PART III.

Vegetable Systems.

Introduction.

From the very beginning my research work in Biochemistry was led by the conviction that there can be no real difference in the fundamental chemical mechanisms of plants and animals. We are all but young buds of the same old tree of life, expression of the same fundamental principles appearing under different disguise.

Results of the last few decades have greatly corroborated me in this conviction. The existence of Vitamins is convincing evidence on this line, as it shows that plants and animals work with the same cogwheels. The substance, Vitamin B_1, instrumental in producing thoughts in our brains, is equally indispensable for the silver membrane of rice. Insulin, the highly specified product of a highly specified organ of our body is found also in the yeast cell. There can be no fundamental difference in two mechanisms, the parts of which are interchangeable.

So if we want to elucidate some fundamental biological principle, it does not matter whether we study a high or low animal, a plant, or the yeast cell. There is no fundamental difference between kings and cabbages.

Vegetable material has many advantages over the animal. Its protein structure is less labile, and often tolerates treatment with anhydric solvents that would denaturate animal protein.

Vegetable tissue might have still other advantages. Animal tissues all live under the identical conditition of their „internal environment". Different plants or different parts of the same plant live under very varied conditions, e. g. under the ground or high up in the air. So we may expect that from

the different oxidative mechanisms the one or the other will predominate, according to the special conditions. Moreover in the plant the picture will not be complicated by specific functions, like muscular contraction etc.

Naturally the vegetable material has also its disadvantages. The chemical changes are on the whole less rapid in the plant than in the animal, and therefore more difficult to measure. Owing to the rigity of the cell wall, the damage done to the cells by our methods is often graver than in the animal. Accordingly it will be still more difficult to correlate the results obtained under artificial conditions with normal cell life.

The Polyphenoloxidase System gives a good example of this. As I will show presently, we can easily demonstrate the presence of this most powerful oxidative mechanism in plants. After having isolated and measured all the single members of this mechanism we are left with the question: has this anything to do with respiration? And we are unable to answer this question. The same applies to the Peroxidase System.

These two oxidative mechanisms just mentioned, the Polyphenoloxidase and Peroxidase System, often dominate the whole appearance of the plant. But in the shadow of these powerful systems we also find cytochrome, which as I have shown, dominates the picture in the animal and carries most, if not the whole of respiration. Thus we are confronted with the question as to whether cytochrome carries the respiration in plants the same way as it does in animals, while the other two systems have some different function. On the other hand, if the Polyphenoloxidase and Peroxidase Systems are involved in respiration, are the same systems not found in the animal cell also?

It is a depressing fact that we are unable to answer these simple and fundamental questions. I am rather inclined to think that all the three systems are involved in respiration of all cells but it will depend on conditions which one will predominate. The conditions of the „internal environment" of the higher animal favour the system of which cytochrome is a member. There are also reasons to believe that the other two, the Polyphenoloxidase and Peroxydase System, are also involved in

animal respiration even if they carry only a small fraction of total oxygen uptake and are perhaps only serving some specefic function of the cell. What makes me think that these systems are involved in animal respiration is that two of the most important members of the „Peroxidase System" have been shown to be of Vitamin nature (Vitamin C and P) and thus to be essential for the functioning of the animal tissue also. The enzyme which is the most characteristic member of the chain, the „Perixodase", has been shown to exist also in the animal tissue. Polyphenols are found in the animal too and are very closely related to polyphenols acting in the vegetable polyphenoloxidase system (Adrenalin, Dopa). The existence of Phenoloxidases has been demonstrated also in animals.

The problem, however, is undecided and my object will be to present briefly the underlying experimental facts. Even if the two great vegetable systems, the Polyphenoloxidase and Peroxidase System have nothing to do with respiration and have no bearing on the higher animal, their study has led to results which are not without importance for animal biochemistry: the isolation of Vitamin C and the recognition of the Vitamin nature of phenyl-benzoγpyrone dyes. In this case my research will be a further example of the fact that even a wrong theory may lead to correct results and any theory is good as long as it suggests experiments.

1. The Polyphenoloxidase System.

It is an age-old observation that certain plants turn brown if damaged. The potato, the apple, the pear, the banana are every-day examples of this. It was *Palladin,* the great Russian botanist, who gave his attention to this phenomenon. He has shown that this discolouration is connected with the function of certain enzymes oxidizing a polyphenol, (a phenol with more than one OH group). In the intact plant, or under anaerobic conditions, the oxidised polyphenol becomes reduced again to the uncoloured compound. If, however, the plant is damaged, this reduction does not take place and the oxidised phenol appears as a „respiratory pigment".

Looking at these observations to-day it becomes evident

at once that these „respiratory pigments" are links in a chain of a respiratory system, at one end of which stands oxygen, oxidising „the respiratory pigment" by the interaction of special enzymes. At the other end stands the dehydrogenase releasing H from the foodstuff, reducing the chromogen to its leuco compound. We are faced here with the same fundametal principles as in the animal respiration: the splitting off of H from the „donator", and its piecemeal oxidation.

The enzymes responsible for the oxidation of phenols have been the object of numerous studies (*Bertrand, Bach, Chodat, Wolf, Robinson, Raper* etc.).

In the study of these enzymes the classical guaiac-reaction played an important rôle. If the cut surface of these plants is wetted with a solution of guaiac resin, a brilliant green colour very soon develops due to the oxidation of the resin. Since the guaiac reaction was known as a specific reagent of peroxides and peroxidases, complicated theories were elaborated about the mechanism of the action of these polyphenol-oxidases. *M. W. Onslow* has shown that the plants giving the guaiac reaction contain catechol or its derivatives. It was thought that these catechols activated oxygen somehow, forming peroxides. I could show, however, that what happened was much simpler: the oxygen, interacting with the polyphenoloxidase, oxidised off two H atoms from the phenolic OH group. The catechol thus became dehydrogenated into an o-quinol. o-Quinols are very powerful oxidising agents and oxidise the guaiac into its coloured compound. (51, 52, 53).

If the phenol is oxidised by the oxidase in the intact plant the quinol is reduced to phenol again. If, however, by damaging the plant we damage dehydrogenases, this reduction does not take place, and the quinol combines with nitrogenous substances present, into highly coloured substances *(Raper* and *Wormall).*[31] The o-quinols themselves are mostly not very highly coloured.

Whether the quinol in the plant is reduced immediately by the dehydrogenase, and whether there are other substances acting as links between quinol and dehydrogenase, we do not

[31] Biochem. J. *21*, 26, 1927.

know. The polyhenoloxidase plants do not contain ascorbic acid mostly in a higher concentration. All the same they contain some and we can expect that in presence of ascorbic acid the quinol will oxidise the ascorbic acid present, and the dehydrogenase will reduce only the dehydro-ascorbic acid. Quinols oxidise ascorbic acid reversibly at a very high rate. (57, 72).

Oxidising enzymes acting on monophenols, like cresol or tyrosin seem to be members of an analogous system.

The phenol-oxidases present a clear case of a vegetable respiratory system. The only trouble is that the system works too well. It calls to mind the detective stories in which suspicion is roused by the fact that the evidence is too plain.

Vietorisz and myself (59) have measured the activity of the polyphenoloxidase in the potato. The inner layers of the undamaged tissue of this plant show no colouration. Thus, if the phenol is oxidised at all, it must be reduced at an equal rate. Now if we squash the potato and damage the tissue, discolouration appears at once. Thus after damaging the cell the phenol is either oxidised faster or is reduced more slowly.

Without any trick it is not possible to measure the rate of the oxidation of the phenol, for its quantity is too small and it is oxidised before the measurement could start. So if we want to measure the rate of the oxidation of the phenol, we have to reduce the quinol all the time. This is easily done by the addition of ascorbic acid. The experiment shows that the squashed tissue takes up at least fifteen times as much oxygen for the oxidation of the phenol as it uses in its normal respiration. Thus even if the oxidase has acted on the phenol in the undamaged tissue, its activity was at least fifteen times smaller than in the damaged tissue, and the potato contains at least fifteen times more oxidase than it needs for its respiration. This seems to be senseless. If no more than one fifteenth of the enzyme could be active in the plant, it could be just as well for the enzyme not to be active at all and have some quite different function than to support respiration. Thus the intact tissue is colourless, not because the quinol is reduced, but because the oxidase does not act.

The fact that damage of the cell releases the phenoloxidase,

suggests that this enzyme might have something to do with the protection of the plant against damage, with natural immunity. Quinols are known to have a strong bactericidal activity and to have a tanning effect on protein. One would think that the polyphenoloxidase together with catechol are stored by the plant to be released only if the cell is damaged. By killing the bacteria and tanning the damaged surface the system will close the gates to further progress of tha damaging influence.

Other oxidising enzymes might have analogous functions. Milk contains a most powerful oxidising enzyme, although has no respiration. This enzyme described by *Shandinger* oxidises aldehydes, producing an equivalent quantity of peroxide. Peroxides are known to kill bacteria and bacteria are known to produce aldehydes.

2. „The Peroxidase System."

M. W. Onslow has termed plants such as cabbages, lemons etc. which do not discolour on injury and correspondingly give no guaiac reaction and contain no catechol, „peroxidase plants". This name was given because the most conspicuous constituent of these plants is a very active peroxidase, the enzyme activating peroxides. In the presence of peroxidase, peroxides will be able to oxidise substances which without peroxidase are not acted upon. It is easy to demonstrate the existence of a peroxidase in a plant juice by adding peroxide and a substance like guaiac resin or benzidine. In presence of peroxidase the rapidly developing colour will indicate the oxidation of our reagents and hence the presence of peroxidase. If there is none present the system will remain uncoloured.

In order to find out something about the respiratory system in which the peroxidase might be involved, I performed many years ago these guaiac and benzidin reactions with the juice of „peroxidase plants". I observed that the reaction occurred only with delay of a second or so, while a purified peroxidase gives the reaction at once. This insignificant delay of the reaction has given me work for many years, for I found it to be due to the presence of a most fascinating substance,

(54) which had strong reducing properties and was capable of reversible oxidation and reduction. This substance, first called O_{x11} by its protocol number, was found to be related to carbohydrates, so I called it ignose, not knowing which carbohydrate it was. This name was turned down by my editor. „God-nose" was not more successful, so in the end „hexuronic acid" was agreed upon. To-day the substance is called „ascorbic acid" (75) and I will use this name.

The delay of the peroxidase reaction in the plant juice was simply due to the fact that the ascorbic acid present, reduced the oxidised guaiac or benzidine again. This went on till the ascorbic acid was used up, which took about a second.

The discovery of ascorbic acid is a classic example of the fact that a wrong theory may yield good results. My studies of oxidation originated from my desire to understand what the adrenal cortex was doing. I thought this gland was involved, by its product, in biological oxidation and so I studied oxidation in order to understand the adrenal gland. It is known that human patients, in whom the gland does not work (*Addison*'s disease) turn brown before they die, in the same way as potatoes, apples or bananas. So my excitement was fully justified when I found that the adrenal cortex contained relatively big quantities of ascorbic acid, the same substances which, if added to polyphenoloxidase plant tissues, prevented pigment formation and which seemed to carry respiration in the cabbage. The adrenal gland contains so much ascorbic acid, that for a long time this gland was found to be the only suitable material for the big scale preparation of this substance. My excitement was increased still more by the fact that Addison patients could be bleached out by the injection of ascorbic acid.[32] (57).

To-day we know that the theory was wrong and the specific product of the adrenal gland is „cortine" and several other tissues like the corpora lutea and the adrenal medulla *(Huszák)*

---

[32] Ascorbic acid prevents only the formation of melanoid pigments. As showed by *P. Koller* (unpublished) embryonic iris tissue growing in a solution of ascorbic acid forms pigment unhampered. Ascorbic acid has thus no effect on normal pigment. This explains why the hopes of solving the colour problem by means of ascorbic acid were disappointed.

(64, 66) contain ascorbic acid in equal concentration. Possibly it serves to protect the autooxidisable hormones.

I could also show later (58) that, along with ascorbic acid, peroxidase plants contain an enzyme capable of oxidising off two H atoms from ascorbic acid which reaction is reversible. The oxidised ascorbic acid will take up H again, if such is offered. I could also show that this „ascorbic acid oxidase" did not act on ascorbic acid just by good luck. The oxidase is made to oxidise ascorbic acid only, and made to act upon it with a maximum velocity, even if only very small quantities are present.

Since peroxidase plants are mostly relatively rich in ascorbic acid, it is natural to suppose that all the three substances, peroxidase, ascorbic acid, and its oxidase are all members of the same oxidising mechanism.

Peroxidase and peroxide do not oxidise ascorbic acid, or do so only at a very slow rate. If, however, peroxide is added to the plant juice containing peroxidase, it will oxidise ascorbic acid very quickly. It is evident that the plant juice contains some other substance also, necessary for the reaction.

The experiments showed that this substance was of aromatic nature. Its function was to connect peroxidase and ascorbic acid. In its presence peroxidase oxidised ascorbic acid immediately. The mechamism of the reaction was this: peroxide plus peroxidase oxidised this aromatic substance into the corresponding quinol, which in its turn oxidised ascorbic acid.

I tried to isolate this aromatic substance from lemon juice years ago (unpublished). The successive purification of this juice left me in the end with a substance apparently belonging to the great group of yellow water-soluble vegetable dyes.

We owe our knowledge of the chemistry of these substances chiefly to the pioneer work of *v. Kostanecky* and *A. G. Perkins.*

The nucleus of the molecule of these substances is a benzo-γ-pyrone. In the most widely distributed representatives of these dyes, this nucleus is substituted at the position 2 by a phenol. Such substances we call flavanones. (Fig. 10.) If there is a

double bond at 2—3 we call the substance a flavone. If in addition to this there is an OH group in position 3, call it a flavonol.

The most common representative of this group is the flavonole quercetine. (Fig. 11.) Like most other members of this group, quercetine has two OH groups on the benzo-pyrone moiety in position 5 and 7. These two OH groups (5 and 7) serve to bind sugar, for most members of this group of dyes are found in the living cell as glucosides. The two OH groups at 3', 4', are free and seem to be connected with the function of the substance, as I will show presently.

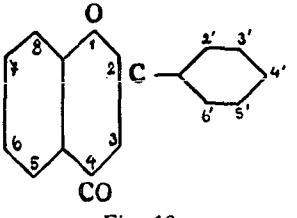

Fig. 10.

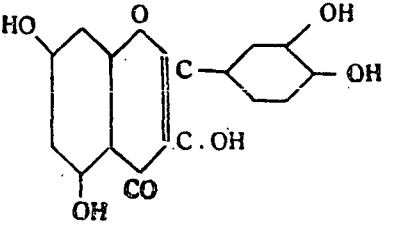

Fig. 11. *Quercetine.*

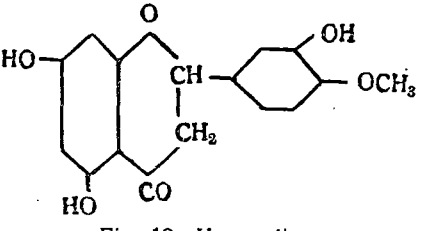

Fig. 12. *Hesperetine.*

Lemons are known to contain the flavanone hesperetine (Fig. 12.) in the form of a glucoside, called „hesperidine". This glucoside has the same structure as quercitrine, the glucoside of quercetine. Naturally in hesperidine there is no double link at 2—3 and no OH at C 3. Morever the OH group at 4' is bound by a methyl. The lemon builds up its store of hesperidine for life at a very early stage, so the unripe lime contains amazing quantities of hesperidine. If one looks at the quantity of hesperidine obtained from one small unripe lime, one wonders where the fruit has been.

The crystalline flavanone fraction obtained from ripe

lemon showed an amazing reactivity, not shared by pure hesperidine which is a very stable substance. The analysis by *Bruckner* (83) and myself has led to the interesting observation that on ripening, the plant demethylates hesperidine, freeing the OH group at 4'. The demethylated hesperetine is called eriodictyol, which dye was not known before as glucoside. By the liberation of the second OH group the dye gets a very high reactivity. The unripe plant seems to store its reserve dye in an inactive, methylated form and demethylates the glucoside in the course of its life according to need. The ripe lemon contains mostly eriodictyol glucoside and little hesperidine, while the reverse is true for the unripe fruit. Hesperidine is insoluble, the eriodictyol glucoside is soluble in water.

St. Huszák (67) has studied these dyes from the point of view of the peroxidase systems. He has shown them to catalyse the reaction between peroxide, peroxidase, and ascorbic acid. They are in this respect active only as far as they have a free o dihydroxy group at 3, 4. Without this grouping they can give no quinols. Accordingly hesperidine is inactive, quercetine and eriodictyol or their glycosides are active. They catalyse the reaction by getting oxidised themselves by the peroxidase plus peroxide and oxidising in their turn ascorbic acid.

This catalitic activity of these compounds was found to be more or less specific. Eriodyctiol, quercetine and their glucosides are about 100 times more active than the equivalent catechol.

Naturally, peroxidase cannot work if there in no peroxide. The question was therefore, as to whether there is any peroxide in plants and if so, where does it come from. This question was answered by *Huszák* (67), who hereby completed the whole system. The peroxide was formed in the oxidation of ascorbic acid by the ascorbic acid oxidase.

If we observe thus a rapid disappearance of ascorbic acid in the cabbage juice, or the juice of other peroxidase plants, this apparently simple reaction has the following mechanism: moleculer oxygen interacts with ascorbic acid oxidase and oxidises off two H atoms from ascorbic acid. The oxygen itself

is hereby reduced by these two H atoms to hydrogen peroxide, while ascorbic acid itself becomes, by the loss of its two H atoms, dehydro-ascorbic acid. The H_2O_2 formed reacts with peroxidase and oxidises a flavone (or flavonole or flavanone). The oxidised flavone in its turn oxidises a second molecule of ascorbic acid. In this way all the four valencies of the O_2 molecule are utilised for oxidation of ascorbic acid. Dehydro-ascorbic acid is reduced by tissues again. It is true that dehydrogenases do not reduce ascorbic acid, but dehydrogenases, acting on hexosephosphate *(Meldrum)* or citric acid (*Banga*, unpublished) reduce glutathion and glutathion reduces dehydro-ascorbic acid. *F. G. Hopkins* and *Morgan*[33] have shown that this reduction is greatly accelerated by the ascorbic acid oxidase. Herewith the system is complete, and represents again a chain of reactions linking up the „Donator" with the O_2 by the piecemeal oxidation of the H.

3. On Vitamin C.

My first observations on ascorbic acid (54) were made in a cellar room of the physiological laboratory at Groningen. The isolation of this substance was made possible by the hospitality of Sir *F. G. Hopkins* at Cambridge. (55). It could be prepered from cabbages, oranges and ardenal glands in small quantities sufficient for the first chemical analysis. (56). I could show that the substance answered to the general formula of $C_6H_8O_6$, demonstrate its reversible oxidisability and show that it was one of the fundamental, widely distributed, reducing agents of the cell, animal or vegetable. Unfortunately the plants mentioned allowed preparation on a small scale only and were unsuitable for big-scale preparation. My raids on greengrocers stores had no result. I could find no suitable material for big-scale preparations. Further progress in the study of the function and chemical constitution of the substance depended on the preparation of larger quantities. The only suitable material for work on a large scale was the adrenal gland. This however was not available

[33] Biochem. J. *30*, 1446, 1936.

at Cambridge and so Professor *A. Krogh* kindly tried to help me by sending big quantities of glands by air from Denmark, but the material on its arrival had deteriorated.

Further progress was made possible by the generous invitation of the *Mayo-Foundation* and the hospitality of Professor *E. C. Kendall.* I was able to use the material of Americas huge slaughterhouses and prepared about 25 gr. ascorbic acid from adrenal glands, working up this expensive material by the hundredweight.

This material enabled me to study the substance from the point of view of vegetable respiration (58), and to find the ascorbic acid oxidase. I could also study its effect on Addison disease (57). My chief concern, however, was the elucidation of the chemical configuration of the molecule. For this reason I shared my material with Professor *W. N. Haworth,* who from the beginning showed a vivid interest in this substance. We both arrived at the same conclusion: this quantity of material was insufficient to make any progress. So the material went and there was no chance of any further preparation. Plants failed as material and repeated preparation from adrenal glands was impossible because the expense was prohibitive. The research was given up in despair and nothing more was left than a small quantity of ascorbic acid in the bottom of one of my tubes.

From the very beginning I suspected ascorbic acid to be identical with Vitamin C but my roaming life was unsuited for Vitamin tests and moreover, somehow or other, Vitamins were my pet aversion. Vitamins owe their great popularity to their paradoxial behaviour, making us ill if we do not eat them, while all other substances make us ill only when we do eat them. What food must contain to be wholesome is a question of primary interest to the cook rather than to the scientist. Accordingly the appreciation of results of vitaminology are often out of all proportion to their scientific importance. Moreover the vitamin nature of ascorbic acid could not contribute much to its real scientific interest, since its importance and presence in plant and animal tissues has been demonstrated already.

Two years later I was condemned to be Professor and sent to the chair (of biochemistry) in *Szeged.* At the same time

fate has sent me a clever collaborator *J. L. Svirbely* who had had some experience in vitamin C test and brought with him the conviction that my ascorbic acid could not be Vitamin C. All the same I made him test my old suspicion to see whether the small quantity of powder left in the bottom of one of my tubes was not the vitamin C. In November 1931 he had definite evidence that it was. At this time also Tillmans directed attention towards the possible identity. We did not publish until we could repeat our experiment on a large number animals. (69—72). Simultaneously *King* and *Waugh* reported the isolation of crystals from lemon juice which had antiscorbutic properties and seemed to be identical with ascorbic acid, that time „hexuronic acid".

By its identity with vitamin C general attention turned towards ascorbic acid. But there is little use in knowing that a substance is interesting if there is none of it. My stores were completely exhaused and there was no chance of further preparation. Big quantities of ascorbic acid were urgently needed to find out the exact configuration, and to perform the chemical work necessary to demonstrate definitely that ascorbic acid really was the vitamin C and the vitamin activity of our crystals was not due only to some enclosed impurity.

Szeged happens to be the centre of the „Paprika" — Hungarian red Pepper industry. This fruit *(capsicum annuum)* was about the only one I had never tested. For some unknown reason nature has supplied Hungarian red Pepper with a most wonderful store of ascorbic acid. In two successive seasons I was able to prepare 3 $^1/_2$ kg of crystalline ascorbic acid from this fruit.

From this substance *L. Vargha* prepared monoacetone ascorbic acid, which in itself is quite inactive, but crystallizes beautifully. After repeated recrystallisations the ascorbic acid could be split off again and was found to be fully active still. This was the first definite evidence that ascorbic acid really was vitamin C. (74).

Most of my ascorbic acid was distributed among all workers who were interested in it. So this material greatly contributed to the rapid elucidation of the structure of the molecule and opened the way to synthesis. Thus it is greatly due to the

Hungarian red Pepper that vitamin C was brought from its mysterious sphere into the domain of cheap synthetic products within the remarkably short period of two years. Now it is produced synthetically at a low price by the hundredweight.

Finding that one of his active substances is a vitamin means a little tragedy to the scientist. Quiet work on basic problems hat to be given up for working out big-scale methods and their dull application. Years have to be spent in hard labour merely to make synthesis possible, which makes all the previous work valueless. Herewith one of the little cycle of hope and disappointment is closed, of which a scientists life is composed.

All the same I am grateful to Pepper for one of the greatest impressions of my life. I am still filled with sincere gratitude for the generous international support, collaboration and comradeship I found in those days. It is encouraging to know that this spirit exists in science. If it prevailed in international politics also, we should all be approaching a more cheerful future.

4. On Vitamin P.

In the previous chapter I have deprecated vitamins and have told why I ran about for five years with crystalline ascorbic acid in my pocket without testing its vitamin activity. I have also given an example of a bad theory leading to good results. Now I want to say a few nice things about vitamins and tell why I was so anxious to find out whether flavones were not vitamins and will give an example of an incident and an error leading to discovery.

The vitamin nature of a substance can also be of great scientific importance. By vitamin we mean a vegetable substance, needed, but not made by the animal, which by its absence will cause disease. This disease often helps us to reveal the existence of such a substance. By demonstrating the vitamin nature of a substance, we prove that it plays an important rôle also in the animal, giving a new evidence of the unity of living nature.

Benzopyrone dyes are of great importance to the plant. These dyes are elaborated in the vegetable kingdom every year

afresh by the ton. It is difficult to believe that these substances should have no importance for the animal, although chemical methods fail to demonstrate their presence in the animal cell. Thus I could only hope to demonstrate their importance if they happened to be vitamins and caused disease by their absence.

The possibility of their vitamin nature was suggested by the fact that the other substance, ascorbic acid, working hand in hand with flavones in the peroxidase system, is also a vitamin. The chances of demonstrating the vitamin activity however, were very small. The most suitable animal for this demonstration seemed to be the giunea pig and the most suitable diet the scurvy diet, although animals on scurvy diet thrive well if supplied with ascorbic acid, which seems to prove that the diet either contains these flavones or else the animals do not need them.

The first indication that flavones might be vitamins was given by an incident. In the early days of ascorbic acid I had a letter from a doctor who was suffering from severe haemorrhagic diathesis. He asked for ascorbic acid to test the effect of this substance on his condition. Not having enough ascorbic acid yet, I sent him pepper in its conserved form („vitapric"). The man was cured. Later the treatment was repeated with pure ascorbic acid: it had no effect. This suggested that some other principle was responsible for the activity. To isolate this principle would have been a hopeless job, had not the idea of the vitamin nature of flavones been in the back of my mind. I set out to isolate the flavanone fraction from lemon juice, which we called citrine and my friend *St. Rusznyák* (80, 81), and his collaborators, *Armentano* and *Bentsáth* tested it on patients. The substance was active, it cured (vascular) haemorrhagic purpura and brought back the fragile and permeable capillaries to their normal state in different pathological conditions. Very fortunately the fragility and permeability of capillaries can be measured fairly accurately by the methods of *Borbély* and *Landis*. There was a sharp difference between diseases in which capillaries answered to citrine (Haemorrhagic purpura, nephritis, sepsis,

nephrosis, polyarthritis) and those in which they did not (diabetes, tuberculosis.

Such an activity suggest vitamin nature but does not prove it. Evidence of the vitamin nature could be obtained only in the animal experiment, and here it was that the error came to help us.

Encouraged by clinical observations we tried what citrine would do to guinea pigs on a scurvy diet, in spite of our bad chances. (82). The result was quite unexpected. Citrine, given in scurvy, not only prolonged life, but prevented rapid fall in weight and reduced haemorrhages. The difference with controls was quite striking and pleaded for the vitamin nature of citrine. So we called the substance vitamin P.[35] The difference between our control animals and the animals receiving citrine was sharp enough to use it as a test, by which the basic facts about vitamin P could be established. *Bentsáth* (86) showed that the intactness of the whole glucoside molecule is essential for the activity, the aglucons (the dyes without sugar) are inactive, but not all members of the phenyl-benzo-pyrone series are active. Both constituents of citrine, the hesperidine and the eriodictyol glucoside were equally active. *(Bentsáth, Rusznyák* and *Sz.)* (84). But quercitrine, the most widely distributed flavonol, though otherwise not devoid of pharmacological activity *(Fukuda, Jeney* and *Zimmer),* was found to be inactive in our experiments. Quercitrine dif-

---

[35] It was called P, because of its effect on *P*ermeability and because of *P*aprika (pepper). I had also another reason to choose P, which was not the first free letter of the alphabet. I realised that vitamin work was full of pitfalls and hoped that in case the vitamin nature of citrine would be disproved, this would happen before vitaminolgy reached the letter P and thus my work would cause no trouble. Moreover I knew orthodox vitaminologists would be teased by my jump in the ABC.

Naturally it would be a grave mistake to call P a „permeability vitamin". This would be much the same as if *Burr* and *Burr* called their substances „anti-tail-drop-off" substance. The *Burrs* have shown rats to loose their tails, if certain fatty acids are missing from their food. The function of these acids is certainly not to keep tails in their place, just as it is not the function of P to keep capillaries in the right condition. That capillaries get ill in absence of this substance is an other question.

fers from the eriodoctyol glucoside only in having a double bond at 2—3 and an OH at 3. This slight variation within the molecule is sufficient to abolish the activity. The position seemed to be analogous to that found in lipochromes, where only a few members of the big group are active as provitamins. *A. Bentsáth* (86) could also establish approximately the necessary daily dose of citrine. This was about 0,2—0,4 mg, for 1 mg had a full, 0,2 a submaximal effect. Together with ascorbic acid vitamin P is not active in quantities of γ-s, like some other vitamins. The daily dose corresponds to the daily dose of ascorbic acid. Lemons contain about five times more ascorbic acid than citrine. The daily dose of ascorbic acid is about 1,5 mg. roughly five times as much as that of citrine.

Thus our test has given valuable results and cleared the basic questions. There was only one great trouble with it. Within one series the experiments gave conclusive results but the activity was not equally reproducible in different series of experiments. The test was a subtle one and there seemed to be unknown factors at play. One factor revealed by *Bentsáth* and *Das* (85) was the importance of the food given prior to the experiment. But even this, if put right, did not assure the constancy of results. The difference between controls and animals receiving citrine became less marked as our experiments advanced. In later experiments citrine failed to prevent haemorrhages, then it failed to prevent loss of weight and in the end, lengthened the life of our animals by only a week only in-stead of a fortnight A laboratory which, at our request, repeated our work with the greatest care on a large number of animals failed to find any difference. All this was very disconcerting. So it was no surprise when *Zilva* published his negative results, obtained with Hesperidine.

A great number of experiments were performed to find out the cause of this discrepancy. Eventually it was found by *Bentsáth* (86) that it was probably caused by an experimental error, without which our results could never have been obtained. This error did not take away the value of our previous experiments, but it explained the discrepancy. *Bentsáth* showed that the difference in results might have been due to the fact that our scurvy diet

still contained traces of ascorbic acid, which were too small to have any effect on the development of scurvy. The flavanones seem to be utilized by the animal only in presence of at least traces of ascorbic acid, or if we want to put it the other way round, traces of ascorbic acid can be utilized only in the presence of vitamin P.

Our peroxidase system could give a simple explanation of this. *Huszák* (67) has shown ascorbic acid and flavanones to act in the same chain of reaction. If this holds for animals, one can expect that the one substance cannot act in the entire absence of the other. So the flavanone cannot act entirely without ascorbic acid. Possibly ascorbic acid cannot act without traces of flavanones.[36]

I am conscious of the difficulties and incertitudes. The first steps in a new field are often somewhat shaky and there is only one way to avoid error with certainty, and that is not to work or at least to avoid new fields.

Whatever the explanation may be, the fact remains that under certain conditions vitamin P has a striking effect. It prolongs life, pevents loss of weight and the development of part of the scurvy symtoms, in the first place haemorrhages. It is hoped that a better test vor vitamin P will be found soon, though the factors, influencing results, are not yet fully known.

One other fact remains. This work has brought to light the activity of a group of substances and seems to have increased the inventory of the doctor by one useful tool to fight disease.[37]

The author has prepared big quantities of citrine for distribution among clinicians, anxious to test it. Naturally these experiments could not clear the problem of therapeutic application. Such problems want very extended experience, and

---

[36] Ascorbic acid is much more readily destroyed than hesperidine and the scurvy diet might contain traces of Vitamin P.

[37] The substance was mostly applied intravenously. One component of citrine, hesperidine, by its insolubility, is unfit for injection, which threatens with dangerous complications. Its other constituent, however, the eriodictyol glucoside, is soluble and can be injected without any harmful effect. According to the nature of the substance it must not be applied in fractions of milligrams but in doses of 25—200 mg. a day.

these first trials were sufficient only to indicate that this substance is a useful one. The effect on resistence and permeability of capillaries is quite definite. Its curative effect in haemorrhagic diathesis (vascular type) is definite too. (81, 82). It acts also on different internal haemorrhages (intestine, kidney, gums) of unknown origin, but its effect seems not to be limited to haemorrhage. The effects obtained in the few cases of acute nephritis were striking *(A. Lajos)* and might lead in the end to the application of vitamin P in any condition threatened with nephritis. Sepsis and polyarthritis are very difficult to judge, but the observations were by no means discouraging.

I am quoting these latter observations only in the hope that they might induce clinicians to try vitamin P in a wider field, for only wide experience will help to establish definite indications.

The fate of Vitamin P in the body will be elucidated only by extensive studies. The first experiments of *Huszák* (68) indicate that this vitamin is not appreciably accumulated or destroyed in the animal. All the same, as *Armentano* is finding in *Rusznyák*'s department, Vitamin P has to be given to man for several days before excretion starts and reaches maximal values. This indicates that a deficit in Vitamin P is a wide occurence. Patients with fever (polyarthritis) who are benefited by Vitamin P excrete this substance at once and seem to be unable to retain it.

5. On Health, Disease and Vitamins.

In the previous chapter I made the statement that Vitamin P cured certain haemorrhagic conditions. The implications of this statement are amazing, if not bewildering. We learn at school that Vitamins have a benificial effect only in case of a shortage. The food of these haemorrhagic patients was in no way different from average human food, thus there was no reason to suppose that their pathological condition was an avitaminosis. But if this is true, then Vitamin P must be looked upon as a therapeutic agent with pharmacological

activity, which again is in contradiction to our generally accepted ideas.

The case of Vitamin P could be dismissed as a curiosity, had not clinical research accumulated a number of analogous observations recently. The isolation and synthesis of the single vitamins enables the doctor to administer these substances parenterally in relatively big quantities to his patients. This application of Vitamins has led to astounding observations. We read reports on the beneficial effect of Vitamin C in pneumonia and myasthenia, of Vitamin B_1 in neuralgia, neuritis and even alcoholic neuritis. I mentioned that Vitamin P has an analogous effect in acute nephritis and restores capillaries to normal resistance and permeability in sepsis. Should all these conditions be avitaminoses? This is impossible. Or should Vitamins act as specific therapeutic agents? This seems equally impossible. It is evident that there must something fundamentally wrong in our ideas.

I believe the trouble is that we were badly misled by the animal experiment. The animal experiment has helped to lay the foundation of our knowledge of vitamins but has misled us in the more subtle question of health and diseae. We made a mistake in calling it „health" if the animals had no scurvy, beri-beri etc. We called it health when a dozen animals sitting in a protected cage grew well. In short, I believe that scurvy and beri-beri are not the first signs of unhealthiness, but are premortal syndromes. Cessation of growth is equally a grave disturbance of health. There is a long way to go from „full health" to the first disturbance of growth, or the first appearance of scurvy symptoms.[38] Accordingly there is a wide margin between the quantity of vitamin sufficient to prevent scurvy and that required to keep us at optimum condition. This partial avitaminosis is a very wide occurrence and I believe that the greater part of humanity is living within this zone. I call the condition „full health", in which health can be improved no more, in which we offer the greatest resistance to noxious influence and in which we can bear strain the best and show the highest ability. A dozen guinea pigs sitting in

[38] This might apply also to the Rumpel-Leede phenomenon.

a protected cage can tell us little about full health. Man living under civilized conditions can be compared to these sheltered animals. He might do quite well and have no indication of his deficiency, but will fail, if suddenly called upon to fight an infection, face some injury, or if by any chance he has some debility like that of the capillary system. The doctor will call the result of this failure „pneumonia" or „nephritis" or the like, and will bless the therapeutic effect of vitamins. But what he has done, in applying vitamins, is only to pay the old debt to nature and give to the body what is due to the body and what it needs for its smooth running.

Accordingly the necessary daily dose of a Vitamin is not the quantity which is needed to prevent scurvy. Applying Sir *John B. Orr's* words to Vitamins: we must increase the quantity of vitamins, till a further increase does not improve health. This is the correct daily dose, meaning by health full health, as defined above.

As an experimental worker I will try to demonstrate the inadequacy of the animal experiment by animal experiment. A few years ago *Jeney* and *Gagyi* reported on the beneficial effect of ascorbic acid in experimental diphtheria. These experiments have since been greatly extended and it is generaly accepted to-day that healthy (not scorbutic) guinea pigs can be protected against diphtheria toxin by massive doses of Vitamin C. This observation is quite analogous to the clinical observations quoted above. The correct explanation of this result is not that ascorbic acid is an antitoxin, but that we made the mistake of calling animals which have no scurvy, normal, healthy animals. If ascorbic acid increases the resistance against diphtheria, this means that it increases the health of the animal. Health, which can be increased is not „full health". This experiment simply shows that massive doses of ascorbic acid are needed to keep the guinea pig in „full health".

I can support this statement with figures. *S. S. Zilva*[39] finds that he needs 2 mg of ascorbic acid daily to keep his animals in perfect condition. At the same time he is amazed to find

---

[39] *S. S. Zilva.* Biochem. J. *30,* 1419, 1936.

that he needs ten times as much to keep his animals at maximum saturation and asks whether this maximum saturation is needed for health? He turns it down. 20 mg of ascorbic acid a day seems to be quite extravagant for a guinea pig, when the dose for man is 25—50 mg. His animals grow beautifully with 2 mg a day, so he thinks this maximum saturation is a luxury. But if he had given diphtheria toxin to his animals, he would probably have found that his saturated animals fared better than the ones kept at 2 mg and would have come to the conclusion, that a health which can be improved cannot be maximal.

But let us consider for a minute, whether 20 mg ascorbic acid a day is such an unreasonable amount for a guinea pig. Let us answer an other question first: What is a vitamin and why is ascorbic acid a vitamin for the guinea pig but not for the rabbit? Guinea pigs in their original, tropical ever-green surrounding had plenty of ascorbic acid all the year round. With every leaf consumed, ascorbic acid flowed into their bodies. Nature knows no luxury. There was no need to make ascorbic acid, so the guinea pig forgot how to make it. This the rabbit could not afford, because he would have died of scurvy in our climate, during the winter when there is no green food and no ascorbic acid is available. Thus the inability of the guinea pig to make its own ascorbic acid is an expression of its adaptation to its surroundings. All animals are perfectly adapted to their surroundings, and this is no more than natural. From the point of view of food „surroundings" means green food for the guinea pig, all the year round. A smallish guinea pig of 300 g. consumes about 120 g of green a day. This contains about 20—60 mg ascorbic acid which is about the quantity *Zilva* finds necessary to keep his animal saturated. This is also the quantity of ascorbic acid necessary to protect a guinea pig against diphtheria, the quantity which will keep it in full health, at which a further increase of ascorbic acid does not increase resistence or raise the ascorbic acid concentration of the body. One cannot do more than saturate an animal.

I expect that the correct daily dose of any vitamin will be found in the end to be that quantity of vitamin which the animal consumed in its original surrounding.

One problem that always puzzles me when I read about „therapeutic effects" of vitamins, is this: What would have happened to these patients, if they had had sufficient vitamin prior to their disease. It is very much easier to prevent than to cure a disease, and had these patients had a sufficient quantity of vitamin, they would probably never have become ill.

This brings me to the highest and most involved problem that ever occupied me as a scientist.

I started my studies with medicine and spent five years studying all those thousands of ailments, from wich we suffer. The next twenty years I spent as a biochemist in silent admiration of the wonderful complexity, precision, harmony and adaptability of living Nature. I cannot help wondering where the contradiction lies. Is man the sole imperfect creation which is kept alive only by the artificial means which his own ingenuity has created? Or is our body not less perfect than that of other living creatures, only put to a use for which it was not made? I think that answering this problem is of more than sentimental or philosophical interest. The answer may influence our attempts to improve health, reduce suffering and increase happiness.

I am convinced that Nature never creates such an imperfect system as our body appears to be. By the rules of life no such system could survive.

I believe that any living object has to be perfect and is strictly adapted to its surroundings (or else it would die). In fact it is not only adapted to but it is part of its surroundings. The limits of life are determined by the quantity of nitrogenous material. This frame is always filled out almost completely and is present in living form. We are badly misled by the apparent feeling of our individuality. Any living system is part of its surrounding, a temporary form of matter pushing to life. The animal in the jungle is only a cell, a cogwheel of this higher organism, the jungle, into which it fits perfectly. Any imperfection in this fitting would entail its ruthless extermination.

I am convinced that man's body is just as perfect as that of his fellow animals, and his apparent imperfection arises from the disharmony between his structure and his surroun-

dings. Man was not born on the pavements of cities. Man was born during innumerable years in some jungle, to the life of which he was strictly adapted. Our civilisation is of recent date and has left no marks on our body. If we want to be healthy, we must put our body back into the surroundings for which it was made.

Naturally we cannot be expected to go back to the jungle. But there is science to help us to find out which factors of our surroundings are essential, and to bring those factors into our homes. These factors are manifold: the quantity of radiation, the purity of air, amount of noise, the amount of muscular work and the reduced chance of infection, etc. etc.

One of the most important links between our body and its surroundings is certainly food. In the form of food the surroundings actually get into our body, flow througt it. And certainly vitamins are one of the most important factors of that coordination.

I am convinced that if our body is put back into the surroundings for which it was made, it will work as perfectly as that of its fellow creatures. Disease is the expression of the disharmony between our make-up and our sorroundings. There is no such thing as healthy or unhealthy. The fish feels uneasy on land, the rabbit under water. It all depends what we are made for.

The story of the island *Tristán da Cunha* is a full corroboration of all this.

I have a strong faith in the perfection of the human body, and I also think that vitamins are an important factor in its co-ordination with its surroundings. Vitamins, if properly understood and applied, will help us to reduce human suffering at an extent which the most fantastic mind would fail to imagine.