XXII. THE ANTISCORBUTIC FACTOR IN LEMON JUICE.

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In a previous communication [1918] we have described an attempt to isolate the antiscorbutic principle by means of adsorption. The attempt proved unsuccessful but we were able to make a series of observations in connection with the behaviour of the antiscorbutic factor in orange juice towards some adsorbents under certain conditions. We have since attempted to study the chemical nature of the factor in question adopting another method of investigation, namely that of fractionating an antiscorbutically active juice. We have chosen lemon juice for that purpose. As is well known the main bulk of the solids of lemon juice consists of citric acid. In the event of the antiscorbutic being associated with the citric acid, good yields of the acid could be obtained from the juice for further investigation. If on the other hand by removing the citric acid the solid residue remained active, the antiscorbutic would then be associated with a comparatively much smaller mass of extraneous matter offering a better opportunity of studying both its chemical and physiological properties. The latter has proved to be the case and we now describe in this communication a few experiments from an investigation in progress on the above lines.

Experimental.

The following procedure was adopted in order to remove the free citric acid from lemon juice. To freshly squeezed out lemon juice an excess of precipitated calcium carbonate was added with constant stirring. The resulting suspension was then treated with two volumes of absolute alcohol and filtered through a fluted filter. The residue remaining on the filter was then pressed out in a hand press and the liquid thus obtained filtered. Both filtrates, which should be clear, were combined and evaporated in vacuo at 35° C. until all

the alcohol had been removed and were then made up with distilled water to the original volume of the lemon juice used. The solution thus obtained is very slightly acid to litmus, yellow in colour, sweetish to taste and retains the characteristic lemon flavour as well as the bitter taste of the lemon. On evaporating the solution to dryness and heating the residue at 110° for an hour the equivalent of 1 cc. of the original lemon juice yields a residue weighing about 15 mgms. The residue after heating becomes caramelised and syrupy and tastes very sweet. It reduces Fehling's solution freely. On incinerating the residue about 3 mgms. of inorganic constituent per cc. of original lemon juice is obtained. The inorganic residue resulting from 10 cc. lemon juice treated as above when dissolved in a known quantity of decinormal H_2SO_4 and titrated back required 2 cc. of $N/10$ H_2SO_4 to neutralise the carbonates produced by the incineration of the small quantities of the salts of organic acids present and this when calculated on the basis of sodium citrate corresponds to 1·7 mgms. per cc. When the decitrated juice was treated with $CaCl_2$ and two volumes of absolute alcohol a scanty precipitate of a few mgms. was obtained from 10 cc. of the solution and this consisted of the calcium salt of some organic acid or acids, as it charred and smelled of burning sugar when heated. A sample of lemon juice evaporated to dryness and heated at 110° for one hour gave a total residue of 105 mgms. per cc., 4 mgms. of which was inorganic.

Experiments were instituted to test the therapeutic and other properties of the treated lemon juice.

Expt. 1. The purpose of this experiment was to test the antiscorbutic potency of the treated lemon juice. Quantities equivalent to 2 cc., 3 cc., 5 cc. and 7 cc. were tried respectively on four guinea pigs. The animals received oats and bran *ad lib.* and 50 cc. of autoclaved full milk per diem. The respective doses of the treated lemon juice were administered daily by hand. The animals were allowed to go on for about eighty days when they were all chloroformed. It will be seen from fig. 1 which represents the weight curves of the animals, that on 2 cc. guinea pig No. 71 just managed to maintain its weight. Although it was unable to grow on that dose it failed nevertheless to succumb to scurvy. The other animals in the experiment actually succeeded in putting on weight. At the post mortem examination guinea pig No. 72, which received a dose of 3 cc. showed slight haemorrhages in the femoral muscles and also slightly enlarged costochondral junctions. The other animals (Nos. 54, 55 and 71) showed no signs of scurvy at all. This experiment shows without any doubt that after removing the free citric acid the residual fraction of lemon juice retains some antiscorbutic activity.

Expt. 2. Our next aim was to ascertain approximately what proportion of the antiscorbutic content of lemon juice remains in the residue after the removal of the acids. It has previously been pointed out by us in another communication [1918] that with our present technique it is impossible to carry out quantitative determinations of the antiscorbutic content of a solution with any great precision. It is nevertheless possible to ascertain roughly the relative antiscorbutic potency of two solutions by administering equal quantities of the solutions to guinea pigs of approximately the same weight kept on a scorbutic diet and watching the clinical developments. We have adopted this procedure in this experiment. Doses of 0·5 cc., 1 cc. and 1·5 cc.

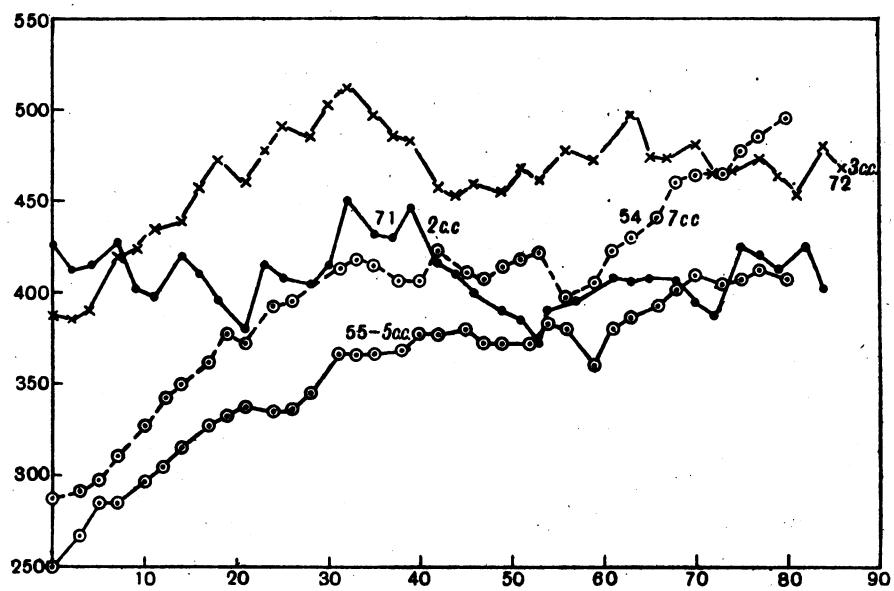


Fig. 1

each of treated and untreated lemon juice respectively were given to six guinea pigs. Animals Nos. 263, 267 and 268 received 0·5 cc., 1 cc. and 1·5 cc. of untreated lemon juice respectively, while Nos. 266, 264 and 265 received 0·5 cc., 1 cc. and 1·5 cc. of treated lemon juice. Fig. 2 represents their weight curves. Guinea pigs Nos. 263 and 266 receiving 0·5 cc. of untreated and treated lemon juice respectively died of scurvy within about a month, showing that 0·5 cc. either of treated or untreated lemon juice is insufficient to prevent guinea pigs from succumbing to scurvy. No marked differential clinical features could be observed in these animals. The behaviour of Nos. 264 and 267 which received a dose of 1 cc. was also very similar. They were both chloroformed

after forty-two days and at the post mortem they both disclosed signs of scurvy. The weight curve of No. 267 which received the untreated lemon juice is however a little better than that of No. 264 which was dosed with the treated juice. Guinea pigs Nos. 265 and 268 received 1·5 cc. of treated and untreated lemon juice respectively. Neither of these animals developed scurvy. Unfortunately No. 268 succumbed after thirty-seven days to an intestinal complaint, which is often the case with guinea pigs receiving high doses of lemon juice. Not the slightest trace of scurvy could however be found at the post mortem examination. No. 265 was chloroformed after forty-two days and was found to be in excellent condition. Nothing abnormal was disclosed by the post mortem examination. It is seen then that the three sets of pigs behaved almost identically, showing that the relative antiscorbutic potency of the treated and untreated lemon juice is practically the same, from which one may safely conclude that little if any of the antiscorbutic is associated with the free acids in the lemon juice of the solids of which they form the main portion.

Expt. 3. We endeavoured next to ascertain whether treated lemon juice is antiscorbutically stable for short periods of time. Small portions of treated lemon juice were stored in the cold room in corked flasks for about a fortnight before being tested. Quantities of 3 cc., 5 cc. and 8 cc. were tried on three guinea pigs. As will be seen from fig. 3 all the animals failed to succumb to scurvy within seventy-four days, after which period they were chloroformed. No. 76 which received 8 cc. and No. 74 which received 3 cc. of the treated extract showed very slight signs of scurvy at the post mortem examination. The other guinea pig was quite normal. As the weight curves show, none of the animals grew satisfactorily. The stored juice then seemed to have deteriorated to some extent during the fortnight's cold storage although it retained the best part of its antiscorbutic potency.

Expt. 4. It was of interest to see whether on evaporating the treated lemon juice the residue would retain its antiscorbutic power. We therefore evaporated a litre of the treated lemon juice at a temperature of 30°–40° C. in vacuo until the residue assumed a thick syrupy consistency. The residue was then transferred to an evacuated desiccator where it was kept during the experiment. Three pigs, Nos. 68, 69 and 70, received 100 mgms., 150 mgms. and 200 mgms. per diem of the residue respectively in addition to the scorbutic diet. The doses were dissolved in suitable quantities of distilled water just before administration. All the pigs died of scurvy within 42–50 days. Fig. 4 represents the weight curves of the above animals. It is seen that the doses given have

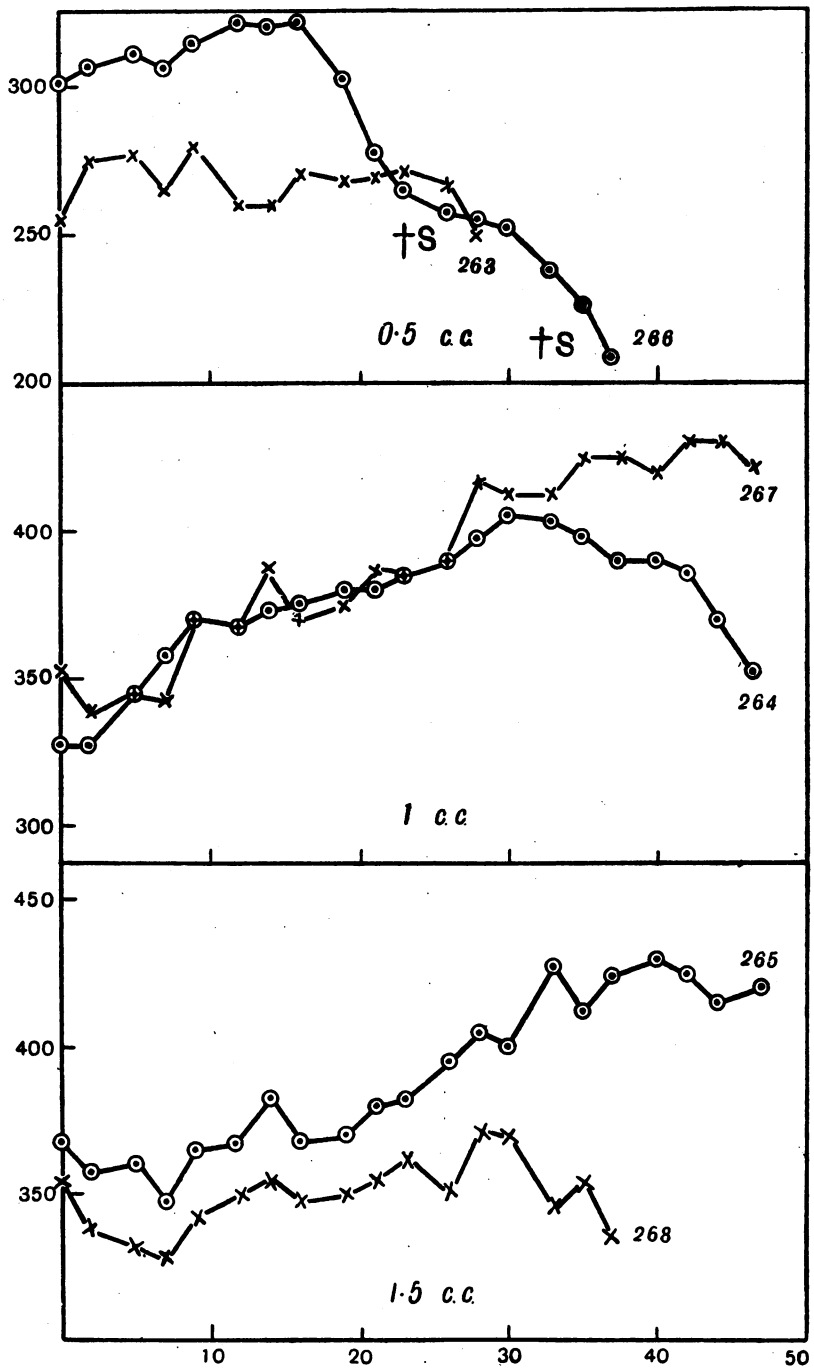

300
250
200
†S
263
0·5 c.c.
†S
266
400
350
300
267
264
1 c.c.
450
400
350
265
268
1·5 c.c.
10
20
30
40
50

Fig. 2

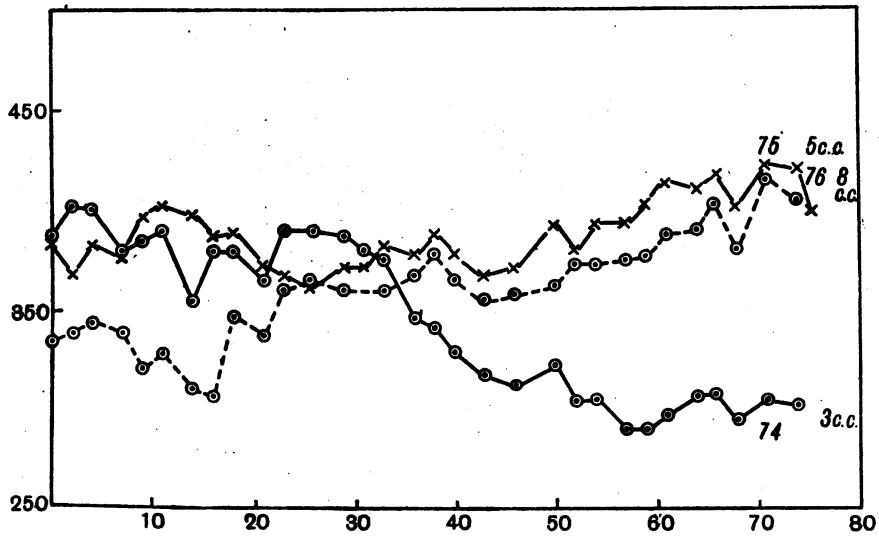
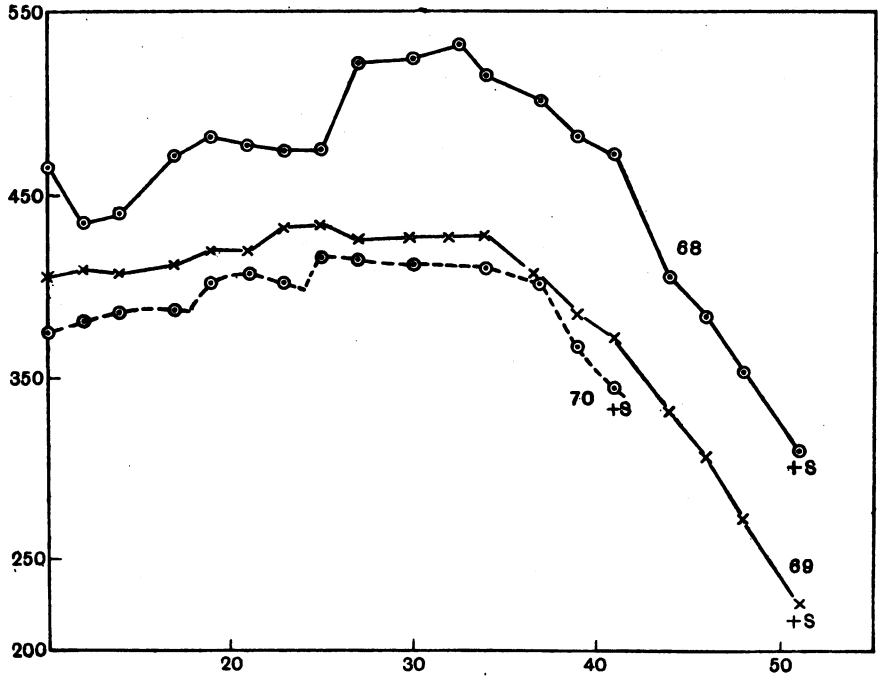

450
350
250
10
20
30
40
50
60
70
80
76 5c.c.
76 8 c.c.
74
3c.c.

Fig. 3


550
450
350
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30
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50
68
70 +S
+S
69
+S

Fig. 4

delayed slightly but not prevented the onset of scurvy. As the doses given were comparatively high it is plain that by drying the treated lemon juice in the way we have just described the best part of its antiscorbutic activity is destroyed.

Expt. 5. From experimental evidence at our disposal obtained in the course of another investigation we decided to modify the process of desiccation by previously acidifying the treated lemon juice. After the precipitation of the free acids of the lemon juice with $CaCO_3$ and alcohol 1 gm. of citric acid was dissolved in the alcoholic filtrate of 1 litre of lemon juice. It was then evaporated *in vacuo* at 30°–40° as before. A residue of 14 gms. was obtained.

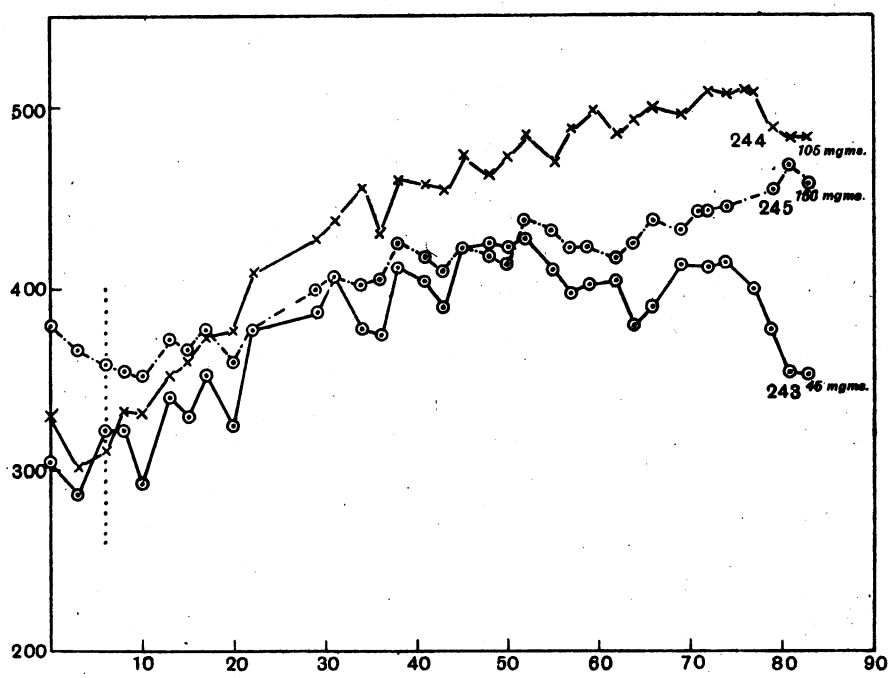

Fig. 5

Guinea pigs Nos. 243, 244 and 245 received 45 mgms., 105 mgms. and 150 mgms. respectively besides the usual scorbutic diet. As before the dry residue was dissolved before administration. As will be seen from fig. 5 which represents the weight curves of these animals they were all increasing in weight. After eighty-three days the animals were chloroformed and at the post mortem examination were found to be in excellent condition. Not the slightest signs of scurvy were to be observed. It is thus seen that by acidifying slightly the

medium, treated lemon juice can be evaporated to dryness yielding a residue of high antiscorbutic potency.

Expt. 6. When pigeons suffering from an acute attack of polyneuritis are treated with subcutaneous or intramuscular injections of highly potent anti-neuritic extracts a cure is brought about even with greater rapidity than when the treatment is carried out *per os*. Holst and Frölich [1912] administered cabbage juice intraperitoneally to guinea pigs subsisting on a scorbutic diet as preventive doses but were unsuccessful in preventing the onset of scurvy from which their animals ultimately died. As the treated lemon juice described above contains very little extraneous matter highly potent doses can be administered. It was of interest to see if by injecting concentrated doses of the treated juice subcutaneously into guinea pigs suffering from scurvy, the animals could be cured. Three guinea pigs were employed for the purpose and the following protocols describe the experiment.

Guinea pig No. 67. When put on a scorbutic diet it weighed 261 g. After subsisting on this diet for nineteen days it received a subcutaneous injection of 15 cc. of concentrated treated lemon juice equivalent to 100 cc. of the original lemon juice. The weight of the animal kept on decreasing in the usual way after the treatment and it died two days later, weighing 271 g. The post mortem examination revealed a very acute condition of scurvy.

Guinea pig No. 58 A. When put on scorbutic diet it weighed 467 g. After twenty-one days it received a subcutaneous injection of 10 cc. of concentrated treated lemon juice equivalent to 100 cc. of the original lemon juice. The weight kept on decreasing and the animal died four days after the treatment. The post mortem examination revealed an acute condition of scurvy.

Guinea pig No. 66. When put on a scorbutic diet it weighed 845 g. After twenty-five days it received a subcutaneous injection of 10 cc. of concentrated treated lemon juice equivalent to 100 cc. of the original lemon juice. The weight of the animal kept on decreasing and it died three days later. The post mortem revealed a very mild condition of scurvy.

It is seen from the above experiments that when guinea pigs are suffering from well declared scurvy subcutaneous injection of concentrated doses of an antiscorbutic does not produce any amelioration in the condition of the animal and the progress of the disease is unaltered.

Expt. 7. This experiment was instituted with the purpose of ascertaining whether by administering high doses of antiscorbutic before putting animals on a scorbutic diet any prophylaxis could be achieved. By using concentrated doses of treated lemon juice very large quantities of very high potency could

be administered within a few days which it would have been impossible to give with the corresponding original lemon juice. The following protocols describe the actual experiment.

Guinea pig No. 80 received within four days concentrated doses of treated lemon juice equivalent to 1280 cc. of original lemon juice. It was then put on a diet of oats, bran and autoclaved milk. Its weight at the commencement of the experiment was 375 g. After fourteen days it began to decrease in weight and died after it had existed twenty-four days on the scorbutic diet. At the time of its death it weighed 259 g. The post mortem examination revealed acute scurvy.

Guinea pig No. 81 received within five days concentrated doses of treated lemon juice equivalent to 1050 cc. of original lemon juice. Its weight at the time it was put on a scorbutic diet of oats, bran and autoclaved milk was 359 g. It began to decrease in weight after eighteen days and died twelve days later. At the time of its death it weighed 199 g. The post mortem examination revealed acute scurvy.

Guinea pig No. 82 received within four days concentrated doses of treated lemon juice equivalent to 900 cc. of the original lemon juice. It was then put on a scorbutic diet of oats, bran and autoclaved milk. Initial weight was 358 g. After sixteen days it commenced to decrease in weight and died eight days later. Its final weight was 238 g. The post mortem examination revealed very acute scurvy.

The administration then of high doses of antiscorbutics previous to a period of deprivation of the antiscorbutic factor does not prevent or delay the onset of scurvy in guinea pigs, as the protocols show that the animals behaved in the same way as animals kept on a scorbutic diet without receiving any previous treatment.

Expt. 8. It was shown by Hart and Lessing [1913] that scurvy can be induced in monkeys. The clinical picture and course of the disease in these animals is almost identical with that of human beings. We too have managed to induce scurvy in monkeys by using a diet which was theoretically complete in every respect but lacked the antiscorbutic element and the description of these experiments will be communicated in detail elsewhere. In view of the fact that all our experiments had been carried out on guinea pigs it was also desirable to study the curative action of the treated lemon juice on a scorbutic monkey. A *Macacus rhesus* monkey, estimated to be about two years of age, suffering from acute experimental scurvy was submitted to the treatment. The details of the experiment are given in the following protocol.

Monkey No. 6 (female *Macacus rhesus*) was put on a scorbutic diet on October 18. Incipient symptoms of scurvy observed on December 29. From that time the disease became gradually worse. On January 29 the animal was in an acute scorbutic condition showing haemorrhaged eyelids, loose teeth with disintegrating enamel. The gums very spongy and haemorrhaged. The animal was almost unable to use its legs. It then received a dose of treated lemon juice by means of the stomach pump. For the following five days it received convenient doses of the treated lemon juice which amounted altogether to the equivalent of 980 cc. of the original lemon juice. On January 24, the day following the administration of the first dose, the teeth became clearer. On January 25 the condition of the gums was markedly improved and the teeth were decidedly firmer. The improvement continued and by January 28, five days after the commencement of the treatment, the gums were normal, the teeth almost firm and the animal was using its legs with alacrity. The condition of the animal soon became normal. It was now receiving a daily dose of treated lemon juice equivalent to 10 cc. of the original juice besides the original scorbutic diet. The monkey throve well on this supplemented diet. On March 16 it was chloroformed and at the post mortem examination, with the exception of a deformed wrist which was evidently the vestige of a fracture which had taken place in the acute scorbutic condition, everything was found to be normal. The histological examination of the costochondral junctions in the ribs did not disclose any perceptible abnormality.

This experiment definitely confirms the potent antiscorbutic activity of the treated lemon juice described in this communication.

Summary.

1. By eliminating the free citric and other organic acids in lemon juice an antiscorbutically active residue is obtained.

2. Comparative experiments show that the best part if not the whole of the antiscorbutic content of the lemon juice is present in this fraction.

3. After storing the treated lemon juice for about a fortnight in the cold room a marked loss can be observed in its potency although it still remains fairly active.

4. By evaporating the treated lemon juice to dryness in acid medium an active dry residue is obtained.

5. Subcutaneous injections of very potent doses of treated lemon juice failed to arrest the progress of well declared scurvy in guinea-pigs.

6. The administration of very concentrated doses of treated lemon juice previous to depriving guinea pigs of the antiscorbutic factor did not prevent or delay the onset of scurvy.

7. The very high curative effect of the treated lemon juice on a scorbutic monkey has been demonstrated.

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