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STUDIES ON CHEMICAL CHANGES DURING THE LIFE CYCLE OF THE TENT CATERPILLAR (MALACOSOMA AMERICANA FAB.), III. SOLUBLE ASH AND SULFATES*

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INTRODUCTION

The composition of insect tissues in terms of fats, carbohydrates and proteins takes into consideration the cells and the products stored in them. Fats are usually stored and may be used either as energy for metamorphic processes or at certain times in the life cycle for maintenance of life processes. Carbohydrates are essential for tissue building and can be used as energy during metamorphosis. The proteins are perhaps most important of the three, since the cells consist mainly of protein materials, and are therefore essential to the growth of the insect, but ordinarily they are not stored for future use.

The decomposition of these different materials (fats, carbohydrates, nitrogenous matter) gives rise to certain waste products, like carbon dioxide, carbonates, sulfates, etc. Gross measurements of these waste products gives a clue to the actual relation between the destruction of the different components, whereas the determination of changes in the amounts of waste products dur-

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ing the life cycle of an insect furnishes data for the interpretation of physiological activities.

This is especially the case when the different stages of development of the insect are compared.

The determination of changes in the amounts of soluble ash (mainly carbonates and sulfates) during the life cycle of the tent caterpillar enables us to determine the sources of energy used during each stage since these waste products will fluctuate and change their relative relation whenever one group of substances is used at a greater rate than the other.

Changes in the carbohydrate contents of the insect may be determined by gross determinations of crude fiber, sugars, etc., but it was thought that indications of the decomposition of carbohydrates, including carbon material from decomposed fats, could be obtained by analyzing the ash for carbonate contents. This reasoning assumes that increased activities (increased carbon dioxide production) would also increase the amounts of acid and true carbonates. If no changes in carbonates would occur, particularly during that part of the life cycle when the larvæ are confined to the egg cases, this reasoning obviously would be incorrect. If changes did occur the interesting question is still to be answered whether the changes are in all cases directly in proportion to the CO_2 output of the insect, but since no attempt will be made in this paper to determine accurately the total amounts of carbonaceous materials used, but rather which substances were used predominately during certain stages, this question can be left for the future. However, the fact that carbonates fluctuated tends to show that measurements of CO_2 output (a measurement commonly employed for energy transformation in insects) which do not take into consideration the accumulation of carbonates are inaccurate.

The object of determining carbonates in the ash content was not only to find possible changes, but also to determine whether or not these carbonates remained, whether their relation to total ash and sulfate content was constant in the different stages of development, and how they changed with the changes in moisture, fat and nitrogen contents of the insect.

Nitrogenous substances (proteins) used in the metabolic processes contain certain quantities of sulfur. Many of the proteins have sulfur largely in the form of cysteine and some in the form of cystine or possibly both. This organic sulfur is excreted largely in the form of inorganic sulfate (in human beings from 75 per cent. to 80 per cent. of the total sulfur of the urine is in the form of inorganic sulfates) and determinations of sulfates would throw some light upon protein decomposition, especially in larvæ confined to egg cases.

Methods and Material

The material used has previously been described (1). Total ash was determined by incineration of 5 to 10 grams dry material. Soluble ash was determined by treating the ash with

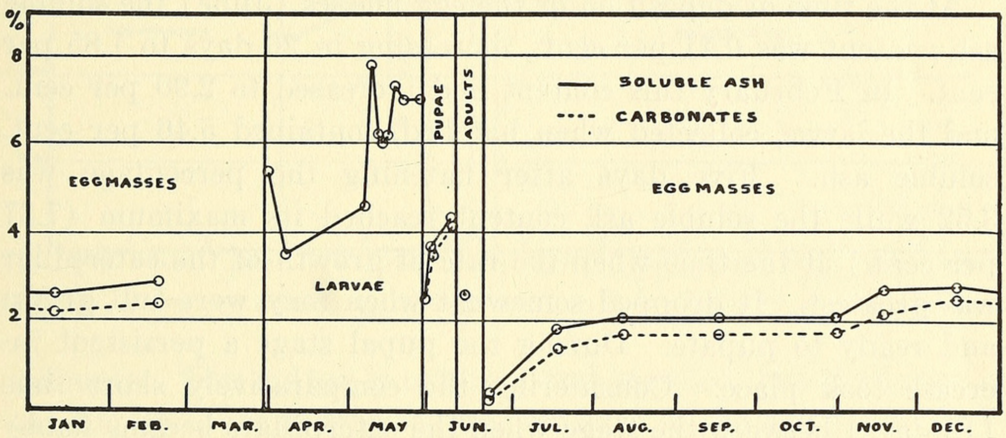

FIG. 1. Percentage soluble ash and carbonates present on a dry basis during the different stages of the life cycle of tent caterpillars.

strong HCl and HNO_3; carbonates by treating ash with dilute sulfuric acid, heating, collecting the gases and determining the CO_2 content of the gas. Sulfates were determined in the usual manner by precipitation with barium chloride. All results were calculated on a dry basis.

Results

Analysis of soluble ash (mainly carbonates) is graphically shown in figure 1. The analyses show that the soluble ash of the egg masses increased rapidly after the eggs were deposited and the larvæ were formed. It remained practically constant during the winter months until shortly before hatching. The soluble ash content of the growing larvæ increased markedly and the same was true for the pupæ. It is evident that every time a new phase of the life cycle started the soluble ash content was lowest, increasing as time went on. It is of interest to place on record that the non-soluble ash contained a substance which behaved like a sodium silicate (glass). Upon heating the material would liquefy, and become hard when cooled. The amounts of this material did not increase with the age of the egg masses and was present and increased rapidly in the growing caterpillar. A quantity of this material is still on hand but has not been analyzed further. It is conceivable that certain ingredients of this substance play a rôle in the maintenance of moisture content by the insect.

At the time of deposition of the egg masses (June) the soluble ash content was 0.41 per cent., increasing in 26 days to 1.85 per cent. In February this content had increased to 2.90 per cent. and the larvæ, collected when hatched, contained 5.46 per cent. soluble ash. Five days after hatching the percentage was 3.52 while the soluble ash content reached its maximum (7.37 per cent.) at the time when the rate of growth of the caterpillar was greatest. It dropped somewhat when they were full grown and ready to pupate. During the pupal stage a persistent increase took place. Considering the comparatively short time (12 days) between the stage when the caterpillars became flabby (ready to pupate) and ready to hatch, an increase from 2.54 per cent. to 4.33 per cent. or a percentage increase of 58.6 is quite remarkable. It seems to explain why the relative fat content of the pupæ increased. Only 10 per cent. of the total fat present was used during the processes of reconstruction; the balance was stored. This brings us to the question, where did the soluble ash increase come from, or in other words what material was used mainly in the processes of reconstruction? The answer will be clearer when, in a next paper, the results on glycogen are presented.

The changes in sulfate content of insects during the different stages of their life cycle are presented in figure 2, expressed in parts per million dry matter. The freshly laid egg masses contained 1600 p.p.m. sulfates. The sulfate content increased rapidly during the period of formation of the larvæ, remained for a time practically constant, and increased again during the latter part of the stage of confinement in the egg cases. During the larval stage (when they were actively feeding) the sulfate content fluctuated but reached a lower level than at the time when the larvæ were still in the egg masses. The sulfate content of the pupæ and adults was comparatively very low. As has been pointed out above, nearly all proteins contain sulfur in small

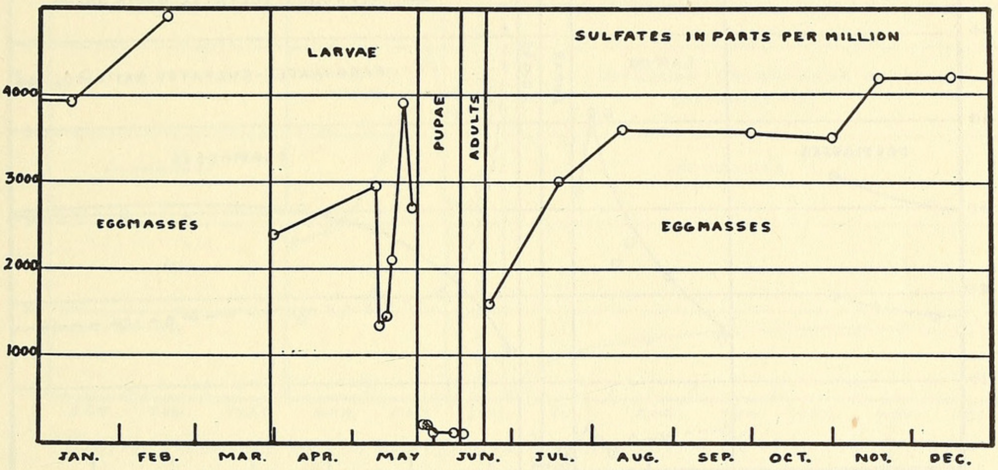

FIG. 2. Parts per million sulfates present on a dry basis in tent caterpillars.

amounts. This sulfur is oxidized and secreted, but the larvæ in the egg cases must deposit these sulfates in the egg cases. It is natural that the sulfates increased rapidly when the small larvæ were forming, if we assume that protein material was changed to amino-acids and the sulfur oxidized.

In a former publication (1) it was stated that part of the nitrogen present in the larvæ ready for pupation was lost during the metamorphosis, and that during the period of "just pupated" to "ready to hatch" the nitrogen did not decrease, but that the fatty substances decreased materially, and the conclusion was drawn that during this period metabolic processes necessary for the maintenance of life were continued at the cost of fatty substances. The low sulfate figures obtained during this period support this conclusion. At the same time the carbonate content of the ash increased during this period giving further evidence.

To enable a somewhat better understanding of the groups of materials which are utilized during the different stages of development, we have plotted in figure 3 the nitrogen-sulfate ratios and the carbonates-sulfate ratios. Keeping the nitrogen constant we see at once that during active larval growth the sulfates are produced at a faster rate than nitrogen accumulates. As soon as the larvæ stop feeding (ready for pupation) the relation is reversed. Since the larvæ in the egg cases had no access to food

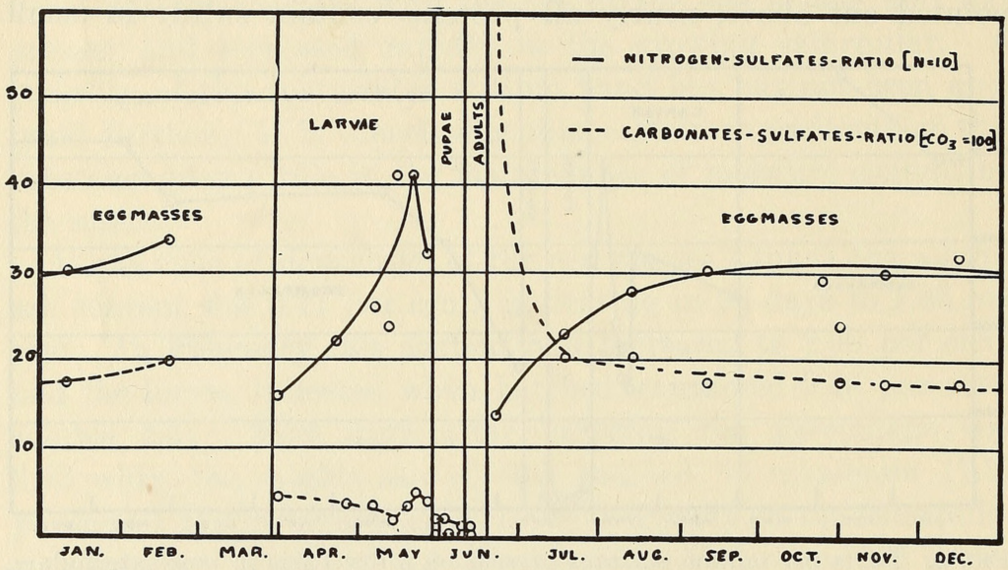

FIG. 3. Relation of nitrogen and sulfates and carbonates and sulfates in tent caterpillars at different stages of their life cycle.

during the second part of this period, the nitrogen decreased at a rate only slightly faster than the sulfates were formed, but during the first part of this stage, when active tissue building took place, sulfates increased far more rapidly than nitrogenous substances disappeared, indicating that the nitrogenous substances were not used for the metabolic processes, but for reconstruction, while in the second part of the stage, when the larvæ were formed, the decrease in nitrogen runs parallel with the increase of sulfates, or in other words, the nitrogenous substances were used for the maintenance of life. It is of interest to note that during the pupal stage sulfates did not increase in relation to the nitrogen content, indicating that if nitrogenous matter was used it was not oxidized but utilized in the transformation processes as building stones without waste.

The relation between carbonates and sulfates (figure 3) changed very rapidly shortly after the eggs were deposited and while the larvæ were formed. During the feeding stage the relation between carbonates and sulfates, both assumed to be waste products but from different sources, remained nearly constant. During the pupal stage the ratios were practically alike for all determinations. The next question is, which of the two groups of substances, carbonaceous or nitrogenous, were utilized at a faster rate? Figure 4 showing graphically the ratios between

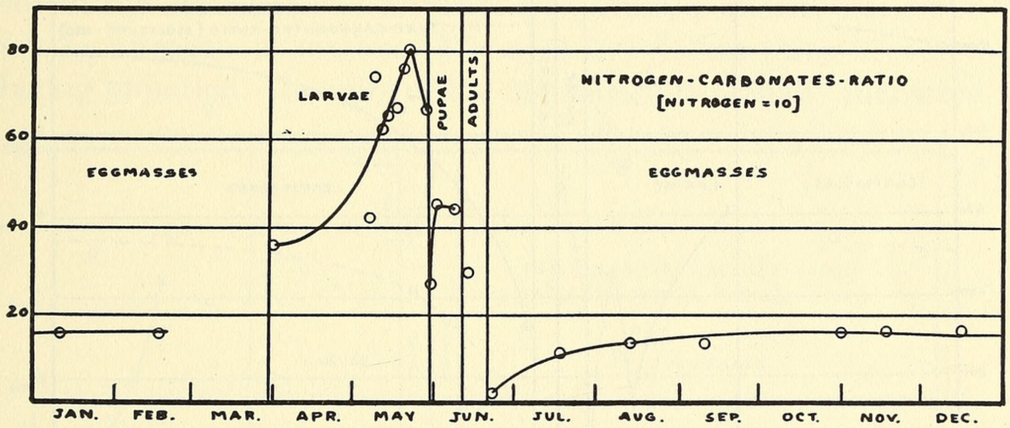

FIG. 4. Changes in the nitrogen-carbonates ratios of tent caterpillars.

carbonates and nitrogen is meant to indicate the answer. If the rate of accumulation of nitrogen had been the same as the rate of carbonates formation, a straight line could have been drawn between the calculated points. If, however, the curve of carbonates rises (when nitrogen is kept constant) the rate of their production must be greater or the rate of decomposition of carbonaceous material is greater than that of nitrogenous matter. It can be expected that results plotted for insects feeding on leaves will show an upward curve for carbonates in relation to nitrogen. During the formation of the larvæ and in the pupal stage this might be less evident if we left out of consideration fatty substances and glycogen. It seems clear however, that during three critical stages of development nitrogenous substances are of special importance, namely (1) when new larvæ are formed, (2) when the insect gets ready for pupation and (3) again when the pupæ are changing to the adult stage.

The relation between moisture content and sulfate formation is graphically shown in figure 5. It is evident that during the first part of the life cycle of the insect (while the larvæ were confined in the egg cases) sulfate formation had a direct relation to the moisture content of the egg masses. When the larvæ were being formed sulfates increased rapidly and the moisture content went down, but the rate of sulfate increase was greater than the rate of moisture decrease. After the larvæ were formed the relation between moisture content and sulfates remained fairly

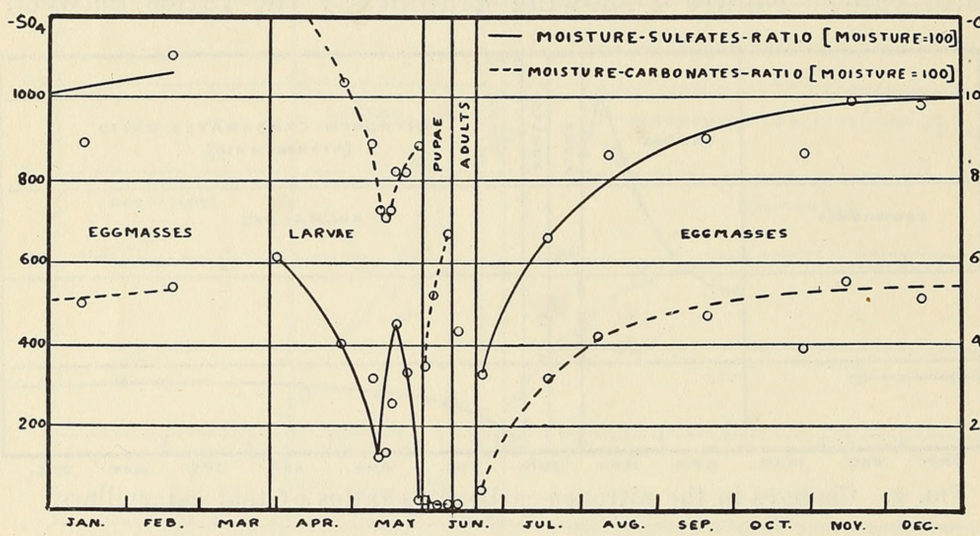

FIG. 5. Relation between moisture and sulfates and carbonates during the life cycle of tent caterpillars.

constant. As soon as the larvæ started feeding the moisture content increased more rapidly than the sulfate content but by the time the larvæ were one-third full grown this relation was reversed. Sulfates increased more rapidly than the moisture content until maturity was reached. As soon as the larvæ were full grown the relation between moisture content and sulfates remained practically constant until the prepupal stage, but changed again from the prepupal to the pupal stage. It remained thereafter constant until the adults emerged.

The relation between carbonates and moisture at the time when the larvæ were formed and remained in the egg cases is quite similar to the relation between moisture and sulfates during this period. When the larvæ hatched the carbonates were very high in relation to their moisture content, but this decreased rapidly until the insects were one-third full grown, similar to the relation between moisture and sulfates. This similarity indicates clearly to my mind that practically all the food intake was utilized for the building of new cells and preservation of life and not for storage, especially in view of the fact that fats did not start to accumulate appreciably until the low carbonate level was reached. The most interesting fact recorded during this part of the life cycle is that, when the larvæ were about full grown the sulfate content dropped in relation to the moisture content, whereas the carbonate content kept on increasing, or in other words, the carbonaceous material was utilized at this stage and not the nitrogenous matter. The next interesting thing occurred during pupation. In spite of the fact that fats actually increased

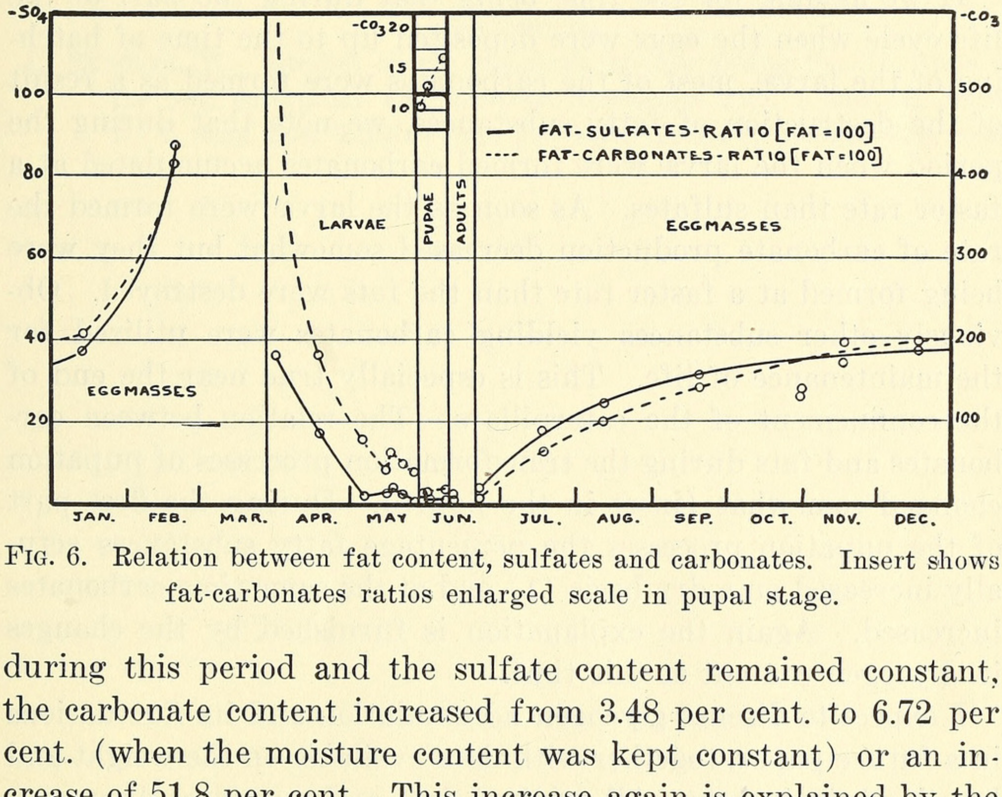

FIG. 6. Relation between fat content, sulfates and carbonates. Insert shows fat-carbonates ratios enlarged scale in pupal stage.

during this period and the sulfate content remained constant, the carbonate content increased from 3.48 per cent. to 6.72 per cent. (when the moisture content was kept constant) or an increase of 51.8 per cent. This increase again is explained by the decrease in glycogen content during this stage.

The relation between sulfates and carbonates and fats during the different stages is plotted in figure 6. At the time the larvæ were formed in the egg cases the relation between fats and sulfates changed rapidly; the sulfates accumulated at a faster rate than the fatty substances disappeared in spite of the fact that fats decreased from 4.45 to 1.71 per cent. during the first twenty-six days. From the middle of August to the middle of January the relation between fats and sulfates remained fairly constant, but during the last months before hatching of the caterpillars sulfates increased very rapidly again, indicating that during this time, when the percentage fatty substances was low, nitrogenous materials were utilized for maintenance of the life processes. During the whole period when the caterpillars were feeding actively the rate of sulfate production was approximately the same as the rate of fat accumulation, while during the pupal stage the rate of fat increase was greater than the rate of sulfate accumulation.

If we assume for the time being that during the part of the life cycle when the eggs were deposited up to the time of hatching of the larvæ, most of the carbonates were formed as a result of the destruction of fatty substances, we note that during the period when the larvæ were formed carbonates accumulated at a faster rate than sulfates. As soon as the larvæ were formed the rate of carbonate production decreased somewhat but they were being formed at a faster rate than the fats were destroyed. Obviously other substances yielding carbonates were utilized for the maintenance of life. This is especially true near the end of the confinement of the caterpillars. The relation between carbonates and fats during the transformation processes of pupation changed somewhat (inset in the figure). During the first part of the pupation processes the percentage fatty substances actually increased on a dry basis (1) and at the same time carbonates increased. Again the explanation is furnished by the changes in glycogen content at this time.

I expect to discuss glycogen accumulation and its fluctuations in a future paper, together with data available on the weight and length of the insects. All original data and a general discussion and summary of the studies will be published.

SUMMARY

Data obtained by chemical analyses on the accumulation and fluctuation of sulfates and carbonates, which are considered a part of the end products of the life processes of the apple tent

caterpillar, are presented graphically and discussed. The relation between sulfates and carbonates during the different stages of development and the fluctuations occurring are compared with the moisture, fat and nitrogen content of the insect.

Nitrogen appears to play an important rôle at three critical stages, namely (1) when the larvæ are formed, (2) when the insect gets ready for pupation and (3) when the pupæ change to the adult stages. Fatty substances are used and stored for energy and do not appear to be of special importance at any critical stage for tissue building. It appears that the moisture content of the insects during the different stages of development is directly related to the rate of their activities.

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