

## CARBOHYDRATE AND LIPID METABOLISM IN SCURVY : EFFECT OF VITAMIN C SUPPLEMENT\*.

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IN a series of publications Banerjee and his co-workers have shown that carbohydrate and lipid metabolism are disturbed in scurvy. Lowered glucose tolerance was observed in scorbutic guinea-pigs (Banerjee and Ghosh, 1947) and in scorbutic monkeys (Sarkar and Banerjee, 1957). A significant decrease in glucokinase activity was present in the tissues of scorbutic guinea-pigs (Banerjee and Ghosh, 1961). Banerjee *et al.* (1958) showed that tissues from scorbutic guinea-pigs had a much higher contents of citric, lactic and malic acids than normal paired fed controls, suggesting a faulty operation of tricarboxylic acid cycle in scurvy. Urinary excretions of citric and malic acids were increased in guinea-pigs when they developed scurvy and these excretions were further enhanced when the animals were fed butyrate (Banerjee and Kawishwar, 1959) or acetate (Banerjee and Ghosh, 1960). In addition to these disturbances in carbohydrate metabolism, both at the level of glycolytic as well as tricarboxylic acid cycle pathways, a few observations were noted suggestive of faulty lipid metabolism in scorbutic guinea-pigs. Banerjee and Singh (1958) showed that total body cholesterol was greatly increased in scorbutic guinea-pigs. Banerjee and Ghosh (1960) fed acetate to scorbutic guinea-pigs and observed lowered total body lipid and increased total body cholesterol. Banerjee and Kawishwar (1961) fed sodium butyrate to scorbutic guinea-pigs and obtained similar results. These observations indicated a preferential channeling of the acetate pool of the body towards cholesterol synthesis in vitamin C-depleted animals. The defects in the carbohydrate and lipid metabolism observed in scorbutic guinea-pigs were due to diminished production of insulin in the scorbutic condition. Scorbutic guinea-pigs had diminished insulin in the pancreas (Banerjee and Ghosh, 1947). If the guinea-pigs were given small doses of injection of insulin during the progress of scurvy, the disturbance in carbohydrate and lipid metabolism could be brought to normal (Banerjee and Biswas, 1959 ; Banerjee and Singh, 1960 ; Banerjee and Kawishwar, 1961). The present report deals with the studies on the effect of vitamin C supplementation to scorbutic guinea-pigs on the metabolism of carbohydrate and lipids. It has been observed that the defects in carbohydrate and lipid metabolism observed in scurvy could be corrected by vitamin C supplement.

### EXPERIMENTAL.

Male guinea-pigs, weighing between 250 g. and 300 g., were fed a scorbuto-genic diet (Banerjee, 1945). They were also fed 5 mg. ascorbic acid every day and

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two drops of a concentrate of vitamin A and D twice a week. The animals which grew well were selected for the study. Animals were placed in separate metabolism cages and 24-hour urine was collected under toluene for 3 consecutive days for the estimation of citric and lactic acids by the methods described previously (Banerjee *et al.*, *loc. cit.*). After the completion of the study, oral glucose tolerance was performed by feeding glucose, 2 g./kg. body-weight, after an overnight fast. Glucose was estimated by the method of Hagedorn and Jensen as described by Hawk *et al.* (1947). Ascorbic-acid supplement was then withdrawn and the above experiments were repeated when the animals developed scurvy, four weeks after the withdrawal of ascorbic acid supplement. Each of the animal was then fed 10 mg. ascorbic daily. Most of them recovered from scurvy and four weeks after the ascorbic-acid supplement the above experiments were repeated. The animals were then killed by decapitation after an overnight fast. About 100 mg. each of liver, kidney, brain, small intestine and testes were removed, spread on previously weighed filter papers and weighed again, dried at 60°C. for 10 hours and extracted in a continuous extractor with petroleum ether. The whole of the skin was carefully removed, weighed, cut into small pieces and dried at 60°C. for 20 hours. The rest of the body was minced, weighed and similarly dried. The dried body and the skin were powdered and extracted with petroleum ether for 24 hours in a Soxhlet extractor. The extracts were made up to a definite volume and a portion dried to a constant weight for total lipid. Total cholesterol in another portion of these extracts as well as in the petroleum ether extracts of tissues was estimated by the method of Abell *et al.* (1952). Phospholipids were estimated by the method of Youngburg and Youngburg as described by Hawk *et al.* (*loc. cit.*).

In another set of experiments, guinea-pigs receiving the scorbutogenic diet and ascorbic-acid supplement, 5 mg. per day, were divided into several groups, each containing of one normal, one scorbutic and one insulin treated scorbutic. Normal animals continued to receive 5 mg. ascorbic acid per day and the other two animal groups did not receive the supplement. The animals meant for insulin treatment were injected with insulin daily from the beginning of the second week of the scorbutic regime, in a dose increasing from 0.1 to 0.3 units per 100 g. body-weight. Paired feeding technique was followed as reported earlier (Banerjee and Kawishwar, 1961). On the fourth week, when the animals deprived of ascorbic acid supplement developed scurvy, animals of all the groups were sacrificed, tissues extracted with petroleum ether and cholesterol and phospholipids estimated.

## RESULTS.

*Glucose tolerance.*—There was no change in the fasting blood sugar values in the scorbutic guinea-pigs and in guinea-pigs recovered from scurvy as compared to the values of normal guinea-pigs. Guinea-pigs in the scorbutic state showed lowered glucose tolerance and the tolerance returned to normal when the animals recovered from scurvy.

*Urinary excretions of citric and lactic acids.*—Guinea-pigs in the scorbutic state excreted significantly more citric acid ( $17.74 \pm 2.51$  mg./day) than in the

normal state ( $5.66 \pm 0.56$ ), and the excretion came down to normal when the animals recovered from scurvy ( $5.91 \pm 0.44$ ). The urinary excretions of lactic acid were: Normal— $1.72 \pm 0.36$  mg./day, scorbutic— $2.05 \pm 0.42$  mg./day and recovered guinea-pigs— $2.76 \pm 0.50$  mg./day. The values did not change significantly.

*Tissue cholesterol and phospholipid.*—Of all the tissues studied, total cholesterol content of liver, kidney and testes did not show any change in the different groups of animals. Brain cholesterol was significantly reduced in the scorbutic animal, insulin treatment did not produce any change but the value returned to normal when the animals recovered from scurvy. Cholesterol content of small intestine significantly increased in the scorbutic guinea-pigs and the value came down to normal in the animals treated with insulin and also in the animals recovered from scurvy (Table I).

Phospholipid values varied from tissues to tissues in the scorbutic guinea-pigs. The value in brain was reduced, in kidney was increased and no significant change was seen in liver, testis and small intestine. The animals receiving insulin treatment had normal phospholipid values of tissues with the exception of liver where the value was increased. Phospholipid content of the tissues of guinea-pigs recovered from scurvy was, however, very high (Table I).

Total cholesterol of the whole body without the skin increased in scorbutic guinea-pigs, but there was diminution in the total lipid and phospholipid contents. Animals treated with insulin and animals recovered from scurvy showed normal concentrations of cholesterol, total lipid and phospholipid of the whole body. As compared to normal animals, the skin cholesterol did not change when the animals became scorbutic but the values increased in the animals receiving insulin treatment and significantly diminished in animals which recovered from scurvy. Phospholipid content of skin increased in scorbutic guinea-pigs, became normal when the animals received insulin treatment but considerably increased in the animals recovered from scurvy. Total skin lipid which diminished in scorbutic guinea-pigs, increased in the animals receiving insulin treatment and in the animals which recovered from scurvy (Table II).

#### DISCUSSION.

The diminished utilization of glucose and the increased urinary excretion of citric acid observed in scorbutic guinea-pigs were corrected both by the insulin treatment of the scorbutic animals and administration of ascorbic acid to scorbutic animals so as to cure them of scurvy. These observations are suggestive of normal insulin production by guinea-pigs recovered from scurvy.

The increased cholesterol of intestine and the whole body without the skin of scorbutic animals became normal when the animals recovered from scurvy. Insulin treatment also diminished the cholesterol content. Skin cholesterol did not change in the scorbutic animals. The overall increased synthesis of cholesterol in scurvy was, therefore, possibly due to the body mass and not due to increased synthesis by skin which is generally accepted as one of the principal sites of cholesterol biosynthesis.

**TABLE I.**  
Total cholesterol (mg./100 g. fresh tissue) and phospholipid (expressed as g. lecithin/100 g. fresh tissue) contents of guinea-pig tissues.

Condition of animal.	LIVER :		KIDNEY :		BRAIN :		INTESTINE :		TESTIS :	
	Choles-terol.	Phospho-lipid.	Choles-terol.	Phospho-lipid.	Choles-terol.	Phospho-lipid.	Choles-terol.	Phospho-lipid.	Choles-terol.	Phospho-lipid.
Normal (5) N	254 ± 20.8	0.853 ± 0.121	348 ± 19.9	0.829 ± 0.088	2,343 ± 105	3.437 ± 0.384	300 ± 19.1	0.589 ± 0.021	208 ± 22.8	0.990 ± 0.044
Scorbutic (5) S	253 ± 24.7	1.068 ± 0.147	326 ± 13.6	1.054 ± 0.029	1,236 ± 91	2.448 ± 0.187	528 ± 22.6	0.509 ± 0.034	216 ± 24.2	1.015 ± 0.199
Insulin treated S (6) ITS	272 ± 19.1	1.227 ± 0.089	359 ± 22.3	0.896 ± 0.109	1,361 ± 42	3.399 ± 0.269	313 ± 15.0	0.551 ± 0.035	239 ± 3.3	1.070 ± 0.025
Recovered from S (9) RS	230 ± 13.5	1.293 ± 0.149	364 ± 42.8	1.780 ± 0.456	2,943 ± 497	5.657 ± 0.662	227 ± 22.0	1.065 ± 0.180	277 ± 79.0	1.804 ± 0.321
Significant P values :										
Between N and S		< 0.05		< 0.005		< 0.001		< 0.05		
Between N and ITS		< 0.05		< 0.025		< 0.001		< 0.025		< 0.025
Between N and RS										
Between S and ITS										
Between S and RS										
Between ITS and RS										

Figures in parenthesis indicate the number of animals. Values are mean ± standard error.

**TABLE II.**  
Cholesterol, phospholipid and total lipid contents of whole body and skin of guinea-pigs.

Condition of animal.	TOTAL CHOLESTEROL : (mg./100 gm. TISSUE)		PHOSPHOLIPID AS LECITHIN : (gm./100 g. TISSUE)		TOTAL LIPID : (gm./100 g. TISSUE)	
	Whole body skin.	Skin only.	Whole body skin.	Skin only.	Whole body skin.	Skin only.
N (5)	113 ± 5.5	247 ± 34.4	0.458 ± 0.010	0.098 ± 0.005	4.675 ± 0.434	4.907 ± 0.329
S (5)	272 ± 15.2	246 ± 25.1	0.268 ± 0.054	0.152 ± 0.006	1.530 ± 0.186	1.904 ± 0.403
ITS (6)	102 ± 8.2	388 ± 28.6	0.449 ± 0.036	0.097 ± 0.013	7.973 ± 1.090	7.008 ± 1.462
RS (9)	105 ± 6.0	140 ± 13.0	0.454 ± 0.047	0.512 ± 0.071	9.590 ± 0.130	9.504 ± 0.973
Significant P values :						
Between N and S	< 0.001		< 0.010	< 0.001	< 0.001	< 0.001
Between N and ITS		< 0.010		< 0.001		
Between N and RS		< 0.025		< 0.001		< 0.001
Between S and ITS	< 0.001		< 0.025	< 0.001	< 0.001	< 0.005
Between S and RS	< 0.001		< 0.025	< 0.001	< 0.001	< 0.001
Between ITS and RS						

N=Normal; S=Scorbutic; ITS=Insulin treated scorbutic; RS=Recovered from scurvy.

Figures in parenthesis indicate the number of animals. Values = Mean ± S.E.

Total lipid content of the body and skin diminished in scorbutic guinea-pigs. When the scorbutic guinea-pigs recovered from scurvy these values were found significantly higher than the normal guinea-pigs. The normal animals were paired fed with the scorbutic animal. But the animals which were allowed to recover from scurvy were fed *ad libitum*. This was possibly the reason of increased body lipid in these animals. The diminished body lipids of scorbutic guinea-pigs indicated diminished lipid synthesis in the condition. This was over corrected when the animals recovered from scurvy. The increased tissue phospholipids of animals recovered from scurvy indicated high turnover of tissue lipids as well as active rebuilding of the damaged tissues, since phospholipids are known to play a prominent rôle in lipid transport and in cell structure.

It will be seen from the above studies that insulin treatment of the scorbutic animals as well as ascorbic-acid supplement to the scorbutic guinea-pigs brought back the defects in carbohydrate and lipid metabolism to normal. Increased hexosamine content of different tissues of scorbutic guinea-pigs was, however, not affected by insulin treatment (Banerjee and Ghosh, 1961a). All the metabolic defects in scurvy, therefore, are not due to hypo-insulinism. Ascorbic acid seems to play a direct rôle in guiding some of the metabolic pathways. The parallel effects of administration of insulin and of ascorbic acid in reversing the metabolism of carbohydrates and lipids in scorbutic guinea-pigs is suggestive of normal insulin production in the animals recovered from scurvy. Injections of small doses of insulin during the progress of scurvy, however, could not influence the onset of scurvy in these animals. The overall defects in scurvy, therefore, are not due to hypo-insulinism observed.

#### SUMMARY.

1. Oral glucose tolerance and the urinary excretions of citric acid and lactic acid were studied in normal guinea-pigs, when the animals became scorbutic and also when they recovered from scurvy after the administration of ascorbic acid. The lowered glucose tolerance and the increased urinary excretion of citric acid observed in scorbutic guinea-pigs returned to normal when the animals recovered from scurvy.

2. Total cholesterol, phospholipid and total lipid were determined in the tissues of scorbutic, insulin-treated scorbutic, paired fed normal guinea-pigs and in guinea-pigs recovered from scurvy. Total cholesterol of small intestine and of whole body increased in scorbutic guinea-pigs and the values became similar to those of normal guinea-pigs when the animals recovered from scurvy. The phospholipid contents of different tissues were differently altered in scurvy but these values increased in all the tissues studied in the animals recovered from scurvy. Total body lipids which diminished in scurvy increased to levels higher than those of normal guinea-pigs, in the animals recovered from scurvy.

3. The defects in carbohydrate and lipid metabolism observed in scorbutic guinea-pigs could be reversed either by insulin treatment or by supplementation of ascorbic acid to the scorbutic animals. Injection of insulin, however, did not affect the development of scurvy in the animals.

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