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Historical Records of Australian Science

Supplementary Material

The contributions of Rupert Best to the modern concept of the nature of viruses

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PRG 232/44

from Dr. Rupert J. Best
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21st September 1977

Professor P.J. Quirk
Director
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Dear Jim,

Some time ago you asked me to let you have a letter setting out the early history of my involvement in work with plant viruses, with special reference to early discoveries on the nature of tobacco mosaic virus. Recently, Bob Symons has asked me to do something of the same sort in view of Fraenkel Conrat's impending visit ; so I propose to write the promised letter to you, and to send Bob Symons a coyp for his information. In order to get it done in time I will keep it brief, and can prepare a fuller account later in the year after consulting what note books have survived, and the relevant published papers.

Perhaps I should begin with a statement on how I had become involved. During the second half of 1933 Geoffrey Samuel called on me for advice on some problems they had encountered in the study of Tomato Spotted Wilt (TSW) virus. When I counselled them to test the relationship between pH values and the activity of TSW virus and also redox potentials and to use TMV as a control system, they said pH and redox potentials were a closed book to them and Samuel invited me to design and conduct experiments along the lines of the chemical approach I had suggested and agreed to supply virus infected plants and quantitative assays on my products. Ever since my honours chemistry years I had been keenly interested in the borderland between chemical and biological systems and gladly accepted the opportunity to work in this area provided that I could address myself to the fundamental question of the nature of viruses and could work with both TSW and TMV which seemed to be at extreme ends of a spectrum in respecy to stability and size. Prof. J.A. Prescott, who was then Head of the Dept.of Agricultural Chemistry, agreed to the collaboration.

We had barely begun when J.G. Bald went off to Cambridge and Geoffrey Samuel was appointed mycologist to Rothamsted Experimental station in U.K. However, from December 1933 to Easter 1934 Samuel and I worked seven days a week, day and night, and produced results that were subsequently published in three papers. These early results were so encouraging that when Samuel left the W.A.R.I. at Easter, 1934, I decided to continue on my own.

Since no-one else at the W.A.R.I. was interested in viruses I changed my approach and concentrated first on TMV with a view to isolating it in as pure a form as possible so that (a) it could be stored in either wet or dry form to use at any time in a ' standard condition and (b) to build up stocks of pure virus to examine chemically and to determine its nature and properties.

This early work was entered in W.A.R.I. note book No 53 with experiments dated from 13 June 1934 to 9 October 1934, and continued in W.A.R.I. note book No 62 now in the S.A. Archives).

I attach xerox copies of pages 153 and 161 and their facing pages (from N.B. No 53) At the top of page 153 is written

' 13/6/34 Tobacco Mosaic Virus '

" Clarification of Mosaic juice ~~c~~ a view to obtaining a precipitate of the virus as free as possible from extraneous substances. "

Then follows a description of the experiment .

On the facing page there is a summary of the lesion counts and the conclusions (inter alia)

" at constant buffer conc. & juice conc. susps at pH 3.4 gave better results then susps at pH 3.1 & 4, 0 " and

" for future work use buffer at pH 3.4. & .08 M & juice conc. 1/10 "

The entry at the top of page 161 reads :

" 11/7/34 Tobacco Mosaic Virus "

" Iso-electric " point of ppt c Y to pH value

The lesion counts, summarised on the facing page,

when plotted confirm pH 3.4. as the iso-electric point .

Lost Records

The techniques and results of chemical tests were entered in smaller octavo size stiff covered note books for greater convenience.

As these octavo books accumulated and the results they contained had been incorporated in published papers they were handed to the administration for safe-keeping, and eventually found their way to the " strong room ". Unfortunately, the strong room became crowded and the contents were vetted from time to time. One of these occasions occurred while I was on study leave and my octavo books were destroyed along with other people's. I was told that some-one had been consulted and had agreed that as the results they contained had been published they were of no more use . The dates on which some results were obtained are therefore lost. However, by the end of 1934 I was satisfied from qualitative tests and nitrogen assays that TMV was predominantly protein, but a complex one with at least two active parts Prescott had gone on study leave in 1934 for nine months and on his return I suggested that I publish a short note on my discoveries (especially the protein nature of TMV and its iso-electric point).

He took a firm stand that the results I claimed were so revolutionary that I should publish nothing until I had water-tight proof. So the investigations went on.

During the second half of 1935 I had decided to publish a short paper in J. Aust. Inst. Ag. Sc. on " The effect of environment on the production of primary lesions by Plant viruses ", and I took the opportunity to insert the following sentence as a footnote to page 160 of that paper :

" The inocula were prepared from a sample of virus purified by precipitating it from clarified plant juice at the iso-electric point of the virus or associated protein (pH 3.4. ± .2)

That journal appeared in December 1935. At the same time I had prepared a longer paper which was submitted to the Aust. J. Exp. Biol. & Med. Sc. on 8 February, 1936 ; and it appeared as the first paper in the March issue.

I was not aware of Stanley's note in Science, 1935, until he himself wrote , on reading my paper in the March, 1936, number of Aust. J. Ept. Biol to say that he thought he and I had isolated the same thing. In those days I read a wide range of specialist scientific journals but confined my reading of populat journals to " Nature ", and did not read " Science ", which is those days was not highly regarded . The degree of agreement and disagreement between my results and those of Stanley and of Bawden and Pirie are commented on in what follows.

In order to complete this account of my early work I will now list the more important papers with expracts to indicate their major significance.

SOME EXTRACTS FROM SOME RELEVANT PAPERS

1935 J. Aust. Inst. Agri. Science . Vol 1 No 4 pp 159-161 (Dec. 1935)

footnote to p 160

"The inocula were prepared from a sample of virus purified by precipitating it from clarified juice at the iso-electric point of the virus or associated protein (p H 3.4 ± .2)"

1936 Aust. J. Exp. Biol & Med Sc. No 1 Vol 14 pp 1-13 (1936)

Precipitation of the tobacco mosaic virus complete at its iso-electric point by Rupert J.Best.

Some extracts from the summary p 13 :

" The point of maximum precipitation is at pH 3.4. where , under suitable conditions , more than 99 % of the virus can be precipitated "

" The precipitate gave positive tests for protein and desiccator dried samples contained 14 p.c. nitrogen ".

1936 Aust. J. Exp. Biol. Med. Sc. Vol 14 pp 323-328 (submitted 26 October 1936)

by Rupert J.Best

The relationship between the activity of tobacco mosaic virus suspensions and hydrion concentration over the pH range 5 to 10.

Extract from the introduction (p 323):

" solutions of purified virus may be kept for long periods (over a year) without any apparent change in concentration of active virus units ".

" It is concluded that inactivation of the virus is associated with the neutralisation of acidic groups. "

The bearing of these results on the nature of the virus is discussed and it is suggested that the groups thus neutralised are an integral part of the chemically reactive prosthetic groups of the virus ".

also on p 327 in the Discussion

" It therefore seems likely that the groups entering into the reactions recorded in this paper are not concerned in the iso-electric reaction and that this latter is determined by some other portion or portions of the complex ".

1936 Report of the W.A.R.I. 1933-36 (printed 1937) This account would have been written in Nov. or Dec. 1936)

Investigations on Plant virus Diseases (Rupert J.Best)

on pp 88

" Although the virus (TMV) remains active between pH values 3 and 4 it precipitates from solutions over this range, and the point of maximum precipitation is at pH 3.4. (3,11). At this pH value practically the whole of the virus in a sample of infective juice may be precipitated, and the product appears to be homogeneous. Chemical tests and the nitrogen content of the virus precipitate showed it to be a protein or protein complex (11) ".

also

" Some observations on the probable nature of viruses.

"As a result of work carried out in the United States, in England and at this Institute, it is now fairly evident that the virus responsible for ordinary tobacco mosaic is essentially protein in nature. Little is as yet known about the make-up of this protein, whether , for example, the activity resides in the protein proper or whether this latter is conjugated with some prosthetic group or groups. On the available evidence and from general considerations the latter would appear to be more likely. Under suitable conditions the virus particles link up into long thin, flexible needles or threads. These show double refraction with straight extinction but have the properties of the mesomorphic state ----- ".

" Viewing the plant viruses as a class and taking into account the properties possessed by them in common with other viruses, of being able to multiply in living cells, and their wide range in size, the most logical picture of them appears to be that of a class of complex organic structures built on a protein base with a large number and variety of active prosthetic groups, these latter entering into the biochemical reactions through which the viruses become evident and which are at the same time concerned with processes which we have come to associate with life and living ".

The paper then goes on to call viruses " living molecules "

1937 (1) Nature Vol 139 pp 628-629 April 10 1937 (date of letter Jan 30)

Visible mesomorphic Fibres of tobacco Mosaic Virus in Juice from Diseased Plants

By Rupert J.Best,

Photographs in ordinary and polarised light to show character and optical properties (extinction)

(2) Nature Vol 140 pp 547 - 548

Artificially prepared visible paracrystalline fibres of tobacco mosaic virus nucleoprotein

by Rupert J.Best

Composition of paracrystalls recorded as 16.6 p.c. nitrogen and 0.52 p.c. phosphorus.

My phosphorus figures are in line with Bawden and Pirie and contrary to Stanley's findings that TMV contains no phosphorus or only small and variable amounts as impurities.

1937 Aust. Chemical Inst. J. & Proc. Vol 4 No 10

The chemistry of some plant viruses

by Rupert J.Best (report of paper read at Conference, May, 1937)

A critical review of my own and other people's work on plant and animal viruses, the last four lines

" viruses may be regarded as living molecules of graded complexity of structure and organisation covering the transition between the architecture of the larger non-living chemical molecules and the architecture of the simplest living cell "

I again argue against Stanley's claim that nucleic acidis not an essential part of TMV and give reasons as to why it is an essential component.

1939 J. Aust Inst. Agri. Sc. Vol 5 No 2 pp 94-102

Virus activitity as a property of some Protein Molecules

By Rupert J.Best,

A paper presented at a symposium on " The relation between chemical constitution and biological activity "at the Canberra meeting of ANZAAS in January 1939.

It was mainly concerned with presenting data on the chemical, strological, and biological properties of viruses and attempting to relate them . The penultimate paragraph contains these words (inter alia) " We may regard the virus molecule as a sort of template. By virtue of its surface electronic forces it would attract to its surface from its environment the essential building blocks which go to make up it structure -------- ".

" It is possible that conditions in this environment ..... may be such that a building block here and there may be slightly different from the standard pattern without detracting from the power of the molecule to multiply. This would readily explain the occurence of mutatious and strains, and variations caused by passage through different hosts. "

In conclusion, my use of the terms " virus complex " ," protein complex ","prosthetic groups " and so on right from the beginning, illustrates that I have always interpreted my results as derived from a macro-molecule consisting of protein plus something else chemically attached to it in some way, and behaving like a " prosthetic group " which was the basis of the specific actions of the virus.

In one 1936 paper my data on the reaction of TMV to pH values show clearly that two quite different parts of the virus are involved , one at pH 3.4. which I ascribe to the normal protein function and the other at the alkaline range where viral inactivation resembles a neutralisation curve which I ascribe to my postulated " active prosthetic group ". I believe I was the first to postulate the two parts of TMV, and to produce evidence for it.

In 1937 I identified the " prosthetic group " with nucleic acid .

I have noted that the Nobel Prize has been mentioned from time to time in the context that although I independently discovered the essential nature of TMV Stanley published aarlier and got a Nobel Prize. These facts are, of course correct; but I would like to make it clear that I have never thought of myself as being " in the running " for the Prize , or of just having missed it. I just didn't think that way. I think the idea may have arisen from the following :

It was reported to me that after Fraenkel Conrat's visit to Melbourne some years ago that he stated there that I had just missed the Nobel Prize. Another possibility is that Fred Bawden was very vocal on this matter ; and I remember him saying at the end of a seminar I gave at Rothamsted in 1950 that my first preparations of TMV were complete virus whereas Stanley's (lacking phosphorus) could not have been and that I should have got the Noble Prize because of my work stood the test of time and Stanley's did not.

In spite of this I still think that since no Australian had received the Noble Prize at the relevant time my chances would have been nil. Since the matter has been raised by others perhaps it would not be considered immodest of me if I now express an opinion : in retrospect I think it would have been appropriate for that particular prize to have been awarded jointly to Stanley, Bawden , Pirie and myself. Having said that, I would like to say that never having expected it I have never felt a sense of loss; but I continue to feel a sense of achievement, and still get great pleasure from the thrills that accompanied those early discoveries.

Kind regards .

Yours sincerely,

Rupert J. Best