VII. THE VITAMIN B OF LEMON RIND.

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IN a preliminary investigation it was shown that the outer rind of the fresh lemon (the flavedo) must contain considerable amounts of vitamins [Willimott and Wokes, 1925, 1, 2]. In the work described in these papers no attempt was made to characterise the vitamins present in the rind but here results are described which show that vitamin B is one at least of the vitamins concerned.

Most of the earlier work on the Citrus family has been devoted to the investigation of the juice rather than the peel; but as the more immediate questions were solved, attention was turned to the important problems presented by the peel. In this connection, the peel of the orange appears to have been investigated almost to the exclusion of that of the other members of the family, and so far as it is known, there is no record in the literature of work carried out on the rind of the lemon. From a study of the alcohol-ether extract of orange peel, Cooper [1920] concluded that this material must contain considerable amounts of vitamin A. Cooper also makes the interesting statement that similar experiments indicate the presence of vitamin A in the peel of the lemon and the grape fruit. Details of these observations were promised but further published records of this work have not been found. A résumé of work done on the Citrus family, with complete experimental details of the methods adopted, is given in the papers first cited, so that little need be said here. The present experiments were planned so that a group of albino rats received a diet adequate in every factor except vitamin B, which was supplied as an alcoholic extract of lemon rind. The growth curves thus obtained were compared with those of a control group receiving a complete synthetic diet.

METHODS.

Inbred albino rats (*Mus norvegicus albinus*) of Wistar Institute origin were used in this investigation. These animals were in good condition at the time they were set up in experiment. The basal diet fed to both control and experimental groups was made up as follows:

Starch (pure potato)	40 %
Sugar (fine castor)	17
Oil (refined palm-kernel)	15
Protein (pure extracted caseinogen)	23
Salt mixture	5

Pure food products only were used and the usual precautions were observed, particularly with regard to the caseinogen, so as to render the basal diet vitamin-free.

For the salt mixture, Hopkins' mixture with some modifications was employed. Mn and F have been increased and Si and Al have been included as additional ions, so that the salt mixture had the following composition:

NaCl	...	...	...	51.8
MgSO ₄ · 7H ₂ O	...	...	...	79.9
NaH ₂ PO ₄ · 2H ₂ O	...	...	...	104.1
K ₂ HPO ₄	...	...	...	286
CaH ₄ (PO ₄) ₂	...	...	...	162
Calcium lactate	...	...	...	390
Ferric citrate	...	...	...	35.4
NaF	...	...	...	0.25
MnSO ₄	...	...	...	5.00
KI	...	...	...	1.00
Potash alum	...	...	...	0.025
Na ₄ SiO ₄	...	...	...	1.00
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The balanced ration so prepared had a nutritive ratio of 1 : 4 and an energy value of 467.5 calories per 100 g. It was thus adequate for the energy and protein requirements of the rat. For the controls the three vitamins were supplied daily as follows:

Vitamin A. Four drops of pure Norwegian cod-liver oil per head per diem.

Vitamin B. An extract of yeast, at the rate of 0.25 cc. per head per diem.

Vitamin C. The filtered juice of fresh lemons at the rate of 0.20 cc. per head per diem.

Distilled water in separate vessels was supplied to all the animals *ad lib*.

The experimental group was restricted to the basal diet with the addition of vitamin A, supplied by cod-liver oil at the same rate as for the controls. The only source of supply for vitamin B (possibly C also) was the extract of lemon rind, which was fed at the rate of 0.5 cc. per head per diem. At this level, this quantity of extract was equivalent to 0.25 g. of the rind. The lemon extract was made from the outer rind of the fresh lemon and in obtaining this material the directions of the British Pharmacopoeia [1914] for Cortex Limonis were followed. Cortex Limonis, B.P., is described as the fresh outer part of the pericarp of the fruit; the outer surface pale yellow and more or less rough, the inner surface should have very little of the white spongy portion of the mesocarp adhering to it. A transverse section shows a peel about 3 mm. thick, of which the outer 1 mm. is yellow and is termed the flavedo and contains numerous oil glands: the inner 2 mm. is white and is called the albedo.

Fresh lemons were first washed, dried and the rind carefully peeled off with a sharp knife so that only the flavedo was obtained. The rind was now cut into small pieces, placed in a wide-mouthed bottle and 90 % alcohol added at the rate of 2 cc. of solvent to 1 g. of rind. The bottle was well corked and shaken at frequent intervals for at least three days, sometimes longer, the extraction being carried out at room temperature. A cloudy liquid resulted which on filtering gave a clear, yellow solution possessing a pleasant aromatic odour. This was the extract fed to the experimental group as a source of vitamin B. Apart from any vitamin present, the oil thus extracted contains the terpenes *d*-limonene, α -terpinene and α -phellandrene, the first being present in largest amount and the three representing 90 % of the bulk of the oil; the aldehydes, citral (minimum 4 % of the oil) and citronellal; the ester geranyl acetate, and from Palermo lemons—employed in this work—linalyl acetate also. The alcoholic extract also contained some colouring matter, whilst a little mucilage was retained by the filter paper. Another important constituent of the peel, the glucoside hesperidin, is insoluble in alcohol and is therefore not present in the extract.

If vitamin B is present in the rind, a considerable amount will be extracted by 90 % alcohol. For the extraction of vitamin B, lower strengths of alcohol are more efficient and from the work of Funk, Harrow and Paton [1922], 70 % alcohol appears to be the optimum strength. Alcohol of 90 % strength was employed as solvent in order that any findings upon the extract could be applied to B.P. preparations in which lemon rind is used. If vitamin B is proved to be present in the extract the case for its presence in the rind itself is stronger, as the solvent employed would not extract all of this factor. The fact of the possible presence of vitamin C in the extract does not interfere with the test for vitamin B, as it is well known that vitamin C is not essential in the normal diet of the rat [Parsons, 1920]. Differences, therefore, in the growth rates between the control and experimental groups turned upon the vitamin B content of the extract.

The animals were fed side by side, under the same conditions. Sufficient of the ration was always provided so that a small amount remained over each day. The control group consisted of 5 male and 4 female albinos, the average weight of which at the beginning of the experiment was 79.5 g. The experimental group was made up of 3 males and 5 females of average weight 69.2 g. In the feeding of this group, the lemon extract was well mixed in with the basal ration before being given to the animals. The alcohol, at the level the extract was fed, was not observed to have any undesirable effect upon the animals. In the third week of the experiment, the daily consumption of food was ascertained by means of back weighings. Although the food was in the form of a dry powder, it was possible to obtain a reliable estimate by taking precautions against the loss due to scattering. Lipped pots, which helped to reduce scattering, were placed on wide metal trays which caught the small quantity of food actually spilled. This amount was carefully col-

lected and added to that remaining over in the feeding pots in the 24 hours for the back weighing. It was found that the food consumption of the controls was on the average equivalent to 48 cal. per 100 g. body weight, whilst that of the experimental set was approximately 46.7 cal. per 100 g. body weight; quantities ample for the calorific needs of the rat [*vide* Donaldson, 1924]. Moreover, the food equivalent of these energy consumptions supplied adequate amounts of protein. Both sets were weighed at the same time at intervals of three days.

Fig. 1. Vitamin B of Lemon rind.

GROWTH EFFECTS.

Each animal in the experimental group grew at a more or less satisfactory rate during the ten weeks of the experiment. There were no deaths. At the end of the experiment, the average of the males was 79 % that of the controls; in the females the average weight was 91 % that of the controls. The mean rate of growth for the whole group when the observations were discontinued was, therefore, not very much lower than that of the control animals. Moreover, the experimental group remained in good health throughout the period, and at the end, exhibited all the characters of normally nourished rats. There is little doubt that had the experiment continued, the animals would have reached the same weight as the controls although by a slower path. The possibility that the animals were obtaining their vitamin B requirement from the faeces, as shown by the work of Steenbock, Sell and Jones [1923], was excluded by the fact that faecal matter fell into trays and was so removed

from the cages. As the diet had proved satisfactory for growth, it was tested for its adequacy in reproduction and rearing. Two of the experimental does were mated with bucks from the same group. Two litters were produced containing 6 and 8 individuals respectively, but they were not reared in either case. The diet, therefore, whilst adequate for growth could not sustain successful rearing of offspring.

At the conclusion of the experiment, a buck and a doe were each chloroformed and autopsied. The internal organs were found to be normal in both cases. In the buck it is noteworthy that spermatozoa were isolated and recognised under the microscope.

CONCLUSIONS.

From the experimental results obtained, it is evident that vitamin B is present in appreciable amounts in the rind of the fresh lemon. When only 0.5 cc. (equivalent to 0.25 g. of rind) of the lemon extract was fed per head per diem as sole source of this vitamin, nearly normal growth resulted. As the albinos were making satisfactory progress, the supply of the extract was not altered, although with a slight increase, it seems probable that complete normal growth would have been obtained. The supply of vitamin B though sufficient for reproduction was inadequate for successful rearing. It is well known that, in contrast to vitamin A, at all periods absence or deficiency of vitamin B is speedily manifested [Osborne and Mendel, 1920, 1]. As growth was maintained for a period as long as ten weeks, it appears certain, therefore, that appreciable quantities of this vitamin were present in the extract. Rather more could be expected to be present in the rind as 90 % alcohol is not the optimum solvent for vitamin B. These results are in accordance with the findings of Osborne and Mendel [1920, 2], who demonstrated the presence of vitamin B in the albedo of orange peel. Particularly so, as it appears very probable that some contamination with the rind (flavedo) of the orange would be almost inevitable, since the preparation of the inner peel involves the removal of flavedo with unavoidable rupture of the oil glands. The likelihood of this interpretation appears to be supported by the quantities of albedo and flavedo respectively which are required by the animal for growth. Allowing for the fact that Osborne and Mendel worked upon the orange, it is striking that these authors used 20 times (5 g.) the quantity of albedo, that I found to be effective of flavedo (0.25 g.) with the lemon, as a supply of vitamin B.

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