ZOOLOGY

THE INFLUENCE OF TEMPERATURE AND KIND OF FOOD ON THE INCREASE IN THE NITROGEN CONTENT OF THE YOUNG WORKER HONEYBEE (APIS MELLIFICA L.) [1])

BY

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Introduction.

During the first five days of the imago life of the worker honeybee, a considerable increase in the protein content of the body occurs, which amounts to nearly 50 %.

STRAUS (1911) was the first one to demonstrate this increase by determining the total nitrogen content of the bee body, but since the content of the digestive tract may vary greatly, so does the amount of nitrogen present there, thus the figures of STRAUS do not give an exact impression of the protein content of the body itself. HAYDAK (1934) and KELLER-KITZINGER (1935) gave a more detailed picture of the changes in the protein content. They determined the amount of nitrogen present in the whole body on successive days of the imago life after removal of the digestive tract. It was shown that the nitrogen increase occurs in the first five days after emergence and then comes to a standstill. According to KELLER-KITZINGER, no decrease takes place after the twentieth day.

The last mentioned author analysed the conditions necessary to achieve the normal protein increase. This happened only if young bees were fed honey and pollen from a comb (the so-called bee bread) at a temperature above 30 C. The same food did not cause an important increase if the bees were kept at 23°. If the bee bread was fed with sugar solution instead of honey, no increase took place, neither at 23° nor at 30 — 34°. Even with bee bread and honey at a temperature above 30° C, the normal increase was not achieved if the food was boiled previously. The enzymes present in honey and bee bread seemed to be indispensable for a normal protein increase. From these results it was concluded that young bees are able to digest proteins only at a temperature higher than 30° and that the normal level of the nitrogen content of bees more than

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5 days old, can be reached only if they are fed honey and bee bread. The findings of a complete lack of protein digestion and anabolism at a temperature only about 10° lower than under natural conditions seemed rather peculiar and a repetition of this observation was considered to be advantageous. Moreover, HAYDAK (1933, 1936, 1937), investigating the value of different nutritious substances as pollen substitutes for bees, found a normal nitrogen increase in the body in the absence of bee bread. From this it may be deduced that the enzymes of bee bread are not necessary.

In the present investigations, it is shown that neither the natural food (honey and bee bread) nor a temperature of more than 30° is a necessary condition for the protein digestion and anabolism of young bees.

Material and methods.

Young bees emerged from a comb with sealed brood in an incubator at 32 — 34° in September 1949. At intervals of 12 hours the bees were caught from the comb and transferred to small experimental cages (12 × 10 × 4 cm), about 50 bees per cage. (The cages were about equal to the Liebefelder experimental cages). The food consisted of sugar candy (4 parts sugar to 1 part tap water) mixed with the bee bread or casein, 20 and 10 % respectively (percentage of the dry sugar). Water was constantly available.

Two experimental groups fed respectively 20 % bee bread and 10 % casein were kept in an incubator at 30°; two other groups fed bee bread and casein respectively were kept in an incubator at 23°.

About 200 newly emerged bees were marked with a dot of paint and added to a colony in an observation hive. Samples of these marked bees taken at intervals served as controls. All experimental bees were descendants of the same queen. The changes in the protein content of the body were followed with nitrogen determinations on samples of 5 bees with the Kjeldahl method. After being killed with chloroform, the digestive tracts were removed, including the honey stomach and oesophagus. This may be done easily by pulling the sting out of the body, then the digestive tract comes out as well and may be removed as a whole. The nitrogen content of the digestive tract and of the rest of the body (including the sting) was determined separately .The bodies were weighed and then placed in an incubator at 80° C for 3 days to determine the dry weight. The destruction was performed with sulphuric acid, potassium sulphate and selenium mixture according to Wieninger. These reagents contained no appreciable amounts of nitrogen. The titrations were performed with 0.1 n sodium hydroxide and a mixture of methyl-red and methylene-blue as indicator. Determinations with ammonium sulphate, glycine and urea showed an error of less than 1.2 %, with an average of 0.5 %.

Results and discussion.

The nitrogen contents of the body and of the digestive tract on successive days are plotted in figures 1 and 2 respectively. Figure 3 shows the changes in the dry weight.

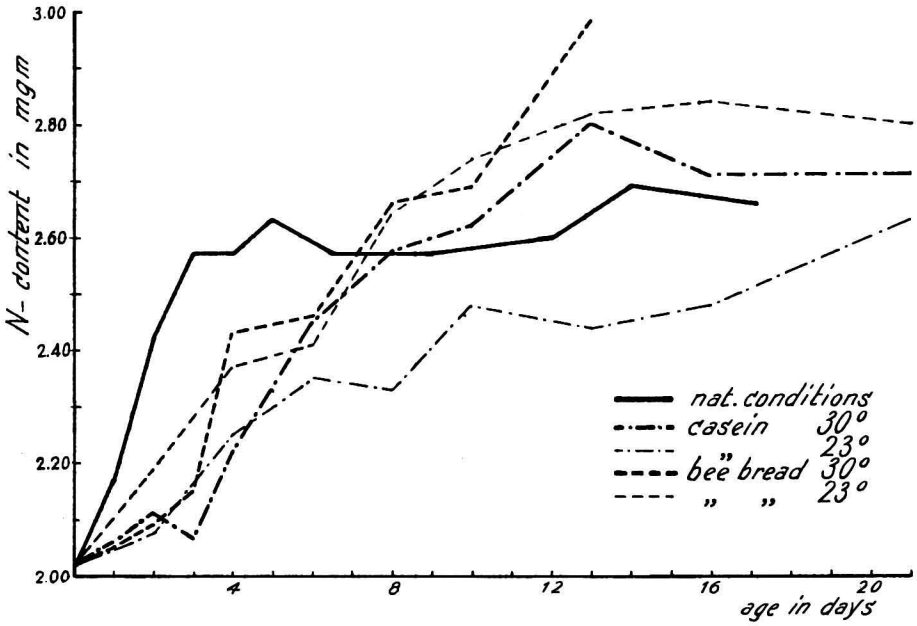

Fig. 1. Increase in nitrogen content of the body of young honeybees without digestive tracts under different conditions. Nitrogen content in mgm. per individual plotted against age in days.

Most of the curves show a rather irregular course, which may partly be attributed to the fact that each point is the result of only one determination. Even so, some important features are clearly demonstrated.

Figure 1 (drawn line) shows a steep rise in the nitrogen content of the body of bees kept in an observation hive which stops at the fifth day when a level of 2.63 mgm. has been reached. After the seventeenth day, a considerable decrease must occur, for the nitrogen content of field bees (descendants of the same queen and of more than 3 weeks of age) lies between 2.27 and 2.51 mgm. with an average of 2.41 mgm. This fact is not in agreement with KELLER-KITZINGER's statement that the nitrogen content remains constant after the twentieth day.

The other lines of figure 1 show a very important increase in the nitrogen content of the bees in each of the experimental cages, resulting in about the same level as under natural conditions (2.5 — 2.7 mgm. per bee) or even somewhat higher. This means that without honey and bee bread, even without a temperature of more than 30°, the normal nitrogen level is reached. This contradiction of the findings of KELLER-KITZINGER who could not obtain this estimation unless honey and bee

bread were fed at a temperature of 30 — 34°, is very striking. In the present investigation, it is proved that sugar and casein are adequate feeding substances to raise the nitrogen content of young bees to the normal level, even at a temperature about 10° below normal. The enzymes present in bee bread and honey appear to be unessential to reach the normal level.

Considering the rate of the nitrogen increase, one observes a considerably slower increase in the experimental bees than in the controls during the first two or three days. From the third till the eighth day however, the incline of the curves is nearly uniform, except for the casein-fed bees at 23°. This means that from the third day, the increase in the nitrogen content of the experimental bees is nearly as fast as under natural conditions.

With the exception of the casein-fed bees at 23°, the normal nitrogen level has been reached at the eighth day, being three days later than normal. This retardation is less pronounced if one considers the very slow rise during the first two or three days. From the second or the third, until the eighth day, the rate and amount of the nitrogen increase in the experimental bees is about equal to that of the controls during the first five days.

The discrepancy in the first three days may be due to the behaviour of the bees when first caged, when they remain very quiet, forming a little cluster and the food consumption is very low (fig. 2). KELLER-

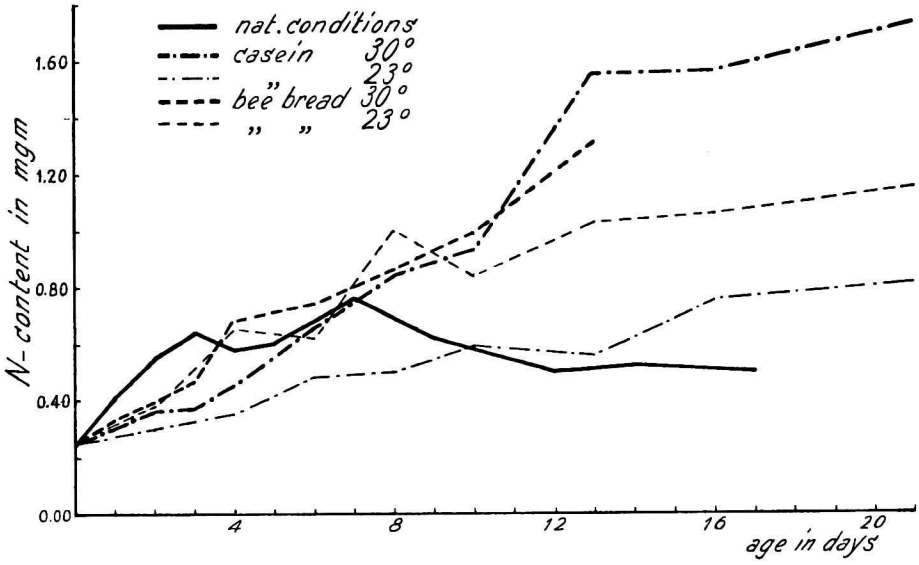

Fig. 2. Changes in nitrogen content of the alimentary canal of young honeybees under different conditions.

KITZINGER noticed that caged bees did not find the food on the first or second day. I am not sure if this is the real cause of the low food intake,

but the fact remains. If this phenomenon is taken into account, then the time in which the normal level has been reached in the experimental bees is only slightly longer than under natural conditions. Thus it seems justifiable to conclude that the retardation in reaching the normal level of the nitrogen content is mainly a consequence of the abnormal behaviour of caged bees and not primarily due to the kind of food or to the lower temperature.

The three curves for the casein-fed bees at 23° (fig. 1, 2 and 3) run somewhat separate from the others, showing a smaller food intake (fig. 2) and a slower increase in the dry weight (fig. 3) and nitrogen content (fig. 1). The bees exhibited a marked lack of vitality as compared with other groups. These differences may be due to the high casein concentration. The fact that a 10 % casein sugar mixture is not a suitable food for bees was shown in other observations in which the mortality was studied, when it was observed that the mortality was much lower if the food contained less casein. (These observations will be dealt with in a further paper. See also MAURIZIO 1950). It is possible that the damaging effect of the 10 % casein food is stronger at 23° than at 30° and this may be the cause of the smaller food intake and the slower increase in dry wieght and nitrogen content.

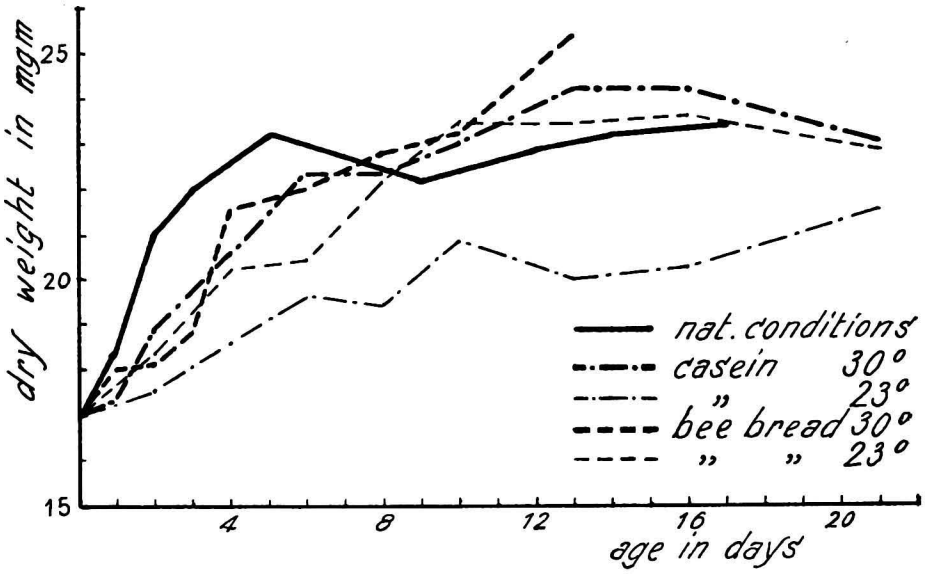

Fig. 3. Changes in dry weight of the body of young honeybees without digestive tracts under different conditions.

The slight influence of the lower temperature on the nitrogen increase is clearly demonstrated by the closely parallel course of the two bee bread series in figure 1. The increase shown by these curves is nearly equal during the first 12 days of the experiment, both in rate and amount. This means that the difference in temperature has no appreciable in-

fluence on the rate of the nitrogen increase: digestion, absorption and anabolism of proteins may occur as well at 23° as at 30°. From this it may be concluded that the temperature is a much less important factor in the metabolism of bees than was claimed by KELLER-KITZINGER.

Finally, it is important to draw attention to comparable figures of other authors. Although the normal nitrogen content of bees more than 5 days old agrees very well with that mentioned in literature, this is not the case regarding the nitrogen content and dry weight of emerging bees. HAYDAK (1934), KELLER-KITZINGER (1935) and LOTMAR (1939) found a nitrogen content of 1.6 — 1.74 mgm. and a dry weight of 14.3 — 14.9 mgm. In the present experiment, (performed in September and October 1949), the amount of nitrogen and the dry weight of 19 samples of newly emerged bees without alimentary tract was determined. The nitrogen content was found to be never less than 2.0 mgm. per bee with an average of 2.06 mgm. and the dry weight never less than 16.6 mgm. with an average of 17.2 mgm. These figures show important discrepancies with those of other authors and which cannot be attributed to a different preparation technique. 27 Samples of newly emerged bees taken from the same colony with the same queen, analysed in May and July, showed averages of 1.78 mgm. for the nitrogen content and 15.2 mgm. for the dry weight which is in good agreement with the figures of the authors mentioned. Thus considerable differences in the composition of the bee body may occur in the different seasons and it is obviously important to mention the season if figures of the body composition of bees are concerned.

Without further information, however, one is not justified in generalising these findings, but it is possible that the higher dry weight and nitrogen content in autumn is related to the preparation of the bee colony for hibernation.

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Summary.

1. Newly emerged honeybees, kept in confinement at temperatures of 23° and 30° C, were fed 20 % bee bread-sugar candy, or 10 % casein-sugar candy. The changes in the dry weight and in the nitrogen content of the digestive tract and the rest of the body, were followed on successive days up to the 21st. day and compared with those of bees living under natural conditions in an observation hive.

2. The statement of KELLER-KITZINGER (1935) that the normal nitrogen content of bees more than 5 days old is only attained if beebread and honey are fed at a temperature above 30° is proved to be incorrect.

3. An appreciable influence of the enzymes present in honey and beebread was not observed, from which it is concluded that these enzymes do not have an important part in digestion and anabolism of proteins. The mixture of casein and sugar appeared to be an adequate feeding substance for the normal nitrogen increase of young bees.

4. At a temperature of 10° below that of the brood nest of the bee colony, the normal nitrogen level may be reached at nearly the normal rate.

5. Attention is drawn to the considerable difference, both in dry weight and in nitrogen content, of emerging bees in summer and autumn, probably related to hibernation.

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