XVII. THE ANTISCORBUTIC REQUIREMENTS OF THE MONKEY.

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THE use of the monkey as an experimental animal in the investigation of scurvy and other deficiency diseases is becoming more and more extensive, owing chiefly to the fact that a great similarity exists between the clinical symptoms of the disease induced in these animals and the symptoms which occur in human subjects suffering from a similar disease contracted through the agency of faulty nutrition. Such experiments, however, are of a prolonged nature and several months must elapse before the results are known. This drawback coupled with the comparative scarcity and high price of the monkey makes this animal in many instances less suitable for experimental scurvy than the guinea-pig. The latter animal is plentiful in this country and results can be obtained with it within a few weeks. One can therefore consider these two animals as complementary in the technique of experimental scurvy, and it is accordingly desirable to establish some quantitative relationship as regards dose and time between them, so that results obtained with one can be translated if necessary into terms of the other.

It is now well established that guinea-pigs which are fed on a scorbutic diet of oats, bran and autoclaved milk manifest well-declared symptoms of the disease after about 15–21 days, although, as was shown by Zilva and Wells [1919], microscopical changes of the teeth may be present as early as 7–10 days after the commencement of the experiment. Our previous experiments [1919] with monkeys have shown that these animals usually manifest symptoms of scurvy after about two months. We have thus an approximate relationship as to the time taken by the two animals to develop scurvy when maintained on scorbutic diets.

It was the object of this investigation to find out the minimum dose of orange juice for monkeys. Orange juice was chosen because it is one of the most common antiscorbutics employed at present in infant feeding and on account of its sweet taste and comparatively low content in acids it can be administered without producing any digestive disturbances, both in human subjects and animals.

It is unlikely that orange juice has a constant antiscorbutic content, since this most probably varies with the variety of the fruit, time of the year and

other factors. This fact must be taken into consideration especially when, as in this case, the experiment is spread over a period of several months. It is at present impossible to standardise an antiscorbutic as regards its therapeutic potency by any chemical method, and the biological method now in vogue requires several weeks before a definite result is obtained and even then the degree of accuracy is not very great. Under these circumstances freshly expressed orange juice was considered to be the most suitable form of the antiscorbutic for our purpose. According to Chick and Rhodes [1918] the minimum dose of orange juice for a guinea-pig lies between $1\frac{1}{2}$ cc.-3 cc. per diem. Our own experience in this laboratory confirms this observation.

Five monkeys receiving 5 cc., 2 cc., 1 cc., 0.75 cc., 0.5 cc. of orange juice respectively were utilised in this experiment. The animals received a scorbutic diet consisting of rice, wheat germ, salt mixture, and butter. The daily ration, which was qualitatively and quantitatively adequate, was made up as follows:

Rice	...	...	300 g.
Wheat germ	...	...	50
Salt mixture	...	...	2
Butter	...	...	5

The orange juice was administered daily *per os* under strictly quantitative conditions, the smaller doses being previously diluted with sweetened water. The orange juice was pressed out fresh every day and endeavours were made to obtain oranges of the same variety throughout the duration of the experiment.

Doses of orange juice of 5 cc. and 2 cc. have proved to be sufficient to ward off scurvy. The monkeys receiving these doses thrive well and gained in weight. The animals on the smaller quantities of juice developed scurvy which in some cases was severe enough to prove eventually fatal. The following are the protocols of the experiments.

Monkey No. 16. *Macacus rhesus*, male. Started on a scorbutic diet on May 13th, 1918 and received 0.5 cc. of orange juice daily. The weight of the animal, which at the commencement of the experiment was 2600 g., was maintained, apart from small fluctuations, until the end of June. After that period the animal commenced losing weight. Scorbutic symptoms became quite definite early in August, when purple and swollen gums were observed. The scorbutic condition developed gradually during the month of August. By the commencement of September the animal had lost the use of its hind limbs. At this stage it was at first intended to cure the monkey but after the administration of a single dose of antiscorbutic equivalent to about 20 cc. of lemon juice the plan was changed and the original daily dose of 0.5 cc. was resumed. The effect of the single high dose was very noticeable as the condition of the animal improved immensely. The pseudo-paralysis of the hind limbs disappeared but the gums and teeth did not clear up. The scurvy, however, soon became severe again and in spite of the continued daily dose

of 0.5 cc. of orange juice the animal declined and died on October 21st, 1918. At the post mortem examination very severe scurvy was found.

Monkey No. 24. *Macacus rhesus*, male. Experiment started July 29th, 1918. Dose 0.75 cc. of orange juice. The initial weight of this animal was 2900 g. Even in the early stages of the experiment the animal showed a slow loss in weight. This decline became very rapid at the end of October and lasted to the end of the experiment. As early as the middle of September the animal showed a tendency to bleed from the gums when handled but no more definite symptoms of scurvy were discernible at this stage. Spongy and purple gums however soon developed and by October 21st pseudo-paralysis of the hind limbs was recorded. The animal declined and died on November 22nd, 1918. At the post mortem examination severe scurvy was found.

Monkey No. 25. *Macacus rhesus*, male. Placed on scorbutic diet on August 26th, 1918. Dose 1 cc. of orange juice. Initial weight 2160 g. This monkey increased in weight until November 9th, 1918 when it reached 2600 g. After that time it commenced to lose slowly in weight. Early in December 1918 the animal developed spongy gums and its incisors became loose. At the same time it lost its liveliness and became dejected. It could however move its limbs without discomfort. As the mild scurvy could not be responsible for this condition it was concluded that the animal was suffering from some other disease. It was therefore decided to stop the experiment and the monkey was placed on a mixed diet on December 12th and a strong dose of an antiscorbutic was also administered. The scurvy cleared up but the animal died five days later. At the post mortem examination no scurvy was found and the cause of death could not be ascertained.

Monkey No. 15. *Macacus rhesus*, female. Placed on scorbutic diet February 16th, 1918. Dose 2 cc. of orange juice. This experiment lasted a year during which time the animal kept in excellent health and gained in weight regularly. The initial weight of the monkey was 3300 g. It weighed 4400 g. on February 19th, 1919 when it was chloroformed. At the post mortem examination the animal was found to be in excellent condition and no trace of scurvy was observed.

Monkey No. 14. *Macacus* (species not determined), male. Placed on scorbutic diet February 16th, 1918. Dose 5 cc. of orange juice. Initial weight 3040 g. This animal increased regularly in weight. After about four months, July 29th, 1918, the experiment was terminated as it became evident from this and the previous experiment that 5 cc. of orange juice was an ample dose. The animal weighed 3450 g. at the end of the experiment and was in good health.

Examining the above results it is seen that the daily minimum dose of fresh orange juice for the monkey lies between 1 cc.-2 cc. No delay in the onset of scurvy was discerned in the animals receiving 0.5 cc. and 0.75 cc. of the juice, compared with those receiving no antiscorbutic. On the other hand, scurvy was definitely delayed in the animal which received 1 cc. of orange

juice. This is plain both from the time of the onset of the symptoms and from the growth of the animal during the early stages of the experiment. Unfortunately this animal succumbed to some other disease and it was not possible to ascertain the extent to which the severity of the symptoms of scurvy could be modified by the daily administration of 1 cc. of fresh orange juice. The higher doses of 2 cc. or 5 cc. have proved an adequate prophylactic. A monkey therefore of 2-3 kilos is protected from scurvy by about the same quantity of orange juice as a guinea-pig weighing 300-400 g. It is interesting to note that while the minimum dose of antiscorbutic required by the two animals to protect them from scurvy is of the same order, the time taken for the development of the disease is very different, being about two months for a monkey and three weeks for a guinea-pig. This suggests that the monkey possesses a higher store of the antiscorbutic factor than the guinea-pig while their daily requirements for metabolism are equal.

REFERENCES.

- Chick and Rhodes (1918). *Lancet*, Dec. 7th, 1918.
Harden and Zilva (1919). *J. Path. Bac.* **12**, 246.
Zilva and Wells (1919). *Proc. Roy. Soc. B*, **90**, 505.