Abstract—Effects of L-ascorbate 2-sulfate (AAS) on lipid metabolism were studied in guinea pigs maintained on diet I with sufficient L-ascorbic acid (AA) supplement or on diet II without AA supplement. AAS (300 mg/kg) inhibited an increase in serum and liver levels of lipids to a greater degree than AA (175 mg/kg), a reference compound, in hyperlipidemic guinea pigs induced by cholesterol feeding with diets I or II. AAS also induced a decrease in serum and liver levels of lipids in guinea pigs which had been previously maintained for 6 weeks on diet II containing 1.0% cholesterol. AA administration significantly increased AA level in various organs of animals maintained on both the diets containing cholesterol. It also rectified the AA level lowered by previous maintenance on diet II containing cholesterol. AAS showed a slight AA replacing effect on the AA level. Both AA and AAS exerted preventive and curative effects on several symptoms due to chronic AA deficiency.

Materials and Methods

Materials: The compounds examined for hypolipidemic effects were sodium L-ascorbate 2-sulfate-3 H2O and sodium L-ascorbate.

Preventive effects of AAS and AA on hyperlipidemia (Experiment I and II): The experimental design is shown in Table 1. Male guinea pigs of Hartley strain, weighing 250 to 300 g were divided into 8 groups of 4 or 5. In experiment I, preventive effects of AAS and AA on hyperlipidemia induced by cholesterol feeding were studied with diet I (RC-4 of Oriental Yeast Co., Tokyo) which was supplemented with 50 mg of AA per 100 g. In experiment II, these effects were examined with diet II (AA-unsupplemented diet of Fuji Mill Co., Shizuoka) which contained 9 mg of AA per 100 g.
TABLE 1. Experimental design of preventive effects of AAS and AA on hyperlipidemia

| Experiment | Period (weeks) | Group   | Dose (mg/kg) | Diet  | Cholesterol in diet (%) | Animal number |
|------------|---------------|---------|--------------|-------|-------------------------|---------------|
| I          | 12            | Control I | ---          | Diet I | 0.5                     | 4             |
|            |               | Control II | ---         | Diet I | 0                       | 5             |
|            |               | AAS      | 300          | Diet I | 0.5                     | 4             |
|            |               | AA       | 175          | Diet I | 0.5                     | 5             |
| II         | 10            | Control I | ---          | Diet II | 0.5                    | 5             |
|            |               | Control II | ---        | Diet I | 0                       | 5             |
|            |               | AAS      | 300          | Diet II | 0.5                    | 4             |
|            |               | AA       | 175          | Diet II | 0.5                    | 5             |

AAS: Sodium L-ascorbate 2-sulfate, AA: Sodium L-ascorbate, Diet I: RC-4 of Oriental Yeast Co., which is supplemented with 50 mg of L-ascorbic acid (AA) per 100 g, Diet II: AA unsupplemented diet of Fuji Mill Co., which contains 9 mg of AA per 100 g.

Each one group from both experiments was administered orally 300 mg of sodium L-ascorbate 2-sulfate-3 H₂O/kg (AAS group) or 175 mg of sodium L-ascorbate/kg (AA group) once a day during the experimental period. The drugs were dissolved in a 20% sucrose solution, and approx. 1 ml of the solutions was given per 500 g of body weight. The remaining groups fed the diet I and diet II with or without cholesterol (Control I and Control II), were given the vehicle only.

Each animal was given 30 g of the respective diet every day. Experimental period was 12 weeks for experiment I and 10 weeks for experiment II.

Curative effects of AAS and AA on hyperlipidemia and latent AA deficiency (Experiment III): Guinea pigs given diet II with 1.0% cholesterol for 6 weeks were separated into 3 groups of 4 or 5. These animals were maintained on diet II without cholesterol for 2 weeks, during which AAS (300 mg/kg, AAS group) or AA (175 mg/kg, AA group) was administered orally once a day. The control group were given the vehicle only.

Analyses of lipids and AA: Analysis of lipids in serum and liver were carried out as described in the previous papers (6, 7). AA in various organs was extracted by homogenization with 1.4N acetic acid in 6% metaphosphoric acid and centrifuging at 3,000 rpm for 15 min. AA in the supernatant was assayed by the method of Roe et al. (8). The results were statistically analyzed using Student's t-test.

RESULTS

Preventive effects of AAS and AA on hyperlipidemia

Experiment I on diet I with a sufficient AA supplement: The growth rates of the 3 groups (AAS-, AA- and control I groups) fed diet I with 0.5% cholesterol, did not differ from that of the control II fed diet I without cholesterol (Table 2). All animals consumed the given diet completely.

The relative weight (g/100 g body weight) of liver and spleen from control I was approx. 2.5 times greater than that from control II. The administration of AAS and AA prevented
the enlargement of liver due to cholesterol feeding by 34 and 20% respectively (Table 3).

By cholesterol feeding, serum levels of cholesterol and triglycerides were increased 9.5 and 1.1 fold respectively, while the contents of cholesterol and triglycerides in liver (mg/g) were 10.7 and 15.8 fold respectively (Table 4). Administration of AAS inhibited increase in the contents of cholesterol and triglycerides in liver by 50 and 47% in the term of mg/g liver and by 63 and 61% in total liver content respectively, while AA administration inhibited only an increase in triglycerides. With administration of AAS and AA, there was a tendency for the increased serum lipid level to decrease but such was not significant.

The content of AA in spleen, adrenal and eye-ball from control I was 18, 13 and 26% lower than that from control II respectively. But the content in liver and testis did not differ between the two groups. Administration of AA increased the level of AA in liver, spleen, adrenal, testis and eye-ball by 40, 42, 82, 36 and 81%, respectively, compared to that from control I. AAS did not alter the levels (Table 5).

Experiment II on diet II without AA supplement: With a 10 week maintenance on diet II with cholesterol, control I showed several symptoms attributed to AA deficiency and included retarded growth, keratinization in limbs, roughness of hair, necrosis in the ear etc.. However, the two groups treated with AAS and AA showed no such symptoms. The average consumption of the diet to the given diet was 97.9% for AAS group, 98.6% for AA group, 95.7% for control I and 99.6% for control II. The growth rate of each group was compared between the experiments with diet I and diet II and results are shown in Table 2. The growth of control I began to show inhibition in week 4 and the body weight started to decrease in week 8. Administration of AAS and AA prevented the decrease of the growth induced by cholesterol feeding in experiment II.

The relative weight of the liver in control I was 2.1 times greater than that from control II. The weight of the spleen, adrenal, kidney and heart also tended to increase and the weight of the testis tended to decrease with cholesterol feeding. AAS and AA prevented an increase in relative weight of the liver due to cholesterol feeding (Table 6).

The serum levels of cholesterol and triglycerides from control I were 6.0 and 1.5 fold higher than those from control II. The contents of cholesterol and triglycerides in the liver (mg/g) from control I was 9.7 and 15.3 times higher than those from control II (Table 7). The serum level of cholesterol, and the contents of cholesterol and triglycerides in the liver (mg/g) were all approx. 60% lower in AAS group than those in control I. In contrast, AA
TABLE 3. Effects of AAS and AA on relative weight of organs in guinea pigs maintained for 12 weeks on diet I with 0.5% cholesterol in experiment I

| Drugs administered | Cholesterol in diet | n  | Body weight | Liver | Spleen | Kidney | Adrenal*2 | Heart | Testis |
|---------------------|---------------------|----|-------------|-------|--------|--------|-----------|-------|--------|
| mg/kg               | %                   | g  |             | g/100 g body weight (mean±S.E.) |
| Control I           | 0.5                 | 4  | 584±30      | 6.94±0.43 | 0.31±0.04 | 0.36±0.01 | 46.3±5.5 | 0.30±0.02 | 0.24±0.01 |
| Control II          | 0                   | 5  | 634±7       | 2.65±0.09** | 0.13±0.01** | 0.31±0.02 | 54.4±5.0 | 0.26±0.02 | 0.26±0.02 |
| AAS 300             | 0.5                 | 4  | 604±18      | 4.60±0.27** | 0.21±0.02 | 0.34±0.02 | 40.0±2.3 | 0.28±0.01 | 0.23±0.01 |
| AA 175              | 0.5                 | 4  | 585±22      | 5.61±0.45* | 0.25±0.06 | 0.35±0.03 | 39.0±6.4 | 0.32±0.01 | 0.24±0.01 |

a): mg/100 g body weight. Significant difference from control I: *P<0.05, **P<0.01.

TABLE 4. Effects of AAS and AA on serum and liver lipid levels of guinea pigs maintained for 12 weeks on diet I with 0.5% cholesterol in experiment I

| Drugs administered | Cholesterol in diet | n  | Serum Cholesterol | Serum Triglycerides | Liver Cholesterol | Liver Triglycerides |
|---------------------|---------------------|----|-------------------|--------------------|------------------|---------------------|
| mg/kg               | %                   | g/dl|                  | (mean±S.E.)        | mg/liver         | mg/liver            |
| Control I           | 0.5                 | 4  | 247±33           | 62.6±5.2          | 46.2±2.9         | 1822±219            | 121.3±8.5 | 4778±549 |
| Control II          | 0                   | 5  | 26±3**           | 56.2±2.5          | 4.3±0.2**        | 72±4**              | 7.7±0.6** | 130±13** |
| AAS 300             | 0.5                 | 4  | 194±31           | 58.4±2.5          | 23.5±2.2**       | 678±95**            | 64.8±8.4** | 1877±307** |
| AA 175              | 0.5                 | 4  | 179±31           | 55.4±5.7          | 37.1±3.6         | 1268±165            | 81.2±10.8* | 2743±333* |

Significant difference from control I: *P<0.05, **P<0.01.
TABLE 5. Effects of AAS and AA on AA level in various organs of guinea pigs maintained for 12 weeks on diet I with 0.5% cholesterol in experiment I

| Drugs administered | Cholesterol in diet | n | Liver | Spleen | Adrenal | Testis | Eye-ball |
|---------------------|---------------------|---|-------|--------|---------|--------|---------|
| mg/kg               | %                   |   | mg of AA/100 g tissue (mean ± S.E.) |
| Control I           | 0.5                 | 4 | 11.8±0.4 | 25.2±3.9 | 48.1±2.2 | 14.5±1.0 | 8.8±0.4 |
| Control II          | 0                   | 5 | 12.4±0.8 | 30.8±1.0* | 55.4±3.6* | 15.4±1.1 | 11.9±0.6** |
| AAS 300             | 0.5                 | 4 | 13.7±1.6 | 23.1±0.9 | 48.9±7.1 | 14.4±1.3 | 11.4±1.0 |
| AA 175              | 0.5                 | 4 | 16.5±1.3* | 35.7±1.8* | 87.4±6.8** | 19.7±1.2* | 15.9±0.7** |

Significant difference from control I: *P<0.05, **P<0.01.

TABLE 6. Effects of AAS and AA on relative weight of organs in guinea pigs maintained for 10 weeks on diet II with 0.5% cholesterol in experiment II

| Drugs administered | Cholesterol in diet | n | Body weight | Relative weight of organs |
|---------------------|---------------------|---|-------------|---------------------------|
| mg/kg               | %                   | g | Liver       | Spleen | Kidney | Adrenal<sup>a</sup> | Heart | Testis |
| Control I           | 0.5                 | 5 | 450±64      | 6.77±0.28 | 0.19±0.03 | 0.46±0.05 | 75.8±12.7 | 0.37±0.07 | 0.19±0.04 |
| Control II          | 0<sup>b</sup>       | 5 | 573±9       | 3.24±0.12** | 0.13±0.01 | 0.35±0.01 | 44.6±3.4  | 0.27±0.01 | 0.25±0.01 |
| AAS 300             | 0.5                 | 4 | 562±66      | 5.10±0.49* | 0.17±0.02 | 0.35±0.03 | 46.0±2.5  | 0.28±0.01 | 0.24±0.01 |
| AA 175              | 0.5                 | 5 | 593±31      | 4.33±0.13** | 0.14±0.01 | 0.30±0.00 | 46.0±3.1  | 0.27±0.01 | 0.24±0.01 |

<sup>a</sup>: mg/100 g body weight, <sup>b</sup>: Diet I. Significant difference from control I: *P<0.05, **P<0.01.
| Drugs administered | Cholesterol in diet | n  | Serum | Liver |
|---------------------|---------------------|----|-------|-------|
|                     | mg/kg  | %    | mg/dl | Cholesterol | Triglycerides | mg/g (mean ± S.E.) | mg/liver | mg/g  |
| Control I           | 0.5    | 5    | 145.9±14.8 | 75.5±5.2 | 33.0±3.3 | 1015±194 | 133.5±22.1 | 4327±1195 |
| Control II          | 0<sup>a</sup> | 5    | 24.4±2.5** | 49.7±2.7* | 3.4±0.1** | 62±3**  | 8.7±0.4** | 160±6** |
| AAS 300             | 0.5    | 4    | 62.2±10.5** | 63.8±4.2 | 13.5±3.5** | 482±159 | 47.5±14.7* | 1720±695 |
| AA 175              | 0.5    | 5    | 129.8±30.0 | 59.7±1.2* | 24.4±5.8  | 643±192 | 82.7±15.9 | 2198±537 |

a): Diet I. Significant difference from control I: *P<0.05, **P<0.01.

---

| Drugs administered | Cholesterol in diet | n  | Liver | Spleen | Adrenal | Kidney | Eye-ball |
|---------------------|---------------------|----|-------|--------|---------|--------|----------|
|                     | mg/kg  | %    | mg of AA/100 g tissue (mean ± S.E.) |
| Control I           | 0.5    | 5    | 7.4±0.4 | 7.9±0.6 | 22.9±1.3 | 1.4±0.2 | 2.9±0.1 |
| Control II          | 0<sup>a</sup> | 5    | 10.7±0.8* | 25.2±1.0<sup>b</sup>** | 35.8±3.3* | 4.5±0.2** | 7.4±0.5** |
| AAS 300             | 0.5    | 4    | 8.6±0.6 | 16.5±3.3* | 26.1±3.6 | 2.4±0.4* | 4.6±0.8 |
| AA 175              | 0.5    | 5    | 14.6±0.7** | 34.7±0.6** | 52.0±3.7** | 9.5±0.6** | 11.7±1.2** |

a): Diet I, b): n=3. Significant difference from control I: *P<0.05, **P<0.01.
### Table 9. Effects of AAS- and AA-treatment on relative weight of organs in latent AA deficient guinea pigs in experiment III

| Drugs administered | n  | Body weight | Relative weight of organs |         |         |         |         |         |         |         |
|--------------------|----|-------------|---------------------------|---------|---------|---------|---------|---------|---------|---------|
|                    |    | g           | Liver                     | Spleen  | Kidney  | Adrenal\(^a\) | Heart   | Testis  |         |         |
| mg/kg              |    |             | g/100 g body weight (mean \± S.E.) |         |         |         |         |         |         |         |
| Control            | 4  | 396 \± 48   | 5.07 \± 0.25              | 0.18 \± 0.02 | 0.46 \± 0.05 | 89.3 \± 21.8 | 0.35 \± 0.03 | 0.19 \± 0.05 |         |         |
| AAS 300            | 4  | 434 \± 46   | 3.90 \± 0.22\(^*\)       | 0.18 \± 0.03 | 0.39 \± 0.04 | 65.8 \± 9.8  | 0.33 \± 0.03 | 0.20 \± 0.03 |         |         |
| AA 175             | 5  | 498 \± 44   | 4.37 \± 0.19              | 0.16 \± 0.01 | 0.36 \± 0.02 | 52.6 \± 6.7  | 0.31 \± 0.02 | 0.22 \± 0.03 |         |         |

\(^a\): mg/100 g body weight. Significant difference from control: \(^*\)P<0.05. Animals were maintained for 6 weeks on diet II with 1.0% cholesterol, and then transferred to diet II without cholesterol for 2 weeks, during which AAS or AA was administered orally once a day.

### Table 10. Effects of AAS- and AA-treatment on serum and liver lipid levels in latent AA deficient guinea pigs in experiment III

| Drugs administered | n  | Serum                  | Liver                  |
|--------------------|----|------------------------|------------------------|
|                    |    | Cholesterol | Triglycerides | Cholesterol | Triglycerides |
| mg/kg              |    | mg/dl       | (mean \± S.E.)        | mg/g/liver  | mg/g        | mg/g/liver |
| Control            | 4  | 133.4 \± 31.8 | 81.3 \± 15.1         | 20.1 \± 7.6 | 330 \± 57   | 71.3 \± 21.0 | 1247 \± 250 |
| AAS 300            | 4  | 63.1 \± 17.5 | 65.3 \± 9.7          | 8.4 \± 2.3  | 127 \± 18   | 34.6 \± 9.2  | 526 \± 62  |
| AA 175             | 5  | 63.4 \± 11.8 | 49.8 \± 2.6          | 14.1 \± 2.5 | 301 \± 58   | 40.3 \± 6.8  | 842 \± 131 |

Experimental details are as described in the legend of Table 9.
decreased significantly the serum level of triglycerides only (Table 7).

The content of AA in liver, spleen, adrenal, kidney and eye-ball from control I was 31, 69, 36, 69 and 61% lower than that from control II respectively. Administration of AA resulted in a remarkable increase of the AA content in liver (197%), spleen (439%), adrenal (227%), kidney (679%) and eye-ball (403%), while administration of AAS increased significantly the level in spleen (209%) and kidney only (171%) (Table 8).

Curative effects of AAS and AA on hyperlipidemia and latent AA deficiency (Experiment III): Administration of AA to animals which had been maintained for 6 weeks on diet II with 1.0% cholesterol resulted in relief of several symptoms, in a few days. However, the appearance of the AAS effect was delayed a few days as compared to AA.

The average consumption of the diet to the given diet during the treatment of 2 weeks with AAS or AA was 86.9% for the control group, 83.3% for AAS group and 95.0% for AA group.

During the induction period of hyperlipidemia and latent AA deficiency, body weight increased at an almost constant rate up to day 38 and then started to decrease. Administration of AA and AAS resulted in recovery of body weight in 5 and 9 days, respectively. The growth rate of the control group did not recover throughout (Fig. 1).

The relative weight of the liver from the AAS group was less than that from the control group, and the weight of the kidney and adrenal also tended to decrease in treated groups.

![Fig. 1. Effects of AAS- and AA-treatment on body weight in latent AA deficient guinea pigs in experiment III.](image)

**TABLE 11. Effects of AAS- and AA-treatment on AA level of various organs in latent AA deficient guinea pigs in experiment III**

| Drugs administered | n | Liver | Spleen | Adrenal | Kidney | Testis |
|--------------------|---|-------|--------|---------|--------|--------|
| mg/kg              |   | mg of AA/100 g tissue (mean±S.E.) | mg of AA/100 g tissue (mean±S.E.) | mg of AA/100 g tissue (mean±S.E.) | mg of AA/100 g tissue (mean±S.E.) | mg of AA/100 g tissue (mean±S.E.) |
| Control 300        | 4 | 6.5±0.3 | 5.0±1.2 | 27.5±6.5 | 3.8±0.2 | 8.0±0.7 |
| AAS 300            | 4 | 8.4±1.4 | 6.4±1.0 | 39.7±4.2 | 4.3±0.4 | 9.2±1.0 |
| AA 175             | 5 | 15.9±0.9** | 17.5±0.6** | 124.1±10.2** | 7.8±0.6** | 22.3±1.6** |

Significant difference from control: **P<0.01. Experimental details are as described in the legend of Table 9.
compared to the control group (Table 9).

AAS and AA administration resulted in a decrease in levels of cholesterol and triglycerides in the serum and liver compared to the control group (Table 10).

AA level in liver, spleen, adrenal, kidney and testis from AA group was 2.5, 3.5, 4.5, 2.1 and 2.8 fold higher than that from the control group, respectively. AAS increased the level in the liver (30%), spleen (28%) and adrenal (45%) respectively, but such was not significant (Table 11).

DISCUSSION

AAS, a naturally occurring substance (1) is reported to be produced from AA in animals with (rat) or without (human and guinea pig) the ability to biosynthesize AA (2–4). The reverse conversion from AAS to AA is also reported in these animals (9).

There are numerous reports concerning the effects of AA on lipid metabolism. AA had been found to exert hypolipidemic effects on hyperlipidemia in humans and experimental animals (10, 11), while there are several reports which deny this (12, 13). In addition, Booker et al. (14) and Spittle (15) reported an increase in serum level of cholesterol by the administration of AA. In animals which biosynthesize AA, exogenous AA may fail to exert such effects (16).

It is known that acute or chronic AA deficiency induces an elevation in serum and liver levels of cholesterol (17, 22) probably due to the abnormal metabolism in the biosynthesis, absorption, distribution and catabolism of cholesterol.

The chronic AA deficient model of guinea pigs recently reported by Ginter et al. seems to provide a good tool for the investigation on the effects of AA on lipid metabolism and atherosclerosis (17). Using this model Ginter showed that AA was involved in the catabolic pathway of cholesterol to bile acids. There is also counter-evidence for this finding (18).

In our experiment I using diet I with a sufficient AA supplement (50 mg per 100 g chow) and cholesterol (0.5%), AAS markedly inhibited an increase in the liver levels of cholesterol and triglycerides. AA administration inhibited only an increase in liver level of triglycerides. Both AAS and AA exerted slight effects on serum lipid levels (Table 4). When the guinea pigs were maintained on diet II without AA supplement and with cholesterol (Experiment II), hyperlipidemia and several symptoms attributed to chronic AA deficiency were observed in animals from control I (Tables 2, 6, 7 and 8). Administration of AAS and AA inhibited an accumulation of triglycerides and cholesterol in liver, but such was significant only in the AAS group. At the same time, AAS significantly inhibited an elevation in serum cholesterol level, while AA inhibited an elevation of serum triglyceride level (Table 7). In experiment III, alleviation of the altered lipid metabolism in guinea pigs which had been previously maintained for 6 weeks on diet II with 1.0% cholesterol was observed in 2 weeks by administration of AAS and AA. In this experiment, AAS and AA also tended to decrease the serum and liver levels of lipids (Table 10). Taking all these data in experiments I, II and III under various conditions, AAS at 300 mg/kg does appear to exert greater lipid lowering effects than AA at a 175 mg/kg dose, the molar equivalent to 300 mg/kg of AAS.
In an attempt to elucidate whether the lipid lowering effects of AAS are mediated by conversion to AA, the level of AA in various organs was compared in the experimental groups. This level was significantly higher in the AA group than in control I (Tables 5, 8 and 11). Administration of AAS, however, resulted in only a slight increase in the level of AA with the exception of spleen and kidney in experiment II where the AA level was significantly increased in these organs (Table 7). These results suggest that the hypolipidemic effects of AAS are independent of the conversion of AAS to AA. In favor of this assumption, there are reports that AAS functions as a sulfate donor to cholesterol (5).

Guinea pigs fed a cholesterol diet consume a larger amount of AA, resulting in a decrease in AA level of organs (14, 19). In our experiments II and III, several symptoms of chronic AA deficiency such as body weight loss, roughness of hair, keratinization in limbs, necrosis of the ear, changes in relative organ weight were observed in guinea pigs fed diet II with 0.5 or 1.0% cholesterol. Administration of AA and AAS alleviated the symptoms. The level of AA in the organs from control I was markedly lower than that in control II fed diet I without cholesterol. Administration of AA and AAS inhibited the decrease (Table 8). Such a replacing effect of AAS for AA in chronic AA deficiency was also observed in experiment III (Table 11), suggesting that AAS is converted to AA in a considerable amount in chronic AA deficient guinea pigs. There are opposing reports concerning replacing effects of AAS for AA in AA deficiency (9, 20, 21). Tolbert et al. report that AAS in a limited dose such as 3 mg/kg effectively replaces AA in acute AA deficiency of rainbow trout and coho salmon, but not in that of guinea pigs, in which AAS is poorly transported through membranes, quickly excreted into urine, and less and slowly converted to AA (9). Our results, however, indicate that a large dose of AAS orally administered possesses a replacing effect as well as hypolipidemic effects, and relieves various symptoms of chronic AA deficiency in guinea pigs (Tables 8, 10 and Fig. 1).

REFERENCES

1) MEAD, C.G. AND FINAMORE, F.J.: The occurrence of ascorbic acid sulfate in the brine shrimp, Artemia salina. Biochemistry 8, 2652–2655 (1969)
2) BAKER, E.M., HAMMER, D.C., MARCH, S.C., TOLBERT, B.M. AND CANHAM, J.E.: Ascorbate sulfate: A urinary metabolite of ascorbic acid in man. Science 173, 826–827 (1971)
3) MUMMA, R.O. AND VERLANGIERI, A.J.: Isolation of ascorbic acid 2-sulfate from selected rat organs. Biochim. Biophys. Acta 273, 249–253 (1972)
4) HORNIG, D., GALLO-TORRES, H.E. AND WEISER, H.: A biliary metabolite of ascorbic acid in normal and hypophysectomized rats. Biochim. Biophys. Acta 320, 549–556 (1973)
5) VERLANGIERI, A.J. AND MUMMA, R.O.: In vivo sulfation of cholesterol by ascorbic acid 2-sulfate. Atherosclerosis 17, 37–48 (1973)
6) HAYASHI, E., YAMADA, J., KUNITOMO, M., TERADA, M., TOMITA, T. AND KINOSHITA, T.: Fundamental studies on physiological and pharmacological actions of L-ascorbate 2-sulfate. I. On the hypolipidemic effects. J. nutr. Sci. Vitaminol. 22, 201–208 (1976)
7) HAYASHI, E., YAMADA, J., KUNITOMO, M., TERADA, M. AND SATO, M.: Fundamental studies on physiological and pharmacological actions of L-ascorbate 2-sulfate. V. On the hypolipidemic and antiatherosclerotic effects of L-ascorbate 2-sulfate in rabbits. Japan. J. Pharmacol. 28, 61–72 (1978)
8) ROE, J., MILLIS, M.B., OESTERING, J. AND DAMRON, C.M.: The determination of diketo-l-gulonic acid, dehydro-l-ascorbic acid, and l-ascorbic acid in the same tissue extract by
the 2,4-dinitrophenylhydrazine method. *J. biol. Chem.* 174, 201-208 (1948)

9) Tolbert, B.M., Downing, M., Carlson, R.W., Knight, M.K. and Baker, E.M.: Chemistry and metabolism of ascorbic acid and ascorbate sulfate. *Ann. N. Y. Acad. Sci.*, Vitamin C Conference, October (1974)

10) Ginter, E., Kajaba, I. and Nizner, D.: The effect of ascorbic acid on cholesterolemia in healthy subjects with seasonal deficit of Vitamin C. *Nutr. Metab.* 12, 76-86 (1970)

11) Sokoloff, B., Hori, M., Saelhof, C., McConnell, B. and Imai, T.: Effect of ascorbic acid on certain blood fat metabolism factors in animals and man. *J. Nutr.* 91, 107-118 (1967)

12) Pool, W.R., Newmark, H.L., Dalton, C., Banziger, R.F. and Howard, A.N.: Effect of biotin and ascorbic acid on the development of atherosclerosis in rabbits. *Atherosclerosis* 14, 131-135 (1971)

13) Samuel, P. and Shalchi, O.B.: Effect of Vitamin C on serum cholesterol in patients with hypercholesterolemia and atherosclerosis. *Circulation* 29, 24-25 (1964)

14) Booker, W.M., Dacosta, F., Jones, W., Froix, C. and Robinson, E.: Cholesterol-ascorbic acid relationship; Changes in plasma and cell ascorbic acid and plasma cholesterol following administration of ascorbic acid and cholesterol. *Am. J. Physiol.* 189, 75-77 (1957)

15) Spittle, C.R.: Atherosclerosis and Vitamin C. *Lancet* II, 1280-1281 (1971)

16) Ginter, E., Babala, J. and Polonyova, E.: Vitamin C und Metabolism der Lipoiden bei mit atherogener Diät gefütterten Kaninchen. *Biologica, Bratislava* 25, 579-586 (1970)

17) Ginter, E.: The role of Vitamin C in cholesterol catabolism and atherogenesis. *Biologické Práce* 21, 1-100 (1975)

18) Kritchevski, D., Tepper, S.A. and Story, J.A.: Influence of Vitamin C on hydroxylation and side chain oxidation of cholesterol *in vitro*. *Lipids* 8, 482-484 (1973)

19) Ginter, E., Babala, J. and Červek, J.: The effect of chronic hypovitaminosis C on the metabolism of cholesterol and atherogenesis in guinea pigs. *J. Atheroscler. Res.* 10, 341-352 (1969)

20) Mumma, R.O., McKee, E., Verlangieri, A.J. and Barron, G.: Antiscorbutic effect of ascorbic acid 2-sulfate in the guinea pig. *Nutr. Repts. Inter.* 6, 133-137 (1972)

21) Kuenzig, W., Avenia, R. and Kamm, J.J.: Studies on the antiscorbutic activity of ascorbate 2-sulfate in the guinea pig. *J. Nutr.* 104, 952-956 (1974)

22) Turley, S.D., West, C.E. and Horton, B.J.: The role of ascorbic acid in the regulation of cholesterol metabolism and in the pathogenesis of atherosclerosis. *Atherosclerosis* 24, 1-18 (1976)