CITRUS VITAMIN ‘P’
(Citrus Bio-Flavonoids)

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Twenty years ago in 1936, Dr. Albert
Szent-Gyorgyi announced the discovery of a
Capillary Permeability factor, vitamin P, iso-
lated by him and his associates from red pep-
per and lemon. At first he believed that vita-
min P was a single chemical substance. How-
ever, several months after his announcement,
he rectified his statement admitting that vita-
min P is a compound of several flavonoids.

Immediately after the discovery of vitamin
P, the California Fruit Growers Exchange em-
arked on an extensive investigation of vita-
m P. The California scientists isolated sev-
eral flavonoids present in citrus fruit and
synthesized some, like methyl-chalcone hes-
peridin. The results of the clinical trials with
their flavonoid compounds were not very en-
couraging, and somewhat disappointing.

By 1946-47, the whole problem of vitamin
P reached a critical state. The work of Szent-
Gyorgyi seemed to be discredited and con-
troversial. The vitamin nature of vitamin P
was denied by some workers, its therapeutic
value questioned, and some workers even
claimed that vitamin P or flavonoids are
neither absorbed nor assimilated by the or-
ganism (Clark). It was under these highly
unfavorable conditions that the Southern Bio-
Research Laboratory in 1947 began the in-
vestigation on citrus vitamin P.

We visualized two possible roads of attack-
ing this problem. One, to follow the steps of
California and to try isolating various citrus
flavonoids, or to return to the original con-
cept of Szent-Gyorgyi and to work with vita-
m in P as a flavonoid complex. We chose the
second approach and decided to work and to
investigate the flavonoid compound we ex-
tracted from citrus wastes. This compound,
composed of the flavonoids naturally present
both in grapefruits and oranges, we called
citrus vitamin P, or C.V.P. It is water soluble,
and it contains several flavonoids, apparently
forming one or two complex flavonoid mole-
cules, as flavonoids tend to do so easily. It
was this compound that we have been inves-
tigating experimentally and clinically for the
last nine years.

The Bio-Assay for Vitamin P

The chemical tests on flavonoids are of a
limited significance. The boro-citrate test, the
Lawrence test and others give an indication
of the chemical nature of a flavonoid but they
do not disclose the biological activity of the
compound. Thus, our second problem was to
work out a reliable bio-assay. Ambrose and
DeEds offered a method of testing capillary
permeability by applying chloroform to the
skin of rabbits and injecting trypan blue dye.
Although this method has some merit, it is
not sufficiently exact for any quantitative test.
Gradually we elaborated a bio-assay technique
which we believe is accurate and dependable.
This method is based on the discovery of Dr.
M. J. Shear of the National Health Institutes
that the polysaccharide isolated from Serratia
marcescens induces an extensive hemorrhage
in the tumors of animals. (1)

The bacterial polysaccharide preparation
supplied to us by Dr. Shear and labeled P-25
is well standardized. A dose of 0.5 mg. in-
jected in a rat, 150 grams weight and bearer
of a tumor two inches in diameter, kills the
animal in 6-7 hours. Death is caused by a pro-
fuse capillary bleeding and a destruction of
numerous capillaries of the tumor.

Our tests with citrus vitamin P have demon-
strated its biological activity. Table I gives
the data pertaining to one of our tests.

When 3 mg. of the citrus vitamin P com-
pound were injected one hour before the in-
jection of bacterial polysaccharide, the animals lived an average of 20 hours instead of an average of 7 hours without vitamin P. However, the dose of vitamin P was increased to 10 mg., 66% of the animals survived and 33% lived an average of 45 hours. (2)

TABLE I

THE PROTECTIVE ACTION OF CITRUS VITAMIN P AGAINST THE HEMORRHAGE-INDUCING ACTIVITY OF BACTERIAL POLYSACCHARIDE, P-25 (*)

<table>
<thead>
<tr>
<th>Rat Nos.</th>
<th>Treatment</th>
<th>Citrus Vitamin P</th>
<th>Result: Death or Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>171-A, m</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 17 hrs.</td>
</tr>
<tr>
<td>171-B, f</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 22 hrs. 25 min.</td>
</tr>
<tr>
<td>171-C, m</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 18 hrs.</td>
</tr>
<tr>
<td>171-D, m</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 19 hrs. 10 min.</td>
</tr>
<tr>
<td>171-E, m</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 20 hrs. 40 min.</td>
</tr>
<tr>
<td>174-A, f</td>
<td>0.5 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 19 hrs.</td>
</tr>
<tr>
<td>174-B, m</td>
<td>0.45 mg P-25</td>
<td>3 mg Citrus Vitamin P</td>
<td>Death in 24 hrs. 30 min.</td>
</tr>
<tr>
<td>174-C, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Death in 36 hrs.</td>
</tr>
<tr>
<td>174-D, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>174-E, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Death in 52 hrs.</td>
</tr>
<tr>
<td>174-F, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-A, f</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-B, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Death in 66 hrs.</td>
</tr>
<tr>
<td>177-C, f</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-D, m</td>
<td>0.4 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-E, m</td>
<td>0.45 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-F, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>177-H, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Death in 26 hrs.</td>
</tr>
<tr>
<td>178-A, m</td>
<td>0.5 mg P-25</td>
<td>10 mg Citrus Vitamin P</td>
<td>Survived</td>
</tr>
<tr>
<td>178-B, m</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 6 hrs. 25 min.</td>
</tr>
<tr>
<td>178-C, f</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 7 hrs. 35 min.</td>
</tr>
<tr>
<td>178-D, m</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 9 hrs.</td>
</tr>
<tr>
<td>178-E, f</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 7 hrs. 30 min.</td>
</tr>
<tr>
<td>178-F, f</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 8 hrs. 20 min.</td>
</tr>
<tr>
<td>178-H, m</td>
<td>0.5 mg None</td>
<td>None</td>
<td>Death in 7 hrs. 15 min.</td>
</tr>
</tbody>
</table>

(*) P-25 is a preparation of Shear bacterial polysaccharide.
Rechecking our results, we found that a dose of 12 mg. gave a complete protection to all animals receiving the deadly dose of 0.5 mg. of bacterial polysaccharide. Thus this dose served us during all our further investigations as the basis of our bio-assays.

During the following years, we had the opportunity to test various citrus flavonoids isolated by us or produced by Californians or some other companies. We found that water-insoluble hesperidin gave no protection against capillary hemorrhage tested by this method, even when given in a dose ten times larger than our protective dose of 12 mg. Taking the index 1.0 (corresponding to 12 mg.) as a departing point for the tests of other flavonoids, we found that methyl-chalcone hesperidin showed a very mild capillary activity with an index of 0.1. The synthetic phosphorylated hesperidin exerted an activity about 0.15. Rutin gave an index of 0.2. On the other hand, the lemon infusion prepared by the California Fruit Growers Exchange has a relatively high index of 0.3, or approximately three times less capillary activity than our Florida citrus vitamin P compound. (Table II)

### Table II

<table>
<thead>
<tr>
<th>Compounds</th>
<th>The minimal dose of flavono-</th>
<th>Index of biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus vitamin P compound</td>
<td>12 mg.</td>
<td>1.0</td>
</tr>
<tr>
<td>Water-insoluble hesperidin</td>
<td>120 mg.</td>
<td>0</td>
</tr>
<tr>
<td>California methyl-chalcone hesperidin</td>
<td>120 mg.</td>
<td>0.1</td>
</tr>
<tr>
<td>Phosphorylated hesperidin, N.D.C.</td>
<td>60 mg.</td>
<td>0.15</td>
</tr>
<tr>
<td>Lemon infusion, excont. California</td>
<td>35 mg.</td>
<td>0.5</td>
</tr>
<tr>
<td>Rutin</td>
<td>60 mg.</td>
<td>0.2</td>
</tr>
</tbody>
</table>

(*a*) Rats, British breed, average weight 150 gm., bearers of Crocker carcinoma, two inches in diameter.

Having asserted and proved to our own satisfaction that the Florida citrus vitamin P compound is biologically superior to the ones produced by Californians, we embarked on the clinical investigations with this compound.

**The Physiology of the Capillary System**

The medical profession fully realizes the important role which capillary dysfunction plays in many diseases. Stefanini and Dameshek (3) in their recent book on hemorrhagic disorders point out capillary fragility as the cause of abnormal bleedings. One must clearly visualize that the essential exchange of body fluids takes place in the capillaries and that the role of the large blood vessels is actually limited to transporting blood to the capillaries. The peculiar paradox of the human organism is that the capillaries are easily injured by numerous bacterial and chemical agents or by metabolic disturbances.

We know by now that increased capillary fragility is a common phenomenon, much more so than we thought ten or fifteen years ago. The work of Griffith (4), Beardwood (5), Greenblatt (6) and many others indicate that the capillaries are abnormally fragile, and therefore might bleed easily in numerous diseases such as arteriosclerosis, hypertension, and particularly so in diabetes (7). When a stroke (apoplexy) occurs, this means that some capillaries of the brain tissue became over-fragile and broke down causing bleeding, often fatal. In many bacterial infections and in almost all virus infections, capillary fragility, localized or generalized, is present. (8, 9, 10). The inflammation of the mucous membrane itself, when one has a sore throat, or swollen gums, or pneumonia, or any other infectious disease is closely associated with the injury to the capillary system. Even in heart failure, with sudden death or coronary thrombosis, one might blame capillary injury for the tragic accident. For in such cases, the so-called intimal capillary, which is located in the wall of the larger coronary vessels is abnormally fragile and might suddenly break down and bleed. If the bleeding is profuse, man dies at once. When the bleeding is very small, a blood clot is formed and coronary occlusion, known as coronary thrombosis, takes place. (11, 12, 13, 14). Older people more frequently have increased capillary fragility than younger ones, and the danger to their lives from capillary bleeding is higher. (15, 16, 17).
CLINICAL INVESTIGATIONS

The clinical investigations, in which ninety-two physicians associated with various hospitals took part, and the results of which were reported in sixty-three papers published in medical and scientific journals, have demonstrated the therapeutic value of the Florida citrus vitamin P compound, isolated by us from citrus waste, in the following conditions where increased capillary fragility or capillary bleeding was evidenced.

Radiation erythema (18, 19, 20)

Tuberculous hemoptysis (21, 22)

Habitual Abortion (23, 24, 25, 26, 27, 28)

Erythroblastosis Fetalis (29, 30)

Bleeding Gastric and Duodenal Ulcers (31, 32, 33)

Cerebral Hemorrhage: Stroke (34, 35)

Retinitis (36, 37, 38)

Dental Diseases and Surgery (39, 40)

Hemorrhagic Cystitis (41)

Hemorrhagic Diathesis (3, 42, 43)

Increased Capillary Fragility (44, 45)

Altogether about 9,000 case histories were collected during the last seven years.

To conclude: The experimental and clinical studies on Florida citrus vitamin P compound extracted from citrus waste, have supplied the evidence of its therapeutic value in increased capillary fragility and capillary bleeding. Indirectly, the data so collected confirms the original findings of Szent-Gyorgyi and his associates concerning vitamin P.

REFERENCES

(15) Perry, D. J. and Linden, L.: Science Newsletter, April, 1953.
(29) Griffith, J. W., Jr., and Linden, L.: Science Newsletter, April, 1953.