LIII. METABOLISM IN SCURVY.

I. THE LACTIC ACID EXCRETION OF SCORBUTIC GUINEA-PIGS.

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As a result of a great number of investigations Bickel [1924, 1, 2; 1925, 1, 2] advanced the suggestion that "avitaminosis" in animals was associated with an "inner hunger." They arrived at this conclusion from the following observations. Animals (mostly dogs) subsisting on a diet free from vitamins gradually lost weight in spite of a liberal consumption of food, manifesting at the same time a decreased oxygen consumption. On the other hand, the C/N ratio of the urine increased. This was attributed to the presence of "dysoxydabler Kohlenstoff" originating from the partially oxidised carbohydrates which normally would have been metabolised to carbon dioxide. In consequence the problem arose of establishing the chemical identity of this "dysoxydabler Kohlenstoff" and reasoning from Neuberg's [1924] scheme of alcoholic fermentation it was considered that lactic acid formed by internal dismutation from methylglyoxal might be one of the probable constituents. With this hypothesis in view Rosenwald [1926] working in Bickel's laboratory investigated the lactic acid content of the urine of dogs on diets free from vitamins.

Rosenwald employed three animals, the first two received a diet of rice, wheat protein, lard and a salt mixture, whilst the third animal was given orange juice instead of lard. In the first two experiments there was about a fourfold to sixfold increase in the percentage and in the total output of the lactic acid in the urine after the animals had been receiving the deficient diet for 2-3 weeks. This increased excretion of lactic acid was not observed in the case of the third animal after 7 days, although the level of the lactic acid in this case was higher. The administration of insulin during the period of high lactic acid excretion brought about an *immediate* decrease in the C/N ratio of the urine, followed a day or two later by decrease in the lactic acid output. The author concludes that the disturbance in the C/N ratio of the urine and the abnormal excretion of lactic acid are due to a vitamin deficiency and that since

the animal receiving the orange juice showed no such deviation (*after 7 days*) it cannot be a vitamin A deficiency. She further expressed the opinion that it is most probably due to a vitamin B deficiency. The immediate diminution of the C/N ratio followed by decreased excretion of lactic acid after the administration of insulin is due, according to her, to the fact that lactic acid forms only one of the constituents of the "dysoxydabler Kohlenstoff."

It is evident that the above experiments are open to criticism in many respects. Concerning the diets, it would be more correct to state that the animals were suffering from malnutrition of a multiple character rather than from "avitaminosis," as their diets were imperfect, apart from the lack of vitamins. Whatever may have been the cause of the increased excretion of lactic acid in Rosenwald's experiments there was no justification for the conclusions drawn from her results.

Similar criticism may be levelled at most of the work done by Prof. Bickel and his school in this domain. To consider manifestations observed in animals existing on diets free from all the vitamins, even though perfect in other respects, as being due to a simple deficiency designated vaguely as "avitaminosis," is obviously wrong and consequently their experiments, apart from other imperfections, fail to lend support to the elegant hypotheses they advance.

In the course of an investigation on the metabolism in scurvy we had the opportunity of studying the lactic acid in the urines of normal and scorbutic guinea-pigs as well as of guinea-pigs suffering from general malnutrition. We were unable to observe any significant change in the percentage of lactic acid in the urine of the animals under the various dietetic conditions and in view of Rosenwald's observations on dogs we think it of interest to put our experiments on record.

EXPERIMENTAL.

The guinea-pigs were kept in modified Hopkins metabolism cages, which permitted the quantitative collection of the urine and faeces. In the scurvy experiments the animals received a mixture of oats and bran *ad lib.* and 40 cc. of autoclaved milk *per diem* and when necessary decitrated lemon juice as an antiscorbutic. A quantitative record was kept of the oats and bran consumed by the animals. The lactic acid was estimated daily in the urine by the Clausen method [1922]. After the preparatory ether extraction, the urine was brought up to 50 % sulphuric acid concentration, distilled at 145° in a metal-bath and the aldehyde estimated in the usual way. Although the quantities of lactic acid yielded in the urines were in most cases just on the border line of the limits of accuracy of the method, we have satisfied ourselves that any increase in the lactic acid concentration would have been registered. Thus the addition of lactic acid in the form of pure zinc lactate in quantities varying from 0.05–0.1 mg. to quantities of urine used in the estimation (2 cc.) produced a corresponding increase with an error of 8–10 % in the results. Hopkins's

thiophen reaction was obtained from ethereal extracts of 2 cc. of urine. The characteristic colour was, however, vitiated by the production of a brown coloration in the heating. An attempt to isolate lactic acid as zinc lactate from the urine of guinea-pigs failed when quantities of 100 cc. were employed. This was not pursued, in view of the negative character of the investigation. The urines gave no reaction for acetone bodies.

The lactic acid excretion of growing scorbutic guinea-pigs.

Six animals were utilised in this section of the investigation and the results are represented graphically in Fig. 1.

Guinea-pig No. 1 was on a scorbutic diet of oats and bran and autoclaved milk from the commencement of the experiment and, as is usual on this diet, it succumbed to scurvy after 25 days. It will be seen that the total daily outputs of urine and of lactic acid ran parallel. On the 20th day of the experiment, while the guinea-pig was declining in weight, there was a marked increase in the excretion of urine and of lactic acid which persisted until death. This phenomenon was observed on several other occasions and it will be shown that it is not due to scurvy. Polyuria in scorbutic animals has been previously observed by Lopez-Lomba and Randoin [1923].

Guinea-pig No. 2. The history and behaviour of this animal are similar to those of Guinea-pig No. 1 except that the polyuria in the premortal phase was not so marked. This animal received water *ad lib.* instead of milk.

Guinea-pig No. 3. This animal received, during the first 12 days, the basal diet supplemented by a daily dose of 5 cc. of decitrated lemon juice and after this time the scorbutic diet alone. Unfortunately the guinea-pig succumbed to an intestinal infection after having subsisted on the basal diet for 14 days only.

Guinea-pig No. 4 was treated as the preceding animal. In this one, however, death was due entirely to scurvy. The urine and lactic acid excretion during the scorbutic period was similar to that observed in Guinea-pigs Nos. 1 and 2 and showed no deviation from that in the prescorbutic period. It will be noticed that the animal did not grow during the time it received the anti-scorbutic. This was most probably due to the unaccustomed conditions of existence.

Guinea-pig No. 5 exhibited polyuria with the concomitant increase of lactic acid excretion to a marked degree in the premortal phase. It is to be noted that death was here due to an intercurrent disease, the animal suffering at the time of death from incipient scurvy.

Guinea-pig No. 6 was investigated during a prescorbutic period of 8 days, followed by a scorbutic period of 19 days, after which time the administration of decitrated lemon juice was resumed. The animal, however, never recuperated properly and had to be chloroformed 15 days later. In this case, as in the others, no evidence of an increased lactic acid excretion due to scurvy could be obtained.

In all the figures the points in the weight curves of the animals indicate the daily weights. The time scale of the other data in the diagrams is the same.

The lactic acid excretion of adult scorbutic guinea-pigs.

Three guinea-pigs (Nos. 7, 11, 12) were used in these experiments. The animals succumbed rather earlier than is usual with guinea-pigs of this weight, but in all cases scurvy was established at the post-mortem examination. The diet and management were the same as in the case of the younger guinea-pigs, except that 10 cc. instead of 5 cc. of decitrated lemon juice were administered daily during the prescorbutic period, and that this was replaced by 10 cc. of tap water during the scorbutic period in order to keep the quantity of liquid consumed as nearly as possible the same. With the exception of 4 days preceding death in the case of Guinea-pig No. 7, and 2 days in that of Guinea-pig No. 12, 40 cc. of autoclaved milk were consumed daily quantitatively. Fig. 2 gives the results obtained with these animals. The tendency to develop polyuria and a parallel increased excretion of lactic acid during the period of decline preceding death is also evident here.

The lactic acid excretion of growing guinea-pigs on a quantitatively restricted diet containing the antiscorbutic factor.

In view of the above results it remained to ascertain whether the tendency to increased excretion of urine during the decline in weight preceding death was characteristic of scurvy. Guinea-pigs were, therefore, placed on a diet of bran, water and decitrated lemon juice. The animals were weighed every day and the quantity of bran offered was such as to ensure a gradual decline in weight until the animal had lost rather less than a third of its original weight. 40 cc. of water and 5 cc. of decitrated lemon juice were administered daily with a pipette so that the volume of liquid consumed was kept constant. All the animals were observed during a preliminary period on a diet of oats, bran, 5 cc. decitrated lemon juice and 40 cc. of autoclaved milk.

The results are represented graphically in Fig. 3. It will be seen from the charts of Guinea-pigs Nos. 15 and 22 that almost simultaneously with the loss in body weight there appeared an increased excretion of urine. Guinea-pigs Nos. 16 and 24, after a period of 10 days on the restricted diet, were given again the mixed diet of oats, bran, cabbage and autoclaved milk. As these animals began to grow the volume of the daily urine also decreased and eventually reached the level observed in the preliminary period on the mixed diet. This is more marked with Guinea-pig No. 24. It is to be also noted that the lactic acid output of the animals Nos. 15 and 16, as in the case of the scorbutic guinea-pigs, ran parallel to that of the urine. In view of this observation the lactic acid excretion of the remaining animals of this set was not investigated further. No significant traces of protein were observed in the urine on addition of trichloroacetic acid during the polyuria in these or in the scorbutic guinea-pigs.

Fig. 2.

SUMMARY.

The concentration of lactic acid (about 0.01 %), as established by Clausen's method in the urine of adult and young scorbutic guinea-pigs, is fairly constant. The decline in weight of the animals in the premortal phase is usually associated with polyuria. There is no increase, however, in the concentration of lactic acid in this condition nor is the polyuria associated with albuminuria. A similar increase in the volume of the urine takes place in guinea-pigs declining in weight on a restricted diet containing a sufficiency of the anti-scorbutic factor. It is, therefore, not specific to scurvy in guinea-pigs.

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