the best, I got no more, and one of the two, which has had no other companion except the other original one since, has produced the following broods: February 22, 3; March 28, 29; April 25, 58; May 27, 28. Judging from her present appearance, she is again an expectant mother!

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Method for Fixing Neutral-red in Supra-vital Stained Blood Smears

IN blood smears from horses stained according to the ordinary methods Giemsa and May-Grünewald Giemsa, we encounter serious difficulties in the examination: first, to differentiate between large lymphocytes and small monocytes, secondly, to determine *qualitative* changes in the white blood corpuscles. In order to remedy this, we have employed as a supplementary method, in an extensive investigation into the white blood picture in the case of infectious anæmia in horses, supra-vital staining with neutral-red.

We mix in a test-tube neutral-red with citrate blood at body temperature in the ratio of 1 : 15,000. The test-tube is placed in a thermostat at 37° for 40 minutes. In the meantime, it is shaken up a couple of times. After that we make an ordinary cover-glass smear, allow it to dry in the air for 24 hours, *fix it with a saturated picric acid solution*, and then stain it with Mayer's hæmalin (that is, Mayer's original recipe without citric acid), and embed in cedar oil.

In this slide thus prepared from *normal* horses, there is a large granulated deposit of neutral-red in monocytes, eosinophiles and basophiles, while no neutral-red grains are discovered in neutrophiles and lymphocytes. The difference between the neutral-red in lymphocytes and monocytes makes differentiation easy. In slides from horses *suffering from infectious anæmia*, there occurs during an attack of fever a more or less large deposit of neutral-red also in the neutrophiles. It is often connected with degenerative changes. The degeneration is demonstrated by vacuoles in the protoplasm and karyopyknosis with strong lobation of the nucleus. We have been unable to discover in the sick horses any changes in the amount of neutral-red deposit in the other white corpuscles.

This method does not, of course, present any opportunity for the study of the live white corpuscles as does the method described by Simpson[1] and by Sabin[2], but in contradistinction to the latter it makes it possible to study the cells at leisure when fixation of neutral-red has been reached. The nuclear staining also largely facilitates the investigation. A great advantage is also the possibilities it presents in the study of qualitative changes in the neutrophile leucocytes.

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