GRW - SS Cohen: Regarding analyses of DNA of bacteriophages and vaccinia; identification of 5-hydroxymethyl-cytosine. [Correspondence]

Publication/Creation

22 May 1951 - 10 April 1954

Persistent URL

https://wellcomecollection.org/works/mdgakhck

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

nature publishing group

NATURE PUBLISHING GROUP
PO BOX 1051 STN MAIN
FORT ERIE ON L2A 6K7

Nature Publishing Group is interested in the delivery of your journal, and we are committed to doing so in a prompt and accurate manner. **PLEASE RESPOND TO THE QUESTIONS BELOW** to help us ensure that you are receiving an excellent level of delivery service. Thank you very much.

Magazine title: NATURE
Issue Date: April 3, 2003

Date of receipt: _____/_____/_____
Month Day Year

Condition of magazine: ☐ **Excellent** ☐ **Good** ☐ **Poor**

PLEASE MAKE ANY ADDRESS CORRECTIONS NEXT TO ADDRESS LABEL

Please fax this sheet to the following fax number:
United States: **+1 212-696-9591**

OR

Send via email to: **fulfillment@natureny.com** and remember to respond to the above questions in your email. Thank you.

PLEASE VISIT OUR WEBSITE AT HTTP://WWW.NATURE.COM

8/2 DIR(G)

068KIWYATTG0064 Exp 0 342
G R WYATT
QUEENS UNIV

DEPT OF BIOLOGY
KINGSTON ON K7L 3N6

PUBLICATION AGREEMENT 70032744

Jan 31, 1953. Letter to Dr. Hampil re results from vaccinia - 'molar quantities - - - general (& quite unexplained) rule in DNAs.'

Jan 31 letter to Cohen - 'ratios are not at all exciting, though they conform to the Chargaff formula'

March 10th, 1954

Dr. S. S. Cohen,
1740 Bainbridge Street,
Children's Hospital of Philadelphia,
University of Pennsylvania,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

Correction! The last sentence in the first paragraph of my letter should read "I could let you have a few more if you need them." Sorry!

Yours sincerely,

GRW/m

G. R. Wyatt.

March 10th, 1954

Dr. S. S. Cohen,
Department of Pediatrics,
Children's Hospital of Philadelphia and
Department of Physiological Chemistry,
University of Pennsylvania,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

The reprints came in just yesterday, and I am sending you about 300. No reimbursement necessary. I have had quite a lot of requests, and am also sending them to a small list of people mainly interested in nucleic acids. I am not sending them to any general list of phage people, thinking that you might be doing that. Could you let me have a few more if you need them.

There is no more word on the fellowship -- official notification is not until April. I wrote also to van Niel after seeing you in October, applying to get into his summer course. He reserves judgement, also promising word in April, but I understand it is quite a hopeful sign that I even got a reply from him!

I have been busy, with very wavering confidence in the value of it, on the composition of silkworm serum, and Silver has been testing the results in concocting tissue culture media. Just this week, for the first time, we have some results that look really promising, with a completely original mixture. With good tissue cultures, insect viruses would be a nice system for biochemical study.

For diversion, I begged some diaminopimelic acid and ran a chromatogram of T6r$^+$. There's not a trace of it in it.

Dr. S. S. Cohen March 10th, 1954

Your thymineless results sound most intriguing. That would be very nice if you could be in Berkeley from next February. I expect to be at the Federation meetings, and look forward to seeing you.

Sincerely,

GRW/m G. R. Wyatt.

UNIVERSITY of PENNSYLVANIA
PHILADELPHIA 4

The School of Medicine
DEPARTMENT OF PEDIATRICS
1740 Bainbridge Street
Philadelphia 46, Pa.

March 2, 1954

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Canada Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

Have the reprints on HMC in the Biochem. Journal come in? If so, I would greatly appreciate having 300, as we discussed earlier. I have a fair number of requests to fill, although I imagine you got most of them.

How are things going? Will you be with Stanier next year on the Guggenheim? I am pointing to a sabbatical leave for next February at Berkeley, and it will be nice if our visits could coincide.

I haven't seen a phage particle in months. We have done a fair number of things on the thymineless bug in the uninfected state and have clarified thymineless death resulting from growth without thymine. The bug works itself into a structural impasse from which it can't back out, a very curious phenomenon which may have some chemotherapeutic interest. We have seen enzymatic adaptation in the absence of thymine.

I hope we will get together soon. Will you attend the Federation meetings?

Sincerely yours,

Seymour

Seymour S. Cohen

SSC/bj

October 2, 1953.

Dear Seymour,

I have exchanged correspondence with Stanier, and there seems to be a fair prospect of my going to Berkeley next summer or fall, which would suit me excellently. The immediate problem is to concoct a project outline for the fellowship application (due by Oct. 15), which I am not finding too easy since it involves such a complete change from the work I have been doing. Fortunately, one is apparently in no way held to the outline in practice, but it is necessary to produce something which at least appears reasonable at the moment. Naturally, I incline to do something with pyrimidines, they being the only group that I know enough about even to make a plausible scheme. It is impossible to plan a project like this in any detail a year ahead, especially when dependent on the literature, a year behind, to know what others are doing, so I thought of trying to make the plan as general as possible, and indicating that I would attempt to contribute to elucidation of the intermediary stages between orotidine and the various nucleosides in some convenient miroorganism. However, I assume that this is exactly the region where you are working this winter, so I want to ask if you care to make any comments now as to parts of the problem that you may expect to concentrate on, or to leave alone. This might enable me to make the outline a little more specific; however, I hope it would be acceptable to follow the more sensible procedure of submitting a general scheme and then picking up the threads wherever they have got to by next fall.

As to methods, I thought of indicating:

(1) To look for compounds which may accumulate during growth of cells in the presence of substances likely to interfere with pyrimidine metabolism (thiouracil, thiothymine, 2,4-diaminopyrimidine, sulfanilamide, methylfolic acid).

(2). To find the effects of cell-free extracts on probable intermediates, especially nucleosides, and perhaps to carry out partial purification of enzymes.

(3) Possibly, in the light of the results of the above methods, to follow the fate of tracer-labelled compounds.

This, no doubt, looks very naive, and comments, if you have any, would be welcome. Also, am I stepping into an over-crowded field here-- I gather that Larry Weed is out of this for the moment at least, but are Gots or others doing all these things? Perhaps there will be nothing left to do in a year's time!

I have finally shaken off the serological project which I have been supposed to be doing for about 3 years, and am starting some analytical work on insect blood. This will probably not be very exciting in itself, but we are hoping it may help to put our attempts at insect tissue culture (which have so far been merely tantalizing) on to a sounder basis, and if that should work, much dull labor would be justified.

I hope your book has made great strides, and that you're not feeling obliged to spend the winter at it. Not likely, I guess!

I am hoping to get to this virus meeting in Detroit Oct. 22 etc., but don't know if it will be authorized. It would be a pleasure to see some faces from the Great Outside again.

Sincerely,

UNIVERSITY of PENNSYLVANIA
PHILADELPHIA 4

The School of Medicine
DEPARTMENT OF PEDIATRICS
1740 Bainbridge Street
Philadelphia 46, Pa.

October 6, 1953

Dr. G.R.Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

I do not think you will be doing anything that I hope to do. I really hope to get to the hydroxymethylation problem eventually and I don't think the steps you mention (orotidine etc) will intersect in a competitive fashion. I may add, however, that I am so encumbered by paper work right now that it is questionable whether I shall ever intersect with anyone again.

Specifically it will be very useful to look for accumulations as you propose. May I suggest, however, that you try to avoid the rut of paper chromatograms which has been so fruitful in the past, and that you really learn bacterial physiology and nutrition from Stanier and Doudoroff? This will last you for many years and even if you flit about and do not get many publications in the first year, it will be highly remunerative later; and of course the enzyme techniques as well. I am really very pleased that you seem to be getting together with Stanier, for the sakes of both of you.

The pyrimidine field is getting to be crowded but there is plenty of room for good work. An aspect which I consider to be particularly important is that of the role of the uridine coenzymes in carbohydrate and protein metabolism. A good start there can provide a line of work for many years to come. Perhaps Doudoroff can make some suggestions.

See you in Detroit.

Sincerely yours,

Seymour

Seymour Cohen

SC/js

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

October 1, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

The enclosed proofs were sent to you at Children's and I opened the envelope suspecting the nature of the contents. I've read them cursorily, noting 2 minor misspellings, and leave them to your careful examination. I should like 300 reprints and wonder how I'm to pay for them. Will you please bill me or something?

We have been continuing in the main with our thymineless bug, since I still have too much paper work to get going again on HMC. But it will begin.

With best regards, I remain

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:KG
Encs.

Marine Biological Laboratory
WOODS HOLE, MASSACHUSETTS

August 3, 1953

Dear Jerry:-

Roger Stanier is to be reached at the Department of Bacteriology, University of California, Berkeley. I know nothing of time, ~~[illegible]~~ and money but Roger can probably steer you to the proper channels. I am seriously contemplating a year on this campus myself, to work with Howard Schachman and learn and relearn some physical chemistry, math etc. and this will probably depend on whether I can shelve certain responsibilities at Penn. I would like very much to do this and it would be very nice to have all the sharp and cheerful people about.

I hope to be in Chicago for the ACS symposium on one carbon fragment metabolism on the way to or from Upsilon. Will you be there? I shall greet Lloyd for you if you are not.

Then back to the salt mines. I have written 4 chapters this summer and have a mere 4 or 5 to go. So that I have the choice of experiments or writing this next year. Which would you guess will win?

Best regards

Seymour

John Simon Guggenheim Memorial Foundation
551 Fifth Ave.
New York 17.

Marine Biological Laboratory

WOODS HOLE, MASSACHUSETTS

July 24, 1953

Dear Jerry:—

The reprints, ~~when~~ if they arrived, are home. I'll slip one when I return.

The $HClO_4$ result is very interesting. I'll bet that this is related to the enzyme resistant nucleotide, which my instinct says has a linkage through the $-CH_2OH$ group. I expect to take a whack at this when I get back - I have all sorts of ideas linking the thiamine problem to this. Why don't you cook up thiamine with $HClO_4$ and formic acid to see if you can get the pyrimidine out? My guess is that $HClO_4$ will destroy, HCOOH will permit the isolation of the pyrimidine.

The swimming is fair the tennis better.

Roger Stanier asked me if you were willing to take a Guggenheim Fellowship to learn metabolism at the Univ. of Calif. He thought he could arrange it.

With best regards

Sincerely

Seymour

July 31, 1953.

Dear Seymour,

Many thanks for your note. As youknow, I'm skeptical about any novel linkage from HMC in DNA, but it would be fun if true. At the moment I'm miserably bogged down in this section on papyrography for the nucleic acid book (now a month overdue), but when I feel like a diverting experiment I may try what you suggest with thiamine.

That is very good of Stanier, and the idea does appeal to me. I had decided not to follow up Weisblat's enquirey, which is undoubtedly a good research opportunity but sounds a little bit nose-to-the-grindstone for my personal inclinations. So we shall probably be here through the coming winter, and I had decided to look around for some sort of fellowship to go somewhere to learn metabolism in 1954, but had scarcely thought of a Guggenheim. What would be the next step in following this up--should I write to Stanier? Is he at Woods Hole now? I take it that he does mean 1954, not 1953?

I got some information on the virus lab now being built by our Department of Health and Welfare in Ottawa which is supposed to open in about a year's time, and sounds like quite a good thing. They have already got Joe Morgan there doing tissue culture, and it sound as if there is a chance for a good program to develop.

Sincerely,

May 25, 1953

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia 46, Pa.

Dear Seymour,

Here is your paper, which I have read with great interest and pleasure. At only one point do I question with your intrepretation—the suggestion that the resistance of T-even phage DNA to DNase enables its survival in the host. However, I hope that you will leave this as it stands, and I can raise the point in the discussion. As for the double band of HMCDR on paper chromatograms, this sounds as if it could be a salt effect from residual perchlorate or phosphate; at any rate, there are several precedents for double spots as artefacts on paper chromatograms.

The final suggestion that perhaps the virus contributes something which makes possible the synthesis of HMC, and similarly with thymine in 15_{T^-}, is quite exciting. It would be nice to suppose that the virus contributes the appropriate genetic unit to make possible production of the required enzyme as an adaptive enzyme; however, I presume your statement that HMC and thymine were synthesized immediately after infection eliminated this possibility.

In my contribution to the discussion, I shall mention the conditions of hydrolysis necessary to get quantitative recovery of bases from DNA, and show a table with base ratios from T6r+ (isolated DNA), T5, vaccinia, and ox spleen, and point out that these tend to support specific pairing of the bases as postulated by Watson, and discuss just how far Watson's theory is helpful in accounting for biological duplication of DNA.

This is not strictly relevant to your line of argument, and if Watson's structure is to be presented or discussed at another session, these remarks might well be transferred there. I shall enquire about this.

Many thanks for letting me see your paper. I feel frustrated with insect viruses we have still no alternative but to use the biological assortment of cells represented by a whole animal, and any biochemical studies are virtually impossible!

Sincerely,

GRW/ s G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

May 12, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

I'm pleased to hear you had an enjoyable, if hectic, trip. I plan to mention the isolation of HMC, and present the analyses on the T-even phages. I shall mention the absence of HMC in the other viruses and perhaps you can give the detailed data on this question. I have already written this section of my paper.

As for the rest, I will discuss the isotope experiments on the origin of HMC, the enzyme degradation work, the isolation of HMC deoxyriboside, assimilation data using HMC and deoxyriboside with B, mutants, enzymatic deamination and possible significance of all this. Finally, I will present our data to show that our thymineless bug makes thymine when infected in the absence of thymine.

Sincerely yours,

Seymour

Seymour S. Cohen

SSC/bj If I get an early copy of the paper which is 5/6 written I'll send it to you as quickly as I can

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

February 24, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Canadian Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

Enclosed you will find one copy of the paper to which I have added certain things, suggested others, and more particularly in the discussion approved of sections which seemed all right as far as they went. I propose elaboration along the lines which consist mainly in posing problems

1. We should present the formulae of 6 pyrimidines

cytosine	HMC	5-methyl cytosine
uracil	HMU	thymine

and indicate that metabolism may go as doexyribosides, not as free base.

2. Note that HMC had added a C_1 unit at the level of formaldehyde.

3. Inquire whether $-CH_2OH$ is the intermediate in methyl synthesis to 5 Me cyt and thymine.

Elsie Harrison

4. Point out that the B-carbon of serine which cleaves to formaldehyde is also implicated in methyl group synthesis.

Elsie Wells deHaan

5. Note that folic acid is implicated in methyl group synthesis and wonder whether it doesn't really function in hydroxymethyl transport rather than formyl - as implied by formyl folic acid. The hydroxymethyl folic acid doesn't seem to have been made yet.

?

6. If so, is this involved in the sulfa inhibition of T2, T4, and T6 recorded by Rutten and Winkler? Even though HMC deoxyriboside doesn't permit T2 synthesis in the presence of sulfa the question isn't answered. Answer to question, Rutten et al didn't test T5.

7. Meningopneumonitis lacks HMC. Sensitivity to sulfa of psittacosis group may be due to need for many folic acid functions (purine, thymine, etc. synth) other than HMC synthesis.

8. Is hydroxymethylation direct to cytosine or derivatives, or is there deamination to U, then formation of HMU, and amination to HMC? No evidence yet, although cytosine does go to HMC. (Weed and Cohen recent data).

9. Is HMU common intermediate to HMC and to thymine?

10. Therefore is HMC a normal intermediate or abnormal endproduct unique to T even?

11. In latter instance does virus provide enzyme to make HMC?

12. It is desirable to have table with host nucleic acids vs virus nucleic acids showing difference in cytosine vs HMC. We may propose the working hypothesis that a T2-virus infected cell can't make or utilize cytosine or necessary cytosine derivatives. This may be due to active inhibition of synthesis by HMC perhaps or HMC wins out competitively since host enzymes degrade cytosine derivatives far more rapidly than HMC. Also virus DNA is more resistant to DNAase and phosphatase than thymus DNA. Therefore cell can no longer make host RNA or DNA, nor compounds associated with these nucleic acids. Thus cell can't grow, make host enzymes, divide.

A lysogenic system can make RNA. Does phage contain HMC? Grapevine says no. Does T7, T5 etc. system lacking HMC make RNA? Published data incomplete, inadequate. In any case, T even virus certainly seems atypical.

Concerning your thoughts expressed in your letter of February 16th. I am opposed to including my data of this year in any detail, since it will take several months of filling out before I shall be willing to commit this data to print. I tried to explore many aspects widely, not deeply, so that I could see which problems to go at deeply.

However on the shift of HMC deoxyriboside during assimilation, this shift is exactly to 2650 in the case of B infected with T2. The further shift to 2525 occurred with W-T2 only and perhaps this is why W can adsorb T2 or T6 and can't make T2 or T6. It destroys HMU deoxyriboside. I hadn't thought of this before.

Concerning your idea of the switchover of C to HMC resulting from a single step mutation being evidence against the idea of differently composed DNA for each gene, at least in these viruses. At first I liked this but it doesn't make too much sense on thinking about it, since I don't really know what genes mean in talking about this system. No one has yet really demonstrated a linear order but only an apparent division of labor based on a pretty obscure crossing technique. The real problem is the origin of T2 itself, since it seems perfectly possible to me that a mutation in B to make HMC at one site within the cell might have carried the entire production of T2 as a consequence, rather than first, the evolution of cytosine-containing T2, then going on to become HMC-containing T2.

I do think we should note, as you suggest, that with HMC we have a special identifier of T even nucleic acid if not of lysogenic phage. Therefore it becomes possible to look for virus nucleic acid at a time before net synthesis of DNA becomes evident, i.e. in B-T2 before the 7 to 10 minute interval in my early curves.

I hope these thoughts are sufficiently detailed for you to use. If you wish me to elaborate, I shall.

Sincerely yours,

Seymour Cohen

Seymour S. Cohen

SSC:KHG

8 May, 1953.

Dr S.S. Cohen.

Dear Seymour,

We have just got back from a most enjoyable, though far too rushed, trip. We visited Lwoff's lab. Siminovitch and Smith have some tracer evidence that there is some turnover in RNA during phage production, but they're cautious about it just yet. Their studies on phage DNA seem still to be in a fairly embryonic stage.

I have heard nothing from the B.J. about our paper, but Roy Markham told me he had received it as a referee, and that his criticisms were of fairly minor points, so I hope we shaln't have too much trouble.

I have been asked to submit a short paper to the C.S.H. symposium in the form of a contribution to the discussion of your paper. It would help very much in writing this if you could let me know briefly what ground you plan to cover, at any rate where HMC is concerned, so that I can avoid duplication.

I hope things have been going well with you.

Sincerely,

G.R. Wyatt

March 10, 1953.

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Pennsylvania.

Dear Seymour:

Many thanks for your provacative suggestions about the discussion. Here is the paper, fairly extensively revised. The discussion is roughly in line with your ideas though I have not gone quite all the way with you. I have slanted it to suggest that the enzyme for making HMC probably comes from the bug, not the virus, though I guess from your letter you might have left the question more open, or slanted the other way. If you have definite feelings or new evidence on this, I'm open to conviction. This is my second draft of the discussion, finished in a rush, and will almost certainly need some changes and improvement of expression.

Specific questions are:

p. 4. Lark's initials and location
" 5. MP - method of preparation correct?
" 21. Rutten and Winkler - I have not seen this paper.
" 27. Is my allusion correct? Reference to Rutten and Winkler.
" 29. I have included names of Sprague and Hampil in the acknowledgement although they both said this was not necessary. Do you approve?

I am leaving today for Europe and shall not be back until May 3. It would be a pity to hold up the paper until then. If you don't mind getting the MS into final form, I'm prepared to accept your judgements. If you will mark any changes on the first copy and return it to Dr. J.M. Cameron at this lab, he will look after it and get any necessary re-typing done. It can

be returned to you for a final wetting if you want, although Dr. Cameron will see that it is put together properly. He will look after its being sent to the Biochemical Journal, and ordering 300 reprints for you.

I shall be more or less inaccessible for the next month, but can be reached after April 6 care of Canada House, Tafalgar Square, London. I look forward to seeing you at Cold Spring Harbor, and hope you can tell us a lot more about HMC by then.

Very best regards,

GRW/h G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

February 19, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

The large project has been completely messed up by the Hospital in the past two weeks, and there is no prospect of its pursuit for this year at least. I'm sorry, because in addition to you, there were two other people and we could have had fun.

I am informed by Dr. Weaver of the National Foundation for Infantile Paralysis that you will receive at least 2 offers in addition to that of Luria, and urge that you be careful to pick the best. If I can give you any assistance in making your choice, please don't hesitate to ask.

With best regards, I remain

Sincerely

Seymour

Seymour Cohen

SC/md

Just got your letter and will try to ship my thoughts + the paper very soon.

16 February, 1953

Dear Seymour,

I'm willing to try to write a discussion for the paper but I need your help in it. I cannot arrive at any reasonable concusions, there seems to be a snag in every generalization or inference one would like to make. Hence I must ask for your ideas, as concretely as possible. Since you have been doing the work on which our conclusions will depend, and have so much more experience in interpreting metabolic experiments, I don't feel I need apologize in asking this. If you can do this, in a letter, and send back the paper, then I think I can do the actual writing so that the paper has continuity of style, and you are saved a certain amount of effort in fitting words together. Is this practicable?

In any case, I should like to in include the quantitative results on T5 and vaccinia, and qualitative results on MP, and this will take a little rearranging of the earlier sections of the paper including the title.

It is up to you how much if anything you want to report from your recent work. Sinsheimer writes from California that he is with Delbruck to work on phage DNA's, so I think some mention should be made of the enzyme resistance. I should almost be prepared to do this from my own single experiment of last spring with DNase and viper venom diesterase. Do you want to give any details of your experiments here, or just a generalstatement to the effect that HMCDR was not released by DNase followed by phosphatase, and that phage DNA left a "core" not dialysable against distilled water much greater than that from thymus DNA.

As for my own ideas about the discussion, as far as I have any, they run roughly as follows: I would point out that it is difficult to assign any critical biological significance to HMC since it has been found only in three viruses which are so closely related that they can be regarded as strains of a species and which do not differ in any fundamental particular so far known from other viruses (such as T5) which lack this base. (Is T5 sulfa-resistant? You don't mention this one). The fact that HMCDR is removed, with spectralshift toward shorter wavelengths, from coli culture medium suggests that it is indeed a normal intermediary metabolite for this bacterium. Since it is untouched by

coli deoxycytidine deaminase, however, and since the spectral shift is to 2525 whereas HMUDR would be expected to have a peak about 2650, the nature of the next step in its metabolism is uncertain and is probably not deamination. Phages T2, 4 & 6 are presumably capable of blocking this pathway at HMCDR, which would then accumulate and be available for incorporation in the phage DNA which is being synthesized. Such blockage is not essential for redirection of a cell's NA synthesis to production of virus DNA, however, for phages lacking HMC also do this. Rather it appears to represent a peculiarity which has presumably arisen in these phages by mutation. HMC differs from 5-MeC in that no DNA has been found to contain a proportion of it along with cytosine--it seems to be all or none. This suggests that the switchover from C to HMC in all the DNA of a virus resulted from a single step mutation, and is presumptive evidence against the idea of differently composed DNA for each gene, at least in these viruses.

Thus, we cannot at present attribute a role in the infective process to HMC. It may, however, be expected to provide a key to research in virus syntheses, by indicating one point where the metabolism of an infected cell will differ from that of the healthy cell. It also provides a label by which virus DNA can be recognized which may be useful--if, for example, a lysogenic phage having HMC were found, the number of particles of this virus carried in a bacterium could be directly estimated from its HMC content.

I am writing this late at night and it may sound complete rubbish by daylight. I'm going to send it off, though, in the hope that it will at least be provocative, and that you will send enough criticism and you own ideas so that I can write something more sensible. We'll have to waste no time to get it off to the journal before I go away.

I'm very sorry indeed if your larger plans have met an immovable obstacle. It was an adventurous scheme. I look forward to hearing the outcome.

All the best

14 Februamy, 1953.

Dear Seymour,

I have just had a letter from Luria concerning a position which may become available in his department next fall, asking whether I am "at all interested in being considered" for it. He mentions a probable salary of $5500 to $6000. This is, of course, not a definite offer, but I would regard such a prospect as quite attractive. So before replying, may I enquire how definite your plans for development have become? Are you in any position to mention dates of starting your expanded project, probable salaries, who is likely to be in your team, or other such particulars? I don't want to be impatient; on the other hand I don't want to reject one potential offer and then find myself left with none.

Incidentally, do you still want your plans kept confidential? I have kept pretty quiet about them so far.

All the best.

Sincerely,

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

February 13, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Canada
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

Now that all the data are in, how shall we proceed? Do you wish me to return the manuscripts so that you can fill in the data and rewrite a discussion? Or shall I write a discussion? Your style is sufficiently different from mine to warrant your efforts on that, to keep the style the same throughout. Anyway, I am lazy and busy both. Of course, if you wish me to exert my creative powers, such as they are, I shall.

We have just completed a few sulfa experiments. Rutten had reported that sulfa-grown B would not support T2, T4 or T6 in the presence of sulfa and other things which do permit the multiplication of T1, T3 and T7. We have confirmed this. Further the fortification of this medium with HMC, HMU or HMC deoxyriboside doesn't permit T4 growth. Other data also suggest that this sulfa effect is not too simple a matter. Nevertheless, I was disappointed with T5 and meningopneumonitis. It would have been so nice.

My teaching is under way and I don't know whether I am coming or going. I have a chapter on pentose biosynthesis for Chargaff's book as well. With respect to HMC, I feel we have cleared away some of the questions obscuring the main problems. My general impression is that the main pathway involves the deoxyriboside rather than the free pyrimidine and there are obvious but difficult things to do here, such as the study of hydroxymethylation and amination. The structural question requires extended exploration. I shall decide soon on which problems to concentrate.

The large project has been fouled up and in my next letter I shall tell you whether it is dead or not. I had written to Delbruck to get you invited to the Cold Spring Harbor Symposium on Viruses in June. I hope to see you then, if not before. I trust you will have a splendid time in Europe with Mrs. Wyatt and hope you will extend my regards to people at the Pasteur.

With best regards, I remain

Sincerely yours,

Seymour

Seymour S. Cohen

SSC/md

February 10, 1953.

Dr. S.S. Cohen,
Childrens Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Penn.

Dear Seymour:

Neither T5 nor MP has any HMC that I can find. The meningopneumonitis virus contained 2.6% P and about 2.5% DNA (by integration from the estimated bases) and very little, if any, RNA. With a total of 17 mg. this didn't give much DNA to play with, so I hydrolysed the whole virus, and after chromatography in two solvents the maximum possible HMC was 3.9% of the C. The products from the non-DNA part of the virus are quite sufficient to account for this figure not being lower.

The T5 contains 2.6% P and DNA bases equivalent to 85% of this, and max. possible HMC = 1.0% of C. To get good base ratios, I isolated some DNA, using N-NaOH in case any RNA was present (I have checked with T6 that N-NaOH overnight at room temp. does not affect HMC), and this comes out as follows from a first analysis:

Adenine	30.4 moles %
Thymine	31.1
Guanine	19.4
Cytosine	19.2

Total bases/P = 0.96

Again, A=T and G=C. There must be structural association of these.

It is too bad the MP results don't contribute to a general theory of anti-virus sulfonamide action. In view of the complexity of the data on sulfa action, though, I suppose

it is perfectly reasonable to postulate a sulfa-sensitive hydroxymethylation for T2, 4 and 6, and something quite else for the psittacosis group. Still, it's a pity this should be necessary.

Knight writes that Fraser has been doing some base analyses and would like to keep his T3. At this stage that suits me very well. I am anxious to get this wound up, and I have to write a section on paper chromatography for a book on nucleic acids that Chargaff is editing. On March 11th my wife and I are going to England and Europe for 7 weeks. This is mainly a holiday (and has to be taken at this time of year because of leave regulations in our department), but we shall get in a bit of visiting, including the Pasteur Institute, I hope. On coming back I am going to do some work on insect viruses. It is tempting to stay with nucleic acids, but this is a pretty competitive field now and I think it is wiser not to try to keep ahead of it here alone, especially when insect viruses are loaded with wide-open questions.

Sincerely,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

January 26, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

I received your letter and the rickettsiae reprints. I'm glad the T2r and T6r+ came out O.K; I couldn't quite believe the earlier divergencies.

I shall be sending a sample of T5 obtained from Gordon Lark (1.5×10^{13} particles) and one of killed meningo-pneumonitis virus from Michael Sigel within several days. Tl will not be coming in. Meningo-pneumonitis (MP) is of interest because it is one of the sulfa-sensitive psittacosis group and it is my guess that these viruses may have HMC. I don't know how clean the preparation is. Microscopic examination is said to show nothing but elementary bodies.

T2, T4 and T6 are sulfa sensitive in the presence of thymine in contrast to the others and I think this reflects the sensitivity of the hydroxymethylation step, presumably as a folic acid requiring enzymatic reaction. So I would prefer to hold off on writing the discussion until we know about MP. However, if you feel urgent, now that the phages are filled in, we can finish off the manuscript.

Concerning the enzyme resistant core. What I call "core" are resistant non-mobile nucleotides after DNAase and phosphatase (30% of the total P) Thymus DNA has about 2% of the P left under comparable conditions. I have just obtained some venoms for diesterase and monoesterase studies.

At this point, our thymineless bug is driving us somewhat crazy. More on it when it's straight.

Sincerely yours,

Seymour

Seymour S. Cohen
SSC:KHG

Did I mention that the cytosine deaminase of yeast which acts on 5 Me cytosine doesn't work on HMC, just like the one from E. coli, which doesn't act on 5 Me cyt.

January 31, 1953

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Penn.

Dear Seymour:

Thanks for your letter of Jan. 26. I shall be most interested to see what T5 and meningopneumonitis have in them, and happy not to have T1. The idea of a sulfa-sensitive hydroxymethylation step in the phages seems very reasonable. If meningopneumonitis has HMC, however, we shall be left with a considerable evolutionary, or genetical problem as to its origin since vaccinia on one hand and rickettsiae on the other have it not. However, we have this problem with T2, 4, and 6 anyway (and perhaps it should be brought out in the discussion of the paper).

Here are final results on vaccinia. DNA is not released by urea, but by leaving 90 mg of virus in N-NaOH overnight at room temp., and deproteinizing with chloroform, I got 1.2 mg. of reasonably clean DNA. This analysed as follows:

Adenine	29.5 moles %
Thymine	29.9 "
Guanine	20.6 "
Cytosine	20.0 "

Uracil and 5-MC, none detectable
HMC, none detectable, max. possible 0.6% of cytosine.

Total bases/P = 1.00

By hydrolysis of 50 mg. of the unfractionated virus, I could not detect any HMC either. There was enough uracil for about 0.5% RNA.

These base ratios are not at all exciting (although they conform to the Chargaff formula) but I think they are reasonably accurate and perhaps worth recording, either in our present paper or elsewhere. We'll see how MP comes out.

No hurry about the paper, the more we can get into it the better.

Sincerely,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

January 2, 1953

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

I have received the manuscript which makes good reading, but if you don't mind I should like to take my time on this one. This won't matter too much since there are the T2r and T6r$^+$ data to complete. A sample of each virus goes out with this letter under separate cover. Concerning the paper proper I would raise the question of whether you would not wish to expand the discussion to consider the possible significance of the pyrimidine from the points of view of parasitism in T-even systems, lysogenicity, and the intermediary metabolism of the pyrimidines. If you wish to avoid speculation this will fall upon me in future papers in connection with my present studies.

I don't think there is any point in waiting for the other phages, since you have already established the precedent of true cytosine in T7. If they come in, all well and good, if not let Ph.D's-to-be be born in a careful dissertation. Incidentally a close perusal of the T7 data strongly suggests that RNA may be synthesized in that system.

I shall greatly appreciate the T7 DNA. I shall try to work with E. coli DNA rather than thymus in continuing the "core" data. I have extended this work as follows:

The nucleotides remaining after DNA ase and alkaline phosphatase (25%) which don't migrate in butand -NH_4OH, have the following composition

adenine	19.1
thymine	17.3
guanine	16.6
HMC	47.1

On dialysis, there is a marked disproportionate loss of guanine and HMC, while the other 2 appear to concentrate. Incidentally I've obtained your ratios on intact virus DNA.

I think you may be quite correct in your speculations on DNA ase action and the relations of guanine and the other pyrimidines. Incidentally, I shall try to repeat this with bacterial DNA ase, if it can be obtained from coli.

Concerning the problem of the genetic core. It seems possible that the role of a core is not to be itself incorporated into phage but rather to provide a model for others, which could incorporate the nonessential degraded fragments. Thus if we started with labeled virus the HMC nucleoside should be much less labeled than the others. Maybe we'll do this experiment, but it's a tough one. Probably best done $\bar{c}$ N^{15}.

Larry and I have repeated the orotic acid experiments and have isolated the 282 nucleotide for counting. Essentially similar results were obtained. He is now analysing B and virus in experiments with β-labeled serine. The bacteria were grown in labeled serine and in a preliminary experiment thymine was labled but not cytosine. These were infected in the presence and absence of labeled serine and the analyses should establish the source of the transferred ring and the $-CH_2OH$ group.

In other studies he finds only HMC in the 282 nucleotide and a 1:1 ratio of thymine and HMC in the 274 compound. He will continue this work since he has to locate the second P in the 282 nucleotide and the number of sugar moieties in the 274 to permit a decent interpretation of the orotic acid studies.

I have been pushing our thymineless bug (with the help of a fine assistant) and have gotten it to reproduce our results. An infected bug will make DNA in large amounts and I now want to know whether this DNA contains HMC and thymine. I will therefore close with the question: How would you do quantitative DNA-base analyses on E. coli?

With best wishes for the New Year,

Sincerely,

Seymour

SSC:KHG Seymour S. Cohen

P.S. The large project seems closer now.

January 16th, 1953

AIRMAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

Many thanks for your letter of Jan. 2 and for T2r and T6r+. These both give base ratios in good agreement with the others, and the following P and N values:

	Bases/P	P/N	DNA P/N
	.96		.309
T2r-3	0.98	0.318	0.315
T6r+-1	0.99	0.306	0.306

Which looks OK to me and will fill out the tables.

Dr. Harpil finally sent a batch of vaccinia. This contains 2.83% P and 2.06% DNA (cf. Hoagland et al. 0.57% and 5.6% respectively)-- that lab seems to rain phosphorus! However, there is plenty of DNA to check for the presence of any odd bases; have not done this yet, but shall let you know of results, and it can go into Table 7 of the paper.

I have sent you about 250 reprints of "rickettsiae". I hope this is enough. I haven't had many requests for it. No reimbursement wanted, just a kind thought for the Canadian taxpayer.

Yes, I agree that the discussion I wrote is a bit skimpy. I was discouraged about speculative discussions after the pruning that had to be done on my insect virus DNA paper to get it published. I was hesitant about mentioning the possible connection

between HMC and stoppage of host NA production as I was under the impression that there is no RNA synthesis in the T7 system. If you feel there is good evidence to the contrary, it might be well to make this point. There are some comments one could make about lysogenicity, but even these may be premature when we don't even know if the stuff occurs in any lysogenic system. When you are through with your comments I shall see if there is anything I feel like adding.

Concerning cores, have you had time and patience to read Markham and Smith's papers on RNA in the latest B.J., which are quite impressive? The "non-dialysable RNase-resistant core" consists of fragments of average length 3-4 nucleotides, which dialyse easily provided sufficient salt is present (2 M-NaCl). Although DNA is more complex, it would be worth knowing if a DNA could be made to dialyse by adding salt too, as this would give some idea of maximum chain length and possibility of genetic value. Also, one should get a direct measure of mean chain length by the amount of P released by phosphomonoesterase. However, it is scarcely fair to suggest jobs for you when I am out of this game!

As for your question of how to do DNA base analyses on E. coli I don't know of any easy, satisfactory way. To make good DNA from coli seems to be inevitably laborious. It can be done more easily with NaOH (Schmidt & Thannhauser and Sevagging), but this gives a preparation with a lot of carbohydrate in it, which messes up the chromatograms. How about removing RNA with NaOH, then extracting the residue with TCA (but more exhaustively than Schneider described, as pyrimidines tend to stay behind), evaporating down the extract and hydrolysing with HCOOH? One would have to check that HMC is unharmed by NaOH.

Sincerely,

GRW/m G. R. Wyatt.

24 Dec., 1952.

Dr S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia, Pa.

Dear Seymour,

Here is a draft of a paper for your criticism and revision. It is set up as for the Biochemical Journal, of which it would take about 5 pages. It just covers the results on HMC and T2, 4 and 6, which makes a compact paper. If, however, T1, 5 (and 3?) are forthcoming soon, it might be well to hold off and include results from them. This would save writing another paper and facilitate the discussion, and I think one comprehensive paper is much preferable to a series of short scrappy ones. On the other hand, would you like to include anything on your results on enzymic degradation of phage DNA, which strike me as pretty important. This being entirely your own work, you would probably prefer to write a separate paper; but I think it might be wise to get something out on this soon, as Smith and Markham are very likely to be doing similar things, and Sinsheimer has expressed to me his intention to work on phage DNA whenever he can get ahold of some to work on.

My queries on specific points are in the margins; please be brutal and suggest any changes, additions or compressions, and send one copy back.

I should still like to have another preparation of T2r for base ratios and one of T6r to check on the anomalous P/N ratio, if it's convenient. Tables 4 and 6 are incomplete.

Beard writes that they have not any T7 left, also that they were unable to get this virus clean by centrifugation alone and had to use filtration which naturally resulted in loss of most of the virus along with the dirt.

I was very interested to hear your results on enzymic breakdown of virus DNA, and its "core". For biological interpretation, it would be desirable to know if DNAase from other sources, eg. Mirsky's "acid DNase" and especially the DNase of coli itself, are resisted in

the same way. Also, if a phage DNA lacking HMC is resistant. In case you want to check this last point, I am sending 7 mg. of T7 DNA, the prep. described in my letter of September 11; this is neither clean nor highly polymerized but should be adequate to indicate whether there is any evidence of resistance.

Is it reasonable to suppose that preservation of a 15% core would be of value to the virus, when the data from transforming factors indicate that the genetic properties of DNA's depend on maintenance of "native" configuration, and also when the second generation transfer experiments fail to distinguish any irreducible genetic core? One might also note that Graham's superinfection breakdown, which he attributes to coli DNase, is more complete with T2, which has HMC, than with T7, which hasn't. So I'm doubtful about biological interpretation of the resistance.

Chemically, however, the enzyme resistance may have considerable significance, both for DNase specificity and for DNA structure. From the UV data you give, it looks as if the core might consist largely of HMC and guanine (G) nucleotides. I should be interested to know if analysis bears this out. Smith & Markham, and Sinsheimer, have noted that a large proportion of the 5-methylcytosine (MC) in wheat germ DNA and thymus DNA can be found after DNase action in the dinucleotide MC-G. Trying to fit this together with the Chargaff constant ratios, whereby G and the total cytosine complex are approximately equivalent in any DNA, I am wondering if it is possible that C as well as MC and HMC in DNA's is structurally associated with G, and on DNase action the C-G link is broken while MC-G and HMC-G are resistant. The high proportion of resistant linkages in phage DNA would then mean that fragments large enough to be non-dialyzable are left. However, this is a wild shot, and the matter is obviously very much more complex.

I'm interested too in your nutritional and enzyme results, and grateful for your trouble in setting them down, but at the moment I have no ideas which would not already have occurred to you. That's good that you have a bug which does recognize HMC. You've certainly been busy.

If I write long letters, it's for lack of opportunity for oral discussion.

It's a bit late to say Happy Christmas, but this conveys my best wishes to Mrs Cohen and you for the new year.

Most sincerely

December 13, 1952.

Dr. S.S. Cohen,
Childrens Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Penn.

Dear Seymour:

Here are the results on Kozloff's T7, all I could do with the rather small preparation (total 3.8 mg.) he sent. Figures are means from two analyses (which agreed quite well), after hydrolysis in HCOOH.

	Moles/100 moles DNA bases
A	24.5
T	30.05
G	21.85
C	23.55
U	None detectable
OC	Note more than 2.5% of C
Bases/P	0.48
P	2.1% of weight
DNA (calc. from this)	10 % of weight

The limit on possible OC could not be got lower because of the small amount of material and the UV absorption of degradiation products non-DNA material in the prep. This non-specific material probably makes the thymine figure too high too. In a preparation of this sort one gets better quantitative results with $HClO_4$, but I did not want to use this as long as there was a possibility of OC. Hence these results are not to be relied on quantitatively; comparing them with the NA of Spizizen's T7, however, suggests they may not be too far off the mark.

Evidently this is a tough virus to make, in spite of the optimistic figures published by Czaky, Beard, etc. I am writing Beard to ask if they have any of those preparations left. Am also writing these results to Kozloff. Actually, however, we have the significant result that there is very little or no OC in this virus, and accurate quantitative figures are probably only worth obtaining if we have good results from at least T1 and T3 to compare them with.

The proofs of the rickettsia paper were sent to our Ottawa office and returned directly from there, along with an order for 500 reprints. I shall be able to let you have about 200 of these if you want them.

What's exciting with you? Best regards,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

December 8, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie,
Ontario, Canada

Dear Jerry:

I have corrected the "Fellowship Fund" back to "Fund" in a letter to Nature. The Rickettsia paper just came out. Could you see about ordering reprints - 300 for me again? If you wish recompense on this, please say so.

Puck has promised T1 and Adams T5. Spizizen says they will ship vaccinia very soon. I have more T2r and others if you wish them.

Concerning the grand plan, we have been practically offered $500,000 for a five year project but the Hospital is loath to accept until it sees its way to cover overhead completely. This is being debated at the moment. When things get more concrete, I shall certainly let you know. The would-be grantors are in quite a hurry to get started.

Let me summarize my main data to date:

A. Isolation of HMC deoxyriboside - cannot be obtained by DNAase and phosphatase. Has been obtained slightly impure by hydrolysis in NHCl, phosphatase, and chromatogrammed in butanol - NH_3. Slight guanine contaminant. Has been obtained quite pure by hydrolysis in NHCl, chromatogrammed in tert. butyl alcohol-HCl, phosphatase on "cytidylic" band eluate, chromatogrammed in butanol-NH_3. In this medium, the R_f is ca .14, UV maximum pH 1 at 282-283. Yield is very poor however, only ca 10%.

B. Nature of virus DNA resistance to DNAase and phosphatase. DNAase depolymerizes thymus and virus DNA (viscosity) and 2600 A increase. On dialysis, thymus DNA loses about 20% of the P, virus $<10\%$. Alkaline phosphatase on DNAase - thymus DNA liberates

93% of P as inorganic P, only 70% from virus DNA. On dialysis ca 2% thymus DNA left, but virus core of 15% left. Spectrum has 2600 max at pH 7; at pH 1 shifts to 2670. I am now beginning to analyse the core. It appears that HMC confers stability to a core of virus DNA and possibly accounts for its survival in infected cells in which DNAase has been liberated (Pardee) and degrades host DNA. Pretty good if true.

C. Nutrition of E, coli strains

1. W cytosineless

a. requires cytosine or uracil, doesn't grow on thymine or HMC. HMCDR not rigorously tested yet.

b. W on infection with T2 is killed, mucleic acid synthesis stops but no virus produced. Not affected by HMC or HMCDR. However, HMCDR disappears with shift to 2525 (possible deamination).

2. 15 - thymineless

a. requires thymine or thymidine, doesn't grow on cytosine, uracil, HMC, HMCDR or 5-methyl cytosine (5 MeC)

b. back mutation rate to thymine independence is low, ca 1 in 10^8, no mutants for 5 MeC replacing thymine isolated. However, we now appear to have a mutant which grows on HMC instead of thymine, suggesting path through hydroxymethyl uracil (HMU). Miller almost has synthetic HMU ready for test. We will really work on this bug.

c. The phage stuff is absolutely weird. Strain 15-thymineless seems to have few receptors. When grown on glucose - thymine T2 virus doesn't adsorb. When grown on broth and infected in synthetic media, a slow adsorption rate prevails but conditions approach the B story. There is a factor in broth required for synthesis of receptors (not amino acids). When the cells are grown in broth and washed, they do not multiply nor make DNA in the absence of thymine. When infected, they make DNA in the presence or absence of thymine. Maybe they are only making HMC cores in the absence of thymine. Here's another phenomenon requiring some effort. We're just learning how to handle this bug properly.

D. Enzymatic Studies

W cytosineless - grown on cytosine on disruption has a cytosine deaminase which doesn't touch HMC or 5 MeC. This enzyme is labile and is readily demonstrable by spectrophotometry only after removal of most cell nucleate by Mn^{++}. The extract also contains a more stable deoxycytidine deaminase which also deaminates cytidine at about 1/3 the rate. It doesn't touch HMCDR.

I plan to get around to base exchange and nucleosidases.

E. Uptake Studies

A. Growing B rapidly chews up cytosine, deoxycytidine with spectral shift as in deamination. There is a barely perceptible uptake of HMC without spectral shift of the same order as the thymine formed in B. A sure proof of this will require labeled HMC. HMCDR disappears at a marked rate with spectral shift. There may be an adaptive deaminase.

t 2650

B. T2-infected B has slower removal of cytosine, deoxycytidine with spectral shift. Curiously enough HMCDR also disappears more slowly with spectral shift. Perhaps in growth, an adaptive deaminase takes the compound to HMUDR which can go in many directions. In infection, adaptation is inhibited. Anyway, it looks as if the deoxyriboside is the key intermediate since HMC seems totally inert in this system.

I always wondered how you kept me so well informed. I find writing a most difficult matter but shall try to overcome this abhorrence in the name of communication. If you have any ideas on what else I ought do and how to interpret all this stuff, please don't hesitate to offer them.

Sincerely yours,

Seymour

Seymour S. Cohen

SSC/md

December 9th, 1952

AIR MAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

I've just finished a first run on your recent prep. of T2r-2, and get rather odd results. Ratios of the bases differ by as much as 10% from those of the other T-evens; total bases are equivalent to only 83% of total P; and P (from hydrolysate) = 2.4% of weight. P/N ratio not done yet. Hence I should like to do another prep. of this virus; I see from your letter of Nov. 25 that you were thinking of making some anyway.

First results on Kozloff's T7: cytosine, no OC (read second chromatogram to check this quantitatively); base ratios very different from T-evens; total bases equivalent to 50% of P; only about 12% DNA in the prep.

Our rickettsial note was in Nature for November 15th. They did not send me any proofs, and I don't know yet whether an order for reprints went through our Ottawa office; have written to enquire.

In haste,

Yours,

GRW/m

G. R. Wyatt.

November 28, 1952.

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia 46, Pa.

Dear Seymour:

Thanks for your comments of Nov. 25. I do mean P/N, as stated, only I slipped with the decimal point. Should be 0.316, etc. Very sorry. I have referred to Kozloff and Putnam, and cannot find any stated figure for P/N nor for P on dry weight, but have calculated from their average figure for P and N per infections unit of broth T6 (J.B.C. 181, 215) that P/N = 0.24. It is probably just coincidence that this agrees with our apparently anomalous figure for T6r+-7. It is a pity I did not get a P/N figure for T6r+-2, which is all finished, and if you care to send a fresh prep. of this virus, I shall get this ratio checked. I have still some T4r+-7, and do not need any more of this virus unless you feel that everything should be checked on at least two different preparations, which is probably superfluous.

I look forward to T2r and T7. They should be here in a day or two.

You are certainly casting your net wide to gather work for me! However, I must overcome inertia and admit that this should pay off, and the results will be more meaningful if this set is analyzed at one time in one lab, instead of individual analyses trickling out each person using his own technique. I only hope the viruses are decently clean, and that I can collect them to run simultaneously.

Our Beckman is in Toronto for overhauling, but should be back next week. Meanwhile I have started writing this up, but the pen is not very fluent.

Best regards,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

November 25, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

Thanks for the letters of November 19th and 20th. I am forwarding T2r-2 and T7 from Kozloff. Sub-numbers refer to different preparations of the same strain and data on T4r-7 and T4r-8 for instance, may be averaged.

Concerning T6r$^+$. I note that there is only one N/P ratio, i.e. T6r$^+$-7, and it is way out of line. Do you wish more T6r$^+$? Also you ought look at Kozloff's or Putnam's figures on this. Incidentally, do you mean N/P, rather than P/N as you have?

On the low and variable P values despite the "correct" N/P ratios. These preparations are lyophilized from relatively dilute solutions, previously dialysed against what we call distilled water. It looks to me as if there is significant salt contaminant in the final solid. There would be no point to column (1) judging from these figures.

On the low N/DNA-P ratio (7% low) for T6r. The non-DNA-P in these preparations would surely bring additional N and account for the low value.

A table of (2), (4) and (5) with the T2r figure as well, would probably be useful. Do I gather from this that more T2r, T4r$^+$ and T6r$^+$ are needed to fill out this table? At least

T6r$^+$, I guess anyway. Our T2r yield was low and we will make more anyway.

Knight has asked me about C_x saying he wished to analyse T3 from Frazier. I suggested he send it to you, but he is unpredictable. I shall write Adams for T5 and Puck for T1.

With best regards.

Sincerely,

Seymour

Seymour Cohen

SC/bj

November 19, 1952.

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia 46, Pennsylvania.

Dear Seymour:

Thanks for your letter of Oct. 28 and phages (T4r+, T4r, T6r+, T6r) sent at the same time. I have done some analyses on these and get no differences in the base ratios. Here is a tabulation concerning P, N, and bases. I don't place too much faith in individual figures, and shall do duplicates of some of these, but am holding off in order to run them along with T2r. The figure of 3.87% for P in T2r+-5 should be pretty reliable except for unknown moisture content so the DNA in this virus must be over 40%. P:N ratios are pretty close to Herriott's. It appears that the T4r preparations contain about 5% and T6r about 10% of non-DNA P, presumably host material. This seems to be in general agreement with your earlier findings from Dische tests (J.E.M. 91, 611). Incidentally, we find T6r+ DNA gives about 3.5% greater Dische color yield, based on P, than thymus DNA, which may be due to the lability of OC.

I'd like to hear your comments on these figures, and whether you feel any of them worth publishing. Perhaps a table corresponding to my columns (2), (4) and (5)?

As for your offer of more T-even DNA's; I'm no more anxious to analyse them than you are to make them, so unless there is some point you would particularly like checked, let's leave it with what we have.

The proofs of the Paris paper came and I've returned them to Lwoff. Since it so largely duplicates the material in the Nature paper, it was decided not to dignify this note as a Contribution from the Division of Forest Biology. This means I can't order reprints

on department account. Can you conveniently do so? If not, I'll order a small number personally. I don't see that we'll need many of this paper--if anyone asks for it we can send them the Nature paper, of which my department will be ordering a vast number.

I wrote to the editors of Nature about 3 weeks ago to ask what their doing with our rickettsial note. No reply yet.

As for Siminovitch and Smith, I don't know Siminovitch, but as long as John Smith is in Paris, his working efficiency which is normally not too good, will be reduced to about 25%. I doubt if you have much to worry about.

I should very much indeed like to get to the C.S.H. symposium, and should be happy if you give any support to my getting invited. This does not entail giving a paper, does it? Are all those attending invited?

Sincerely,

GRW/h

G. R. Wyatt.

Properties of T-even phages. Analyses to Nov. 19/52.

Preparation	No. of analyses	(1) % P	(2) DNA bases / P	(3) % DNAP	(4) P / N	(5) DNAP / N
T2r$^+$-5	4	3.87	0.99	3.87	3.16	3.16
T4r$^+$-7	1	3.31	0.98	3.28	3.19	3.16
T4r -7	1	3.35	0.95	3.21	3.22	3.09
T4r -8	1	2.86	0.94	2.72	3.36	3.19
T6r$^+$-2	1	3.75	0.99	3.75	-	-
T6r$^+$-7	1	2.88	0.96	2.79	2.44	2.36
T6r -6 (rec'd May/52)	1	2.73	0.92	2.53	3.14	2.92
T6r -6 (rec'd Oct/52)	2	4.21	0.86	3.66	3.43	2.98

(1) Calculated from P content of hydrolysate used for base estimation; subject to error (maybe $\pm$ 10% on an individual estimation) because of uncertainty in hydrolysate volume. Virus from desiccator but not specially dried.

(3) $= (1) \times \frac{(2)}{0.99}$, 0.99 being mean bases: P ratio for isolated DNA.

(4) P and Kjeldahl N from virus suspensions made up for this purpose.

(5) $= (4) \times \frac{(2)}{0.99}$

Institut Pasteur
28 RUE DU Dr ROUX . PARIS XVe
TEL. SEGUR 01-10

PARIS, le 18 novembre 1952

Dr. Seymour S. COHEN
The Children's Hospital
Dept of Pediatrics
1740 Bainbridge Street
PHILADELPHIA
Pennsylvania

Mon cher Seymour,

J'ai bien reçu les épreuves de votre mémoire avec Wyatt et corrigées par celui-ci. Le nombre de tirés à part n'était pas indiqué. Voudriez-vous m'envoyer un petit mot avant une huitaine de jours, si possible, pour me dire combien vous-même et Wyatt désirez de tirés à part.

Bien amicalement,

A. Lwoff

A. LWOFF

Dear Jerry:- We want 300 here and I shall so tell Lwoff. Please give him your own order.
I have a T7 from Kozloff to be lyophilized for shipping as well as T2. We will send them next week. I assume you got our other samples but I haven't heard. Things are going slower than I had hoped. The mutants are messy.
Incidentally I was wrong about enzymatic examination of Cx. I've done it over elegantly and obtained clean negatives. The enzyme prep. contained RNA which we'd to get.
Sincerely
Seymour

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

October 28, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

I've just returned from another grueling week through the Midwest. Now I should be able to work for several months relatively undisturbed.

I have isolated the C_x deoxyriboside by hydrolysis in N HCl, freezing out the guanine at pH 8 and chromatographing on paper in butanol-NH_3. It is slightly contaminated with guanine; but in the future, I will first remove guanine and adenine on a resin. Both C_x and deoxyriboside are deaminated by an extract of E. coli which deaminates cytidine, deoxycytidine, cytosine, and 5 methyl cytosine. We are now beginning some phage growth and DNA synthesis studies with these compounds.

I am mailing about 20 mg samples of T6r$^+$, T6r, T4r$^+$, and T4r. We are now in position to make T2r and will ship this out as soon as I have it. I have still to get any virus from Sharp & Dohme but they promise some. Concerning reporting P analyses, do you have such figures? I would be interested in seeing them in view of Herriotts report. I don't know yet whether it would pay to publish them.

Would you feel better if you had the rest of the DNA preparations? I could do it but it does take some work.

I have a promise of T7 from Kozloff and perhaps T5 from Luria and a lysogenic P from Bertani. I'll keep after them.

I got a letter from Siminovitch outlining all the things he and Smith would be doing on the cytosine problem in lysogenic systems. These people are

entirely amoral. Why not? The competition will set me on edge, make me work for a change, and increase error-proneness, but I shall develop a calm detachment.

With best regards,

Sincerely yours,

Seymour

Seymour Cohen

SC/md

You still haven't told me about the Rickettsial DNA note. I haven't seen it - will it come out? Also the paper to Swoffl - have you gotten the proofs?

There is to be a virus symposium at Cold Spring Harbor in early June. Delbruck will be inviting. If you wish I could ask him to be sure to include you, which he might have done anyway. Also it is not entirely clear that my suggestion would be efficacious.

Best

SSC

October 24th, 1952

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
Philadelphia 46, Pennsylvania. USA.

Dear Seymour:

I have done some more analyses on the preparations still on hand, and to illustrate the sort of results I now get, enclosed is a table of all the analyses done so far under the new conditions. A slight systematic difference remains between results from DNA and whole phage, but this is undoubtedly an artefact, and it does not look as if any differences between mutants are showing up with the greater precision. As I can run four analyses at a time, it will not take long to fill out the table when more preparations arrive.

Spizizen's second shipment of "T7" (T7, lot # 1, 2.6 gms.) has 3.36% P and 4.0% DNA, so is not much better than the first. It contains cytosine and no measurable OC; I did not bother to isolate DNA or measure the base ratios. If you have access to a better source of T7, I'd be glad to do a quantitative analysis.

I see Herriott gets T2 with 50% DNA. Do you think we should report % P content, or N:P ratio, as characterization of the preparation analysed?

How are things going?

Sincerely,

GRW/m

G. R. Wyatt.

October 14, 1952.

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia 46, Pa.

Dear Seymour:

Thanks for your letter of October 8 which crossed my last. Since you have those preparations available, we could reverse what I suggested and present analyses of the series of six whole phages, and just 3 or so isolated DNA's to illustrate lack of difference. For this, you would not have to send any more DNA, but I should like some of all the phages except T2r+. I have also T6r, but, as I said, it seems to have some extra P, so it would be good to try another batch. Then I shall get these analyses done and written-up as quickly as possible.

You seem to be wasting no time with the metabolic work. It's interesting that the pyrimidineless mutants can't use OC; I wonder what they'll do with its deoxyriboside. It is perplexing that one shouldn't get the deoxyriboside enzymically--you remember I got the same result from DNase and snake venom phosphodiesterase. I got a faint spot with the correct position and spectrum for OC deoxyriboside, but mainly a diffuse streak of substance (or substances) having a spectrum close to that of cytosine deoxyriboside (pH 1, 279; pH7 and 13,272). It looked as if the OC nucleoside was decomposing on the paper. Is it possible that the nucleoside is even more sensitive to oxidizing conditions in acid than the free base? If so, one might get better results by using a neutral solvent on paper, or resin columns.

I've just been to a symposium on "nucleoproteins" at Laval University in Quebec. Zamenhof gave an excellent paper on his work with transforming factors. They are very sensitive, any treatment which breaks a few hydrogen bonds being enough for inactivation. J.A.V. Butler states that urea depolymerize's DNA. This might explain the vegative result of your phage transformation experiment; I suppose, however, with present ideas as to the specific adsorptive function of the virus skin, one would hardly expect it to work.

Some more T7 from Spizizen has arrived in the Soo, but I haven't got my hands on it yet; shall let you know of results.

Congratulations to Larry.

Sincerely,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

October 8, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

In looking through my desiccators I have turned up some dried preparations of T4r$^+$, T4r, T6r$^+$, and T6r. We are making some T2r$^+$ and T2r now. If you wish some of the former I'll send them right off. You don't really need the isolated DNA, do you, for these final analyses?

We have begun work seriously on C_x. We have pyrimidineless mutants of E. coli, one for cytosine or uracil, one for thymine, neither of which use C_x. We are scanning a few other mutants, having scoured Tatum, Lederberg, Ryan, Davis, etc for them, and will have a more complete nutritional story soon which will put us in a better phage and enzyme position.

I have made deoxyribosides from thymus NA via DNase and intestinal phosphatase, finally separating them very nicely on paper. Virus DNA doesn't liberate the C_x nucleoside by this method which is rather interesting and will be checked.

We are almost set to do isotope experiments on the origin of C_x, following the ring with labeled orotic acid and methylol with β-labeled serine. These will get started in a while and will take longer. When these are out of the way we'll be ready for enzymes. Larry will participate when he's settled after his marriage taking place next week.

I've been to Michigan and Wisconsin and everyone is quite properly excited about C_x as were the people at the phage meeting. Incidentally, Miller is having a difficult time with the uracil derivative. Also I have a promise of legitimate T7, for you, if you want it.

With best regards, I remain

Sincerely yours,

Seymour

Seymour Cohen

SSC/aws

October 7, 1952.

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia, 46, Pa.

Dear Seymour:

Thanks for your letter of Sept. 16. I hope your trip and talks went off well. I'm glad you're isolating OC nucleoside, and shall be interested to hear if it goes smoothly.

For the T7 DNA I used your urea method, and got a yield not far from the total DNA content of the material. I looked for OC in some of the unfractionated "T7" too, and couldn't find any, and as Spizzen says there should be 10^{15} active particles per gram in this stuff, I feel reasonably satisfied there is no OC in T7. Spizzen says he is making a better batch. I re-examined an insect virus too, and it has all genuine cytosine.

Another finding about hydrolysis. It is important to avoid an oxidizing atmosphere. This turned up when I tried using the volume of formic acid I had found to be optimal (0.25 ml) in larger test tubes for convenience and got results right back where they were before with less acid. It is a question of air volume, as I confirmed by substituting N_2 or HCOOH vapor for air, which gave better recoveries. This may possibly explain Larry's loss of all bases when he tried HCOOH.

Thanks for your promise of more material. I should welcome both the phages (T2r, T4r, and T4r+) and the DNA's (T4r, T4r+, and T6r) mentioned in your letter. The prep'n. of T6r-6 phage which you sent at the beginning of June gives about 10% lower recoveries in terms of P than the other phages (including T6r+) but as the ratios are the same, I imagine this prep. picked up

a trace of extra P somewhere. Also, I have now run out of T6r+ phage. However, I now get virtually identical figures from whole phages and from DNA's and feel it would be satisfactory for publication to present analyses of the six isolated DNA's and say 3 or 4 whole phages to illustrate the lack of difference. OK?.

Well, I seem still to be in the analytical business whether I like it or not, and after spending so much on development of technique I should probably be foolish not to get the most out of it. So when you have T1, T5, lysogenic phages, prophages or whatnot, send them along if you care to. The results are still interesting enought to overcome the boredom of cutting up bits of paper, it would be good if we could do the whole T series, in view of the biological differences that are being turned up. Have you seen Graham and Co's. results on superinfection breakdown, which he interprets as due to re-arrangement of host DNase, and in which T2, 4, 6, and 5, behave very differently from 1, 3, and 7?

I see that the majority of letters now appearing in Nature are dated about April; as ours was early May, it should not be long now. I hope they give the OC note some priority.

Incidentally, I never asked for reprints of your papers on carbohydrate metabolism (J.B.C. 188, 509, and 189, 617), and should be grateful for copies of these, if they are still available.

Best regards,

GRW/h

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

September 16, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario
Canada

Dear Jerry:

Thanks for your note of September 11th. This plan I mentioned is proceeding in the appointed channels but things won't get genuinely definite for several months. I'm glad you are interested; it could be both fun and profitable.

I returned eager to get to work and one of the first things I am trying is the isolation of the desoxyriboside following enzymatic hydrolysis. S. & D. promised me a large batch of phage but I don't suppose I'll get it for some weeks. Meanwhile I'm going through the procedure with thymus DNA and 12 mg. of T2r DNA. I've written to Larry but haven't heard his plans yet. He's getting married next month; will see him Monday

Oct. 7 Have:
DNA Phage
T2r
T2r+ T2r+
T6r+ T6r

Concerning your wish for more phages. I'll be glad to supply it when we make them. I'm breaking in a new assistant and it will be some weeks before T2r, T4r, and T4r$^+$ will be coming through. Do you also want the DNA of T4r, T4r$^+$, and T6r?

I'ts nice that the numbers now add up to 100%. It's usually true that you discover how to do something right when you are at the end, rather than the beginning. This is known as experience. Anyway, if you wish to write it up with results based on the new method and new materials, I am quite willing to wait since the note is in. This is up to you. By the way, what happened to the Rickettsial note?

Do you still consider yourself to be in the analytical business? I shall undertake T1 and T5 and some lysogenic phages this year and would be just as happy if you took over this job.

Concerning T7. How did you isolate the DNA? There is no guarantee this would work for T7 and although the result is such as to make you wish to have a decent sample of T7, I wouldn't trust it in any other way. Spizizen was all excited over the absence of OC in T7. But it would be interesting if it were true.

Finally, I'm sorry but I won't be able to visit your laboratory, since I do have many things, including teaching to get back to. But I trust we will get together soon again.

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:KHG

September 11th, 1952

AIRMAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia 46, Pennsylvania. USA.

Dear Seymour:

You are probably wondering by now what I have been up to for the past 3 weeks. Our original note finally came back from Nature last week, and the revised note has been sent in, with Sprague's suggestions incorporated, under date of Sept. 3rd. Nature seems to be jammed with notes, so we just have to wait.

Spizizen sent 1.75 g. of so-called "T7". It had 4.4% P and 3.6% DNA, and so cannot be more than about 10% virus. From 1 gram of this I isolated 45 mg. of crude DNA which contains cytosine and apparently no hydroxymethylcytosine (OC). Maximum possible OC, by chromatography in two solvents from 18 mg. of crude DNA is 0.5% of the cytosine, and the eluate had no peak at the proper wavelength, so there is no evidence that there is actually any OC. Ratios of the other bases are:

A	26.5
T	30.4
G	16.8
C	26.2

P recovery = 95%

But as these probably represent a mixture of T7 DNA, coli DNA, and maybe a little RNA, we'd best forget them. The lack of OC is a little disturbing to ones assigning any crucial significance

Dr. S. S. Cohen September 11th, 1952

to this base, if T7 stops RNA synthesis as effectively as the T-evens. Of course the preparation was dirty, but if there was any OC it should have shown up. I've written these results to Spizizen, and asked if he did activity titrations, or has any evidence of how much T7 is in that mess. Do you think it's worth trying to get hold of any clean preps of T-odd phages?

I started to go through my notes with the idea of putting together a paper, and realized that for phage DNA I have always used 0.05 ml. formic acid per sample of 0.5-0.7 mg., and for whole phage 0.15 ml. per sample of 1.5 mg. The 0.05 ml. was arrived at by scaling down proportions I originally found satisfactory when working with animal DNA's and to much less exact standards than we aim at now: an experiment I did 2½ years ago suggested that the amount of HCOOH was not very critical. The 0.15 ml. for whole phages was to take up the extra organic matter. Now it occurred to me that the amount of HCOOH might have something to do with the greater yield of OC from whole phage. So I did an experiment with 0.7 mg. samples of T6r$^+$ DNA and 0.05 to 0.75 ml. HCOOH, and found that the amount of acid is exceedingly important, yields reaching a maximum with about 0.25 ml. or more. OC is affected most, but guanine and adenine slightly too. Presumably it is the liberated phosphoric acid which is harmful and must be diluted.

Here are some results with the new procedure, using 5 times as much HCOOH as before:

	Moles per 100 total nucleotide				Bases
	A	T	G	C or OC	P
My ox spleen DNA	28.0	27.5	22.6	20.5	101%
My T6r$^+$ DNA, from Spizizen's prep.	32.2	32.2	18.3	17.3	101
Your T6r$^+$ DNA	32.7	32.9	18.4	16.0	102
Your T2r-2 DNA	32.4	32.1	18.1	17.4	102

These are the first results from phage that one can really regard as quantitative, and, incidentally, they bring phages within the fold of the magical Chargaff formula A/T = G/C = 1.0 approx., only with OC for C. I am now trying different amounts of acid with whole phage, in the hope of getting similar results.

Dr. S. S. Cohen September 11th, 1952

Well, I am happy to have at last results which can be published without apology, but I am thoroughly fed up with myself for not having tried this a year or more ago, and saved dozens of false analyses and much material. Now I have left of your DNA's only T2r, T2r+, and T6r+, and of whole phages T2r+, T6r, and T6r+. It is scarcely fair to ask for more material at this stage when we were to be packing this project up, and I don't imagine you have time to prepare anything for a few weeks anyway, but if you do feel it is worthwhile in order to get results presentable without apology or explanation, I am prepared to do the analyses. Meanwhile, I shall continue with the remaining material on hand. How long does it take one to learn to take nothing for granted, and treat each problem as new and potentially different?

I had a letter from Angus Graham today, back from Paris, saying the phage conference was very good, and asking for more information on our new base. I am sending him a copy of our note for Nature.

I hope this gets to you before you leave on your trip, and that the latter is successful. Dr. Bergold asks if I could persuade you to come up to the Sault. We are only 400 miles north of Madison, and you would certainly be exceedingly welcome here! Unfortunately, our department does not, as a matter of policy, ever provide expenses to bring visitors, so we cannot extend a very hospitable invitation.

Most sincerely,

GRW/m G. R. Wyatt.

DIVISION OF ENTOMOLOGY

SCIENCE SERVICE

CANADA

DEPARTMENT OF AGRICULTURE

Sept. 10, '52.

Dear Seymour,

It is not from lack of interest that I have not replied to your note before this. I am exceedingly interested in your ideas about animal virus work. Certainly a big proportion of the animal virus work being turned out now is of a rather messy sort, and the application of the more rigorous methods and concepts that have grown up with phage should produce results. At any rate, it would be great fun to try, and I should count myself very fortunate ~~[illegible]~~ to be associated with such a project.

~~So I look forward to hearing how your scheme develops.~~

So ~~If~~ if it does materialize, ~~I should welcome any opportunity to take part in it~~ and you feel there is a place for me in it, either in tissue culture or in biochemistry, I shall seriously consider any offer. ~~While my wife and I are not reconciled to remaining in the Sault indefinitely, we are not impatient to move just yet, and have come to the conclusion that it is best to wait for a really attractive opening.~~ I have no experience of tissue culture but ~~I could learn~~ am interested in the method and could learn. ~~have some interest in it especially since my wife has~~ It does appear to be the most promising system for study of animal viruses under controlled conditions of cell number, cell nutrition, etc (although it does still seem to involve a certain mystique!). Indeed it is with this in mind that my wife is

developing methods for culture of mixed tissues here now.

~~There is probably no~~

So I am very happy that you have told me about this scheme, and look forward to hearing how it may develop. Incidentally, where would the proposed lab. be located — in the new building at Chelsea's?

All the best.

Sincerely

Table I

Yield of bases from ox spleen DNA

after hydrolysis in $HClO_4$ (70%, 15 µl./mgm. NA) at 100°

Material analysed	Hydrolysis time	Moles per 100 moles total bases*				P accounted for, per cent	N accounted for, per cent
		Adenine	Thymine	Guanine	Cytosine		
Purified DNA	1 hr.	28.2	26.3	23.0	20.7	95	97
"	2 hr.	28.5	26.0	23.2	20.7	98	97
DNA 15%, albumen 85%	1 hr.	27.8	27.0	22.7	20.7	99	-
"	2 hr.	27.8	26.9	22.7	20.9	100	-

*Allowing 1.3 for methylcytosine, not estimated in these experiments.

Marine Biological Laboratory

WOODS HOLE, MASSACHUSETTS

Aug. 18, 1952

Dear Jerry:-

There is not a word I choose to change. It is a beautiful paper. I am returning one copy, forwarding one to Sprague for his remarks (which I will ask him to make to you directly - I dont think he will have any - although he may switch the names of Sprague and Miller) and keeping one. As far as I am concerned it may be submitted when you are satisfied with it.

I dont know whether I ever discussed with you my notions on going into the animal virus business. It is conceived of as an integrated group of studies involving an animal and egg lab, a tissue culture lab, a physical chemistry and biophysics lab (electron microscope, centrifuge, electrophoresis) and a ~~molo~~ biochem lab. The effort would be essentially to reproduce the phage ~~results~~ data on some virus such as vaccinia, with an eye to chemotherapy. I have never pushed it for fear of the administrative chores involved.

However I may talk myself into it and some Foundation into a commitment of $500,000 over a 5 year period. Indeed I have just chatted with Rivers who chairs the Virus Committee for the Polio Foundation and he liked the proposal, indeed was most optimistic. Further they guarantee salaries on an increasing scale for the 5 years.

If I try to push this, with an appropriate lab set up, would you be interested in running one of the labs?, perhaps tissue culture? Someone would have to learn this and I picture this as a biochemical effort in nutrition and metabolism of the host, essential to and prior to setting up infected cells, and not as an exercise in obtaining clean glassware on which an explant "grows." What do you think? It wouldn't come into being, if ever, in under 1½ years.

With best regards I remain

Sincerely yours

Seymour Cohen

August 15th, 1952

AIRMAIL

Dr. S. S. Cohen,
Marine Biological Laboratory,
Woods Hole, Massachusetts. USA.

Dear Seymour:

Here is a rehashed note on 5-hydroxymethylcytosine (we'll have to call it OMC or something!). I'm sending 3 copies so you can send one on to Sprague after correcting it. I wrote to the editors of Nature on Monday, asking if we could retract the earlier note, and it would be well that we should have a new version ready to submit on hearing from them. I re-determined ε with the synthetic material (which was here when I got back) and got 9650, practically the same as Miller did, so am re-calculating results on this value. Examined with our equipment, the synthetic material has identical spectra (peaks agree to 0.5 mμ) and Rf's with the natural, so that's OK.

Please make any corrections or changes you like in the MS, and in particular, check that the acknowledgements to Spizizen, Sprague, and Miller are in appropriate form. I hadn't the figure for the N analysis, but have written to Sprague for it.

I'm embarking on a full paper on this now, but guess it will take a couple of weeks at least. I'll do it in the form of the Biochemical Journal.

Dr. S. S. Cohen August 15th, 1952

I enjoyed the trip to Philadelphia and am grateful for your part in making it possible. Although not very exciting, the meeting was probably worthwhile in the end for all parties.

Best regards,

Sincerely,

CRW/m G. R. Wyatt.

July 29, 1952.

Dr. S.S. Cohen,
Marine Biological Laboratory,
Woods Hole, Massachusetts.

Dear Seymour:

No word from Sprague yet, but I am hesitant to rush him after keeping him waiting myself what may have seemed an unreasonably long time that it took me to prepare the substance. I am sure synthetic work, like analytical work or any other sort of work, always takes longer than one hopes. The elementary analyses, however, should be routine, and if we don't hear by the end of the week it might be well to write him. I'll let you know as soon as I hear from him, but I expect he'll write you at the same time. I only hope my sample is really pure, as it appeared to me, and the analysis significant.

Our Kyildahls unfortunately did not give N on a dry weight basis, as I used only 0.8 mg. of Cx, weighed on the ordinary balance, for the experiment.

Here are some figures on recovery of Cx heated with formic acid in the presence of thymus nucleic acid and separated by two-dimensional chromatography:

	Yield from DNA		Recovery of added base C:	
Hydrolysis	Adenine Mols./mol.P	Cytosine Mols./ mol.P	Mols./ mol.P	% of unhydrolys
100° 2 min.	.260	-	.146	(100)
165° 30 min.	.252	.191	.112	77
170° 30 min.	.251	.192	.106	73
175° 30 min.	.247	.189	.099	68
175° 30 min., with added protein	.249	.186	.066	45

It looks as if we have been losing 25-30% of Cx from the isolated DNA's. The result from the added protein (thymus DNA + bovine serum albumen 1:1) is a complete surprise. I had expected loss to be reduced by presence of protein, since I get higher and more consistent yields from whole phage than from phage DNA. I can only suppose that it makes a difference whether the nucleic acid is bound *in situ*, and am not sure how to determine the true recovery of Cx from whole phage. Any ideas? Well, I only completed this experiment today, and shall ponder further over the results.

Sincerely,

GRW/h

G. R. Wyatt.

Marine Biological Laboratory

WOODS HOLE, MASSACHUSETTS

July 23, 1952

Dear Jerry:-

I was very pleased indeed to hear about your isolation of C_x in decent yields and that Sprague had 13 mg for analysis. Do you feel that I ought to write Sprague urging speed with the hydroxymethyl pyrimidine and the C H + N? I will if you get any impression of a lackadaisical attitude on his part, although I do imagine he will realize how wise for him it will be to hurry. I suspect the synthetic work is always slower than one hopes.

You are really to be congratulated on having done a splendid job and I am very fortunate to be associated with you in this. It will be great fun to start the metabolic work in the fall and learn some new chemistry and procedures.

I trust the heat wave has left you alone

Sincerely

Seymour

Incidentally, what % N did you get for C_x in your Kjeldahl? Cytosine has 37.8 % N, CH_2OH-cyt - 28.4 %

July 19th, 1952
File:

Dr. S. S. Cohen,
Marine Biological Laboratory,
Woods Hole, Massachusetts. USA.

Dear Seymour:

I have just sent off 13 mg. of C_x to Dr. Sprague. This was isolated by chromatography in two solvents, purified through the picrate (in spite of my earlier remarks, it does form a nice insoluble picrate under the right conditions), and 3 times crystallized from water. I have another roughly equal amount in various stages of preparation, and should be able to get another crop of crystals. Once clean, it forms beautiful prisms very easily, and it is very satisfying to have some in a bottle!

The purified C_x has a molar extinction coefficient (based on Kjeldahl N, and assuming 3 atoms N per molecule) of 10,000 at the maximum at pH 1. Cf. 10,500 for cytosine and 9800 for 5-methyl cytosine. This means an upward correction of 5% to the estimates of C_x — not enough to affect the total recovery much. Now that I have some of the pure base, I shall determine exactly how much is lost under conditions of hydrolysis.

In a letter of July 1, Dr. Sprague said they were attempting to prepare the 5-hydroxymethyl pyrimidines, but I have not heard anything further about this yet.

Dr. S. S. Cohen July 19th, 1952

The note on rickettsiae was received by Nature in early May (I find by enquiring from our head office, through whom all this is done), and acknowledged, but apparently nothing said about acceptance or non-acceptance. Perhaps that is all that Nature does let one know? I think we can assume it has got in; at any rate, the manuscript has not come back.

Enclosed is a copy of the final version of the note on C_X, which has been sent in to Nature too. I hope it won't be long now before we can write something more substantial.

I hope you're enjoying Woods Hole.

Sincerely,

GRW/m G. R. Wyatt.
Encl. 1

Marine Biological Laboratory

WOODS HOLE, MASSACHUSETTS

June 31, 1952

Dear Jerry: —

Enclosed you will find the ever-so-slightly corrected manuscript.

Concerning the multiple publication, it is my feeling that it is justified in this instance since the paper to the phage meeting will not be published for years, judging from the tardiness of other French publication of symposia. And this will have a wider audience. And presumably the extended paper will have elementary analyses and an identification (I hope). But in general it is undesirable to have multiple publications of the same data. Oh well.

Incidentally I never heard about the rickettsial DNA note. Does it get in?

Sincerely

Seymour

June 27, 1952.

Dr S. S. Cohen,
Marine Biological Laboratory,
Woods Hole, Mass.

Dear Seymour,

Here is a draft of a note for Nature. It says practically the same things, with slightly different words and emphasis, as your paper for Lwoff. Do you feel this is undesirably close duplication, especially as some of it will have to be said again eventually in a more extensive paper? The figure referred to is to be the same figure of relative R_f values. Please make any changes you think desirable.

Sincerely yours

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

June 27, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

Thanks for the prompt reply. Your chromatograms are beautiful but I think you ought to use them in the Nature or other paper. As you've noted, this one for Lwoff is all jammed up now. They are so pretty I will post them around the lab.

Concerning the number from Ottawa, I will ask Lwoff to correspond directly with you on everything involving publication, so that you can introduce the number in proof.

Congratulations on getting crystals of C_X. I have spoken to Dr. J. Sprague, Head of Chemistry at Sharpe and Dohme, Inc., West Point, Pa., who will do C, H, and N analyses on 8-10 mg when you get it. He will send you a note confirming this. You ought to deal directly with him since I'll be at Woods Hole. Also, they just ran the last step on the 5 methylol uracil yesterday, and he will send the stuff to you when and if they get it.

I hope you have a nice summer. I'll come back in the Fall raring to work on C_X.

With best regards, I remain

Sincerely yours,

Seymour

Seymour Cohen

SC/mw

June 24th, 1952
File:

AIRMAIL
SPECIAL DELIVERY

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

Here is your MS, returned the same day it arrived here, which is probably a record for me. I think it covers the ground well. I have substituted T2r+ figures for whole phage, and altered the T6r+ figures by averaging them with a second analysis done recently, and this happens to bring the three very close together. I have made some slight alterations in UV data and added figures for 4-methyl cytosine.

As for the text, I have made some minor changes most of which you are at liberty to change back if you feel strongly about them. I thought the idea of minimizing the number of molecular species, in your first sentence, a little off the main theme at the moment, and may confuse the reader. Also, we are stretching Lwoff's 500 words considerably, so any shortening may be good.

I'm enclosing also duplicate prints of a couple of demonstration chromatograms, which you can include with the paper or not as you think best.

I have some Cx dry and crystalline now, and if, after it has been recrystallized a couple of times, there is enough left to weigh, I shall send it to you. Some more is in preparation.

Sincerely,

GRW/m

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

June 20, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste Marie, Ontario
Canada

Dear Jerry:

Do whatever you wish to this but do it fast. Do you have $T2r^+$-whole phage figures you wish to use here in Table 1 instead of the figures used Figure 1 will be your figure minus R_f values given in the Table. Please fill in references 4 and 5.

I'll send off a photograph of the Figure when I get the changed manuscript back. I will probably be leaving for Woods Hole June 27 or 28, and just possibly, your corrections will be back here before that date. I hope that I'll be able to get it off to Lwoff by then.

Sincerely yours,

Seymour

Seymour S. Cohen

SSC/mw

P.S. I think one figure adequate, no slides needed. And I wouldn't wait on the note to Nature, although I'm expecting some 5-methylol uracil possibly next week

Box 490
June 14, 1952.

Dr S.S. Cohen,
Children's Hospital of Philadelphia,
Philadelphia, Pa.

Dear Seymour,

Thanks for Sharpe & Dohme's second batch, and for your letters of June 2 and June 4. The latter took 8 days getting here: I think it is worth using airmail and putting our P.O. box number (490) in the address.

Here are some analyses on the two Sharpe & Dohme batches:

	% P	% DNA (Dische)
Prep. 1. "T6 concentrate"	1.92	18.7
Prep. 2. "T6r+"	2.05	20.1

I am surprised at the small difference between the two, and the lack of extra P, and shall have the estimations repeated, but I have no reason to suspect them. The new material I am treating in smaller batches, keeping the temperature down, and not trying any shortcuts. From 7 g. of the material I have removed most of the protein and purines, concentrated in vacuo, hydrolysed in formic acid (15 min. at 165°), and spread the hydrolysate on 8 sheets of Whatman's No. 3. A pilot chromatogram indicates that there must be a total of 25-30 mg. of CX of the papers. The other 5 g. of the batch are still intact. I think the big loss in the earlier lot came when I evaporated the formic acid hydrolysate to dryness on a water bath: CX was thus exposed to 100° in the presence of phosphoric acid, and didn't like it.

Thanks also for the T2r+ and T6r for analysis. I left off the +. Sorry. One run with these does not confirm the differences suggested by the runs on isolated DNA's, and substantiates my view that they are artefacts. I must send you more of the quantitative results, but should like to do another run or two first.

I have done some estimations of maximum possible CX in thymus DNA and coli, and cytosine in phage. Results in enclosed table. This was done by eluting the C + CX band from big chromatograms in

- g" to you, but if Sprague ---- a note to Nature now.

isopropanol-HCl, concentrating in vacuo, and running in butanol-NH_3, and measuring the extinctions of the eluates of the appropriate areas. In no case was the spectrum of the eluate characteristic of the substance sought; with the whole coli the absorption was probably due to a trace of cytidine, which has RF's close to CX in both solvents. So I have actually no evidence for the presence of any CX in thymus DNA or coli, though of course the possible presence of minute traces can still not be excluded.

5-methylol cytosine sounds a good suggestion. It had actually occurred to me, and I rejected it on the following grounds: the shift in UV spectra caused by a 5-substituent appears to be a general damping effect, and so should bear some relation to the mass of the substituent group. Methylol, being bigger than methyl, might be expected to have a bigger effect on the spectrum, whereas actually CX stands between 5-me cytosine and cytosine. However, this is a naive sort of reasonging, based on the assumption of similar effects from dissimilar things, and does not provide an alternative suggestion. So 5-methylol is certainly well worth trying, especially in view ofits chemical relationship to 5- me cytosine and thiamine.

Well, I wondered what sort of results Hershey was getting. It's good he wrote you. I'm very happy to have you write the paper for Lwoff, and enclose some RF data. Ithink they are most meaningful when expressed as fractions of the RF of cytosine. This might make a good lantern slide. Do you want any more detailed UV data? How about a slide of the curves, cytosine vs. CX, at 3 ph's? If you think this worthwhile, I coudl send the figures or draw the curves. I should be glad to draw them, and the RF ones too, for uniform style. I shall run a couple of demonstration chromatograms and send you prints.

Yes, I suppose it would be wise to send a note to Nature now. I'll write something and send it to you, but if Sprague is likely to produce 5-methylol C soon, we might hold it off for that. From the uncommunicative tone of a recent note from John Smith, I gather that he didn't like our note on rickettsiae (of which I sent him a copy) and they are competing with us on CX. I'm sorry this has come about; I suppose I should have made the note less pointed.

Sincerely,

Maximum possible cytosine and CX contents, by UV absorption of appropriate areas eluted from chromatograms.

Substance sought	Amount of starting material	Moles per cent of CX	Moles percent of cytosine	% of weight
Cytosine in T6r+ phage	45 mg.	0.2	-	0.004
CX in *E. coli* crude DNA	400 mg. *coli*	-	0.6	0.02
CX in whole *E. coli*	270 mg.	-	0.2	0.002
CX in thymus DNA	18 mg.	-	0.2	0.014

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

June 4, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

I am sending 12 gms of a lyophilized T6r$^+$ under separate cover. I have not estimated DNA, imagining that you would do it anyway and I should like to know this since the activity measurement suggests either considerable impurity or much inactive virus. This comes from Sharp & Dohme and I don't know how much salt is in the preparation. However, it did have 1 decent differential centrifugation cycle. I'm shipping the remaining 2 gms to Larry.

I have just received a note from Hershey telling me of a new non-cytosine spot destroyed by perchloric acid, and asking what it is. In this event I would suggest that we have to submit a paper to Lwoff. However, this would eventually appear in the Comptes Rendus of the Phage Congress and would not appear for at least a year. Therefore, I would further suggest, if you feel it wise, that you write up a note for Nature. Also, that I undertake the paper to Lwoff, with you as senior author on both, and my version to you first of course. I think I know the data fairly well and would bring out phage points with less difficulty.

However, if you wish to compose it for Lwoff, I have no objections. Indeed, I almost prefer it since I do not have the R_f data at all completely and there would be a point in showing one of your prints of virus vs thymus DNA with the absence of cytosine and presence of C_X in virus.

Concerning 5-methylol cytosine, I don't think I elaborated on it. It could be a formaldehyde addition compound, on the way to becoming 5-methyl cytosine. Furthermore, it then becomes a relative of

the pyrimidine of thiamine, which raises a point in the polio-myelitis problem. Sprague has the 6-methyl 5-methylol uracil to test and will make others. I'm told he seems somewhat excited and indeed I'm just getting to be myself. I'll try to keep Hershey slightly informed.

Sincerely yours,

Seymour

Seymour Cohen

SSC/dk

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

June 2, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

Your recent analyses on our so-called phage DNA batches have suggested to me that urea and Sevagging are not entirely satisfactory, at least in their present form, and I shall putter with this in the fall, knowing that it won't help you now. I shall have to get after Sharp & Dohme for their batches which haven't come in yet. Larry wants some too.

I sent T2r$^+$ and T6r as was requested in your letter of May 7. Your letter of May 23 calls for T2r and T6r. Which is correct or did you forget the + on the 23rd?

It has been suggested that the 5-methylol compound may be it, and I am hunting it down. If I can't get it from Johnson's old stock, Sprague, who runs organic chemistry at Sharp & Dohme, has offered to make it and analogues. I now have him sold on the importance of the problem and I think he will be quite helpful. He has a lot of experience in pyrimidines.

Weed has agreed to whatever we wish about a paper. I've written to Lwoff asking whether he would read a brief note and should hear soon. He has okayed it and will present a 500 word note for us. How does this sound?

We are clearing up our "arabinokinase" problem. It looks as if this is 2 enzymes, the pentose isomerase which catalyses D-arabinose $\rightleftharpoons$ D-ribulose, and a specific ribulokinase which catalyses D-ribulose+ATP $\longrightarrow$ ribulose phosphate + ADP.

This last will now take some work but we have now separated it from the isomerase and can follow the phosphorylation separately.

With best regards, I remain

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:ms

May 23rd, 1952

Dr. L. R. Weed,
Department of Bacteriology,
Army Medical Centre,
Washington 12, D. C. USA.

Dear Larry:

I have been meaning to write you for a while, but you know how fast time goes when you're busy in the lab. Seymour sent a massive amount of T6 and I'm working on isolation of enough of the supposed new base "CX" for analysis.

Seymour tells me you got a small amount of a substance with max. in acid at 278 by evaporating down the 282 fraction in HCl, but lost everything by autoclaving in formic acid. When hydrolysing a nucleic acid, I do not lose more than 5% of guanine, adenine, cytosine, or thymine in formic acid at 175° in 30 minutes. "CX" is more labile - the loss may be 20 or 40% - I have not yet had enough of the pure stuff to check this. Also, the loss may be more from a purified nucleotide than from a whole DNA. Anyway, if you would care to send a sample of your 282 column eluate, I should like to try hydrolysing under our conditions and compare the product (if any!) with my CX. I should be very interested to see if I can get anything out of the 274 fraction too, which from your data looks very mysterious.

Incidentally, the ratio D_{278}/D_{262} I got for CX is 1.93 - satisfactory aggreement with your 1.88, I should think.

Does your bug really have no thymine? This will shake nucleic acid and chemistry to its roots. If only Levene were alive!

Best regards,

Sincerely,

GRW/m

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

May 22, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

I am sending a preparation of T6r and T2r$^+$ under separate cover. They have been lyophilized but not dried over P_2O_5.

A large batch of purified T6r$^+$ will follow any day now. A new suggestion for C_x is 5-methylol cytosine and I shall explore obtaining it.

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:ms

May 23, 1952

Dr. S. S Cohen,
Childrens Hospital of Philadelphia,
Philadelphia, Pennsylvania.

Dear Seymour:

Thanks for your letter of May 13, which I did not get until after I had written you. I am happy to know that the first batch from Sharpe and Dohme was not their final and definitive preparation, as its impurity made operations considerably more difficult. I left it in urea (360 g./l.) overnight, and depotenized by the Sevag method, then added 1.5 vols. of alcohol and a little acetic acid, but only about half the nucleic acid came down. Over prolonged contact with urea break up DNA, or can bacterial enzymes have been active? I should not have thought they had a chance in 36% urea. I have not had much success at recovering the degraded DNA from the urea solution. The faction which did precipitate was satisfactorily freed of purines and hydrolysed, but CX did not come down on addition of picric acid, so it is now running on chromatograms. Cytosine purate would have precipitated under these conditions, and this may point to a difference in solubility. The chromatograms should yield enough for the experiments I have in mind, but probably not sufficient for really clean material for analysis, so I'm glad there is another batch on the way, and I shall profit from the experience of the first lot. Is urea and Sevagging really the best way to isolate DNA from phage? Have you ever tried detergents?

I look forward to small preps. of T2r and T6r DNA's for quantitative analysis. One reason I think the apparent reciprocal relations of CX and G in phage DNA's are artefacts is that they are <u>not</u> in line with the Chargaff thesis.

He points out (and the insect viruses agree) that A:T and G:C are relatively constant ratios, whereas A T:G C is the most variable ratio characteristic of the NA's source. With the T-evens, on the other hand, A T:G C is constant, and G:CX appears to vary.

I have looked at Larry's notes and am returning them. You must be amused at our rude remarks about each other's methods of hydrolysis. CX is certainly far more labile than the other pyrimidines. I'm writing him now to see if he would care to send me some of the column fractions so I can try to get the CX base from one or both.

Please don't feel apologetic about not pulling your weight in the lab. work. You did not ask for this problem and have obviously got plenty to do, and are supplying material we could not otherwise get.

Yes, I think it might be a good plan to send a note to Paris. What would be the deadline date?

Sincerely,

GRW/fl

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

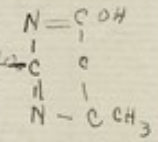
Department of Pediatrics
1740 Bainbridge Street
Philadelphia 46, Pa.

May 13, 1952

Dr. G. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canda

Dear Jerry:

Thanks for your letter of May 7. Too bad about 5-OH uracil. I am sending, under separate cover, a sample of 6-methyl uracil. 6-Me uracil is really the old 4-Me uracil In our hands it has a maximum at pH7 of 260-261, but at pH13 it is 275-278. I am sending it anyway since I don't know exactly how you make up your solutions, and you would wish to know the R_f values. Since the isotope data indicated cytosine $\longrightarrow C_x$, there may have a flaw in the addition of the methyl to C_6 instead of C_5 i.e. virus infected bacteria are drunk, blind, or can't pin the tail on the donkey.



Also, I am sending with the 6-methyl uracil the first produce of Spizizen at Sharp & Dohme. He lysed lots of B (our strain) with T6r$^+$ (our strain), but didn't clean it up properly. Thus, he centrifuged the lysate at high speed, before a low speed run and lyophilized the pellet, which of course contains the bacterial debris. However, from the phage assay this stuff would contain at least 2.5gms of phage DNA and, if you think you can get anything out of it, it is all yours. I suspect you can.

In any event, within the week there will be several grams of phage DNA in decent phage lyophilisate. I have seen the unlyophilized preparations. You will be quite busy very soon, as if you weren't already. I shall send you, as soon as I can make them, lyophilized preparations of T2r$^+$ and T6r. I gather that the differences with which you are concerned are within the apparently reciprocal relations of guanine and C_x, i.e. in the numbers you sent me $G+C_x$ appear to add up to a constant in all of them, even as A+T. Again a point tying C_x to cytosine and another link in the Chargaff thesis.

I am told that substitution at C_6 labilizes the nucleoside link i.e. uridine is much more labile than uridine etc. This extends to synthetic 6-Me uracil derivatives. Perhaps you can detect C_x after mild acid hydrolysis of phage DNA. This would suggest a C_6 substitution

About the public notice. I prefer your second suggestion. I am in favor of a paper summarizing the analyses on the phages and pointing to the existence and properties of C_x, alluding to the existence of Larry's data and briefly describing the coincidence which led to calling a nucleotide fraction "cytidylic acid". If you think Weed's data should go in this paper that is allright with me, but I think the paper would become bulky and difficult to put together. If and when C_x is isolated and identified, Larry's nucleotide data could more properly go in then with all the details about elution patterns etc. This eventual second paper would not be concerned with Hershey's ideas as much as with the terrible 3, paper, resin, and Beckman. I've written to him, asking if this is acceptable.

Your plan to estimate maximal amounts of C_x in B and thymus DNA sounds very good. Do you need more B?

Finally, I am enclosing Larry's last letter to me and experiments which seem lucid enough when read after his last note to you, as well as his accompanying notes. I should like these back after you've digested them. Also it seems to me that if you wish to get C_x out of his 282 compound isolated by his methods you ought take a whack at it. You will note that he obtained a small amount of 278 compound from the nucleotide and this isn't really enough evidence on this score. Anyway you ought to establish direct communication with him.

This is the first time I have been involved in a venture of this sort, in which I did not pull my weight in the laboratory work. Simply, at this point I can not. Besides teaching and four other projects, I am to have a review in at the beginning of June and have barely started it. However, I do intend to work seriously at C_x in the fall.

Incidentally, there is a phage congress in Paris in August. There might be a point in announcing the analyses and C_x in a brief note to be sent to Lwoff for reading. What do you think?

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:ms

May 16th, 1952
File:

AIRMAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

Thanks for the magnificent surprise parcel.

6-CH_3 uracil is interesting to compare with deaminated CX but is not the same. In a chromatogram solvent of low water content (e.g. 85% butanol), 6-CH_3 uracil moves faster than uracil, whereas deaminated CX moves slower. This parallels the behaviour of 5-methylcytosine, and is what I anticipated. The UV maxima are as follows:

	pH1	pH 7.5	pH 13
6-CH_3 uracil	261	261	276

It is identical with deaminated CX in acid and neutral solution, but different in alkali. Comparing this with uracil, it appears that a 6-substituent reduces the extent of the shift from neutral to alkaline spectrum. A 5-substituent, however, (e.g. thymine) does not. Since deaminated CX has the same alkali shift as uracil, it seems likely to be the 5-position in which it differs.

The T6 concentrate is so large that I propose to put it through a chemical fractionation instead of chromatograms. I hydrolysed one sample whole and ran a chromatogram, and it was a mess from extraneous substances, so it seems it will be best to isolate the DNA. Then I

Dr. S. S. Cohen May 16th, 1952

propose to split off the purines with dilute HCl, and dialyse them off, making "apurinic acid" (Chargaff), then hydrolyse with formic acid, and precipitate CX as the picrate. This should separate CX from everything except any cytosine which may be present.

Sincerely,

GRW/m G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4

THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street
T. F. McNair Scott, M. D.
Research Professor

May 2, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Jerry:

Some bits of news. Weed has written to say that he has isolated a nucleotide, apparently homogeneous, which on hydrolysis gives C_x. He is excited about an organism he has produced with Cu which lacks thymine (he says, having done it thrice) and wonders what kind of phage it will produce. Our strains do multiply in it.

Spizizen of Sharp and Dohme believe he can have large amounts of T6r$^+$ for me before long.

A student of Ryan at Columbia has found that carefully washed bacteria require a nutritional factor for T2 multiplication. The factor is in broth, in lysates, and in hydrolysed DNA, but not in any substance of many supplied by Waldo Cohn. It is possibly C_x.

Have you obtained 5-OH uracil from Hitchings? 5-OH cytosine was made last by Johnson and McCollum JBC 1 446. If deaminated C_x is 5-OH uracil, I will undertake to make 5-OH cytosine, or get it made.

With best regards, I remain

Sincerely yours,

Seymour

Seymour S. Cohen

SSC:ms

May 7, 1952

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia, Pennsylvania.

Dear Seymour:

Many thanks for your letter. Hitching's 5-OH uracil arrived yesterday, and I am sorry to say that it is not the same as deaminated CX. Nor is barbituric acid. Here are the UV data:

	Maxima		
	pH 1	pH 7-8	pH 13
5-OH uracil	278	298, 239	304
Barbituric acid	No peak above 210	257, 224	260
Deaminated CX	261	261	285
Uracil	259	259	283
Thymine	264	264	288

This is very disappointing. Clearly, a polar group in the 5 position has a much greater and completely difficult effect from a non-polar (e.g. methyl) group, and deaminated CX remains closer to uracil than any other compound we know of. Looks like uracil with deuterium in it! Yet, what substituent or other structural change could cause the shift in partition and leave the spectrum so little changed? Deaminated CX differs from uracil in its chromatographic

movement just as CX does from cytosine--faster in solvents with much water and slower in those with little water. Right now I have no other formula to offer, but I shall let you know if any ideas arise from more reading and consideration.

What else can we try out? I shall look up the color tests for pyrimidines and see what is known about their specificity. We have now a paper electrophoresis set-up--I shall try this: it should show whether there is any ionizable group in addition to those of cytosine, and if so, its pK. Whatever is to be tried, we need more of the stuff. I have had made two large chromatogram tanks which will greatly facilitate preparation. If you can obtain sufficient phage, and wish to entrust me with it, I am prepared to isolate a sample for elementary analysis. One gram of T6r$^+$ should yield 10-15 mg. of CX. I assume you could get the analyses done.

Bergold, as I mentioned, was in England recently, and he visited Markham's lab. Markham and Smith have been growing phage again, and, as far as I can gather, have got the nucleotide of CX and find it has different electrophoretic movement from desoxycytidylic acid. Bergold did not get any details, but it seems they are working on it. When I first found there was something peculiar about phage "cytosine" last summer, I wrote and told John Smith, since we had done the earlier work together. He had little to say about it then, and as late as March when I heard from him he said he hadn't done anything more with phages. So they probably haven't done much with it yet.

I wonder if it would be wise to publish a note saying that T-even phages contain no cytosine but another substance whose properties we could describe briefly, without waiting to identify it? Weed could be included with his data on the nucleotide. I do not like the idea of this, but since we don't know how long it will take to identify it, it just might be advisable. Perhaps the best thing will be for me to complete the quantitative work (check on hydrolysis loss and extinction coefficient), and if then we still do not know what CX is, to write a paper with quantitative results and some properties of the apparently new substance.

Incidentally, in reply to my sending him reprints, Hershey sent me a mimeographed MS entitled, "Independent functions of viral protein and nucleic acid in growth of bacteriophage", and a note saying they are using my chromatographic method with good results. His paper, which I presume you have seen, certainly throws a heavy burden of genetic specificity on the DNA, and makes it especially important to check on possible quantitative differences. I should like to confirm whether the small differences

I got are artefacts. The two strains which showed the greatest apparent difference from one another are T2r+ and T6r. If you care to make and send new preparations of these two, I shall analyse them simultaneously, and estimate N, and thus check on possible difference and on my hypothesis that the presence of protein impurity protects CX from destruction in hydrolysis.

Nucleic acids have given too many surprises.for one to say you cannot have a bug without thymine, but it would certainly be pretty staggering. I am also interested to hear about Ryan's students nutritional factor. By hydrolysed DNA as a source I presume he means thymus. With my new tanks I can run a two-dimensional chromatogram with a massive amount of thymus DNA, and get a figure for maximum possible CX. Same with E. coli.

Hope you have patience to read all this. I wish the days were twice as long!

Most sincerely,

GRW/f

G.R. Wyatt.

April 5th, 1952

AIRMAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
Philadelphia, Pennsylvania. USA.

Dear Seymour:

The note on rickettsiae has been passed around this lab for comments, and the last paragraph has come in for some criticizm as being obscure. How about the following modification:

"If, for as yet obscure reasons, these proportions have to be maintained in DNA structure, this would greatly increase the probability of two distinct DNA's having like composition by chance. This may account for the similarity of the R. burneti and chick nucleic acids".

I see that Biochim. Biophys. Acta publish short notes in about 3 months less time than Nature, and wonder how you would feel about sending it to them.

Sincerely,

GRW/m

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4
THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street

Dear Jerry:

Fine. See you at Childrens Friday and/or Saturday, April 18 + 19. I'll try to get Weed to come here then.

Sincerely yours
Seymour Cohen

P.S. Manuscript is OK.
SSC

KINGSLEY 6-2700

FOUNDED 1855

KINGSLEY 6-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA

1740 BAINBRIDGE STREET

PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

March 24, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario

Dear Jerry:

Thanks for your note and manuscript. On the latter I have made several small changes which you may rearrange as you wish. On p.2, it would be well to include the reservation in some form that rickettsial DNA may not come from the host at all. Concerning the last two paragraphs, I wonder about the significance of the magic ratios and try to cover my uneasiness with the qualifications you see introduced.

I intend to be in New York on Monday and Tuesday, April 14 and 15. I teach Wednesday and Friday mornings and shall try to avoid returning to New York on Wednesday unless some exciting papers are to be heard. It would be very nice if you could come to Philadelphia on Friday or Saturday perhaps, when we might plan our campaign on Cx, unless of course we can meet in New York and discuss things then. Since Cx doesn't seem to be in E. coli, I should like to begin thinking seriously about critical phage experiments. Also we ought to plan getting Larry Weed into our discussions.

Sincerely yours,

Seymour

Seymour Cohen

SC/drm

March 22, 1952

Dr. S.S. Cohen,
Research Dept.,
Children's Hospital of Philadelphia,
Philadelphia, Pa.

Dear Seymour:

Many thanks for your letter, Dr. Weed's summary, and the T6r$^+$ and *E. coli* preparations.

Enclosed is a draft of a note on rickettsial DNA's, which I have written with Nature in mind. In order to keep it brief and to the point, I have included only data that seemed strictly relevant. Please be harsh with it, and make any alterations you see fit. I gather that if my name comes first, it will have to be submitted through our head office and possibly even dignified with a contribution number. The Dische results do not seem to indicate any significant difference between *prowazeki* and NaTN, although the difference one would expect from the base ratios, 2-3%, is probably on the margin of sensitivity of the method with small quantitites.

E. coli DNA contains cytosine and no CX. This is based on a crude DNA prep. made with NaOH. Now I am running some hydrolysed whole bacteria as a check.

Weed's data do not seem to exclude the possibility of a nucleotide different from desoxycytidylic acid. If this is confirmed, the best way to isolate enough CX for analysis may be through the nucleotide, since you can get nucleotides quantitatively with viper

venom phosphodiesterase (Hurst, Little, and Butler, J. B. C. 188, 705), and they behave so nicely on resin columns.

From 40 mg. of T6r$^+$, I have isolated about 0.5 mg. of CX. With this and the remaining phage, some further characterization can be done, though I am feeling rather void of bright ideas now, and look for analytical figures as a guide. I shall also push on with base ratios on the last series of DNA's. Presumably we shall be able to apply an correction for hydrolysis loss later.

Sincerely yours,

GRW/f

G. R. Wyatt.

KINGSLEY 6-2700

FOUNDED 1855

KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA

1740 BAINBRIDGE STREET

PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

March 11, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario

Dear Jerry:

I agree with the suggestion of a note on the provazeki DNA, containing reference to Smith's hypothesis. I don't particularly care where it's sent; perhaps Nature, certainly not Science. Why don't you write it up?

Concerning the phage DNA, I am in favor of elegance and think it would be more elegant to have the analyses on isolated DNA of all strains. It does seem likely that the low P recovery is due to poor "C" recovery, and that eventually figures on ratios will require some modification. Nevertheless, assumptions can probably reasonably be made concerning the quantitative recovery of adenine guanine, and thymine and data on similarities of the strains expressed in terms of these three bases. (Precovery is still 15-20% better than Marshak + Chargaff on T2 + T4

I am dialysing some T6r+ we just isolated and I shall send you this batch which should come to about 100 mg. as well as some of our strain of E. coli. They will be lyophilized but not defatted. We will not be able to supply any of the small phages; we are not set up for work with them.

Weed came up from Washington and we went over the isolation. As you say, he took severe losses by hydrolysis in N HCl. Nevertheless, he separated purines, "cytidylic" and thymidylic on paper and extracting from the paper he showed the proper absorption spectra for the "cytidylic". He has ion exchange data which suggest some inhomogeneity in the "cytidylic" but had overlooked these at the time. He will send you a summary of his data (via me) and will re-examine the properties of the nucleotide from T6r+ which I will supply. If you concentrate on the free base and/or nucleoside presumably progress will be expedited, while he works on the nucleotide

I really don't know whether I shall get to the Federation Meetings but it will be desirable for us to get together at that time there or in Philadelphia. When my own program is clarified, I shall write to you.

Meanwhile, I shall explore the possibility of getting really large amounts of T6r+ from Sharp and Dohme.

Sincerely yours,

Seymour

Seymour Cohen

SC/drm

March 1, 1952

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
Philadelphia, Pennsylvania.

Dear Dr. Cohen:

Many thanks for your letter and promise of more material.

To consider simplest things first, there is the prowazeki DNA. I shall get a Dische test on this to check on its slightly different purine-pyrimidine ratio from thymus DNA, and then perhaps wershould write a note on it to counter John Smith's rather rash inference. What do you suggest?

As for the phage DNA's, the different guanine ratio in the whole phage has no biological significance, but is due to interference by protein degradation products on the chromatograms. I did not attempt to correct for this, nor for hydrolysis loss, as I considered our main interest was comparison of strains.

I could easily run off analyses on the additional DNA's you are sending and confirm, within a certain experimented error whether they are all the same. But it is rather unsatisfactory to be estimating an unknown (call it CX), and even with the HCOOH method the yields of it are not as reproducible as those of the other bases (this shows up in the table I sent). This may point to some loss, and have something to do with the low P recovery. Hence, the first job seems to be to find out wherein the chemistry of phage DNA's differs from that of other DNA's, and we had better push on with that.

My results seem to me to point to a new pyrimidine like cytosine substituted with a hydrophilic group in the 4 or 5 position. Yet this would be so startling that I feel very cautious about it just as yet. I should get some confirmation, and shall try to isolate the corresponding nucleotide which should not be difficult in paper chromatogram quantities. I am ordering some alkaline phosphatase for this. Incidentally, I am not clear from Weed's papers how he hydrolysed to get desoxycytidylic acid. The yield from acid hydrolysis must be very poor. Meanwhile, I shall get experience with ion-exchange columns (which I have not used as yet) with a view to isolating greater quantities of the substance.

Yes, it is certainly worth checking E. coli, and I should be happy to receive a hyophilized or ether-dried sample (they will have to be de-fatted anyway). If they contain OX, they will be a cheaper source than phage. I don't think they do, because Smith and I examined coli for 5-methylcytosine, and the identity of at least part of the cytosine would automatically be checked in that procedure. It seems most unlikely that phage would contain a pyrimidine not found in coli. Only alternative that I can think of is that phage has cytosine somehow differently linked so that it is not fully liberated on hydrolysis- but this does not seem to fit the data so well.

I should also like to check a T-odd phage, because Marshak says T3 contains cytosine, and his method only picks up the real thing. It would be interesting if these were both.

If it does become a case of identifying a new base, I wonder if you would be able to help with it yourself, or if you could get the cooperation of some other good chemist. I am a biologist, not a chemist, by training, and I know nothing of organic syntheses, and have to learn by experience and mistakes in this sort of problem. So I feel it would be saving time and possible waste of material to have an organic chemist in on this.

I hope to be at the Federation meeting, in New York in April. Will you be there? If not, I can probably arrange a trip to Philadelphia.

Sincerely yours,

GRW/f

G. R. Wyatt.

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

February 25, 1952

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario

Dear Jerry:

The data in your last letter are very exciting and I trust that you will find it possible to continue work on this new "cytosine" at least. I am sending under separate cover the isolated DNA of 4 phages which you haven't had yet in the isolated state, T6r+, T6r, T4r, and T2r. I think you should want analyses on these to fill out the data and confirm certain observations such as the slightly higher guanine in isolated DNA as contrasted to that obtained with intact phage. Do you feel these differences have any significance?

Concerning finishing off some things.

1. The R. prowazeki data are interesting and should be reported before people say that rickettsiae get their DNA from the host.

2. With additional data from the additional DNA, it is clearly important to be able to say that 6 genetically related but different phages have identical base composition. Whether this can be said is up to you since you are the expert and senior author. Further, it is believed in two laboratories that there is about 1/3 less DNA in T4 than T2 and T6. Therefore, the deletion is of a fragment similar in composition to the whole DNA.

3. If you wish to determine the nature of the apparent "cytosine", I will undertake the preparation of the equivalent of 1 gram of DNA from some phage, e.g. T6r+. This can be quite arduous with our limited facilities, and I wonder if you would let me explore assistance from a commercial house, whom I probably would have to tell about a new "pyrimidine", in strictest confidence of course.

Dr. G. R. Wyatt February 25, 1952

As you know numberous people, Chargaff, Marshak, yourself, have reported cytosine in E. coli. Was it really cytosine? Will you wish some lyophilized E. coli, strain B. to determine this?

If the bacteria do not have the new "C", the problem of its origin and role in phage synthesis is of considerable importance and we shall certainly wish to pursue this, as I am sure you will in seeing whether "C" exists anyplace other than the phages.

Finally, I shall obtain the precise data from Weed on his characterization of desoxycytidylic acid in T6r$^+$. He is in the Army and is stationed in Washington but visits fairly frequently.

With best regards, I remain

Sincerely yours,

Seymour Cohen

Seymour Cohen

SC/drm

February 20, 1952

Dr. S.S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge St.,
Philadelphia, Pennsylvania.

Dear Dr. Cohen:

Thanks for your letter, which was timely, as I have been putting off writing to you day by day for just a few more results. The quantitative results are summarized in the enclosed tables, but the phage DNA's have some peculiarties which I still don't understand. Here are the main points:

(1) Your NaTN does not differ in composition from my own sample of beef spleen DNA. I have used two methods of hydrolysis (72% $HClO_4$ 100° 60 min., and 88% HCOOH 175° 40 min.) since these give consistently slightly different yields and each has advantages for certain problems. The "P recovery" from $HClO_4$ is liable to be erroneously high because of adsorption of P to the insoluble hydrolysis residue.

(2) I have done your epidemic rickettsial DNA by both methods too, and get reproducible results, with good P recovery. There is a trace of uracil in this preparation, but scarcely enough to estimate. The composition is obviously different from Smith's burneti but (Table II) the "constant ratios" observed in other DNA's seem to hold good, and it looks as if the Rickettsiae might form a series the way the insect viruses do. The similarity of burneti and chick DNA is probably coincidental.

(3) With the phage DNA's it is impossible to get decent results with $HClO_4$. I wasted much of your earlier batch of

phages this way, since this method was proving so good with the insect viruses. The "cytosine" is partially destroyed in $HClO_4$, and the yield varies greatly with precise experimental conditions. With HCOOH, much more reproducible results are obtained, though the yield of "cytosine" may not be quite quantitative as there is some reduction on prolonging hydrolysis time. P recovery is about 10% lower than with thymus DNA. Consistent results can be got by cooking whole phage with HCOOH too, but these differ somewhat from those from the isolated NA because interference by degradation products. The figures from the various T-even strains are very similar. Earlier, I should have suspected small differences might be revealed by repeated analyses for increased accuracy. But since doing the insect viruses, I have inclined away from the idea that each mutation may be accompanied by a change in DNA composition, and I should not be surprised now if the T-even phages have actually identical DNA composition.

(4) Now the part I don't understand. The "cytosine" liberated from T-even phage DNA by HCOOH or $HClO_4$ is not the free base cytosine, which one gets from other DNA's, including that of typhus rickettsiae. It has the following properties which, in addition to its lability in $HClO_4$, distinguish it from cytosine, cytidine, and desoxycytidine.

(a) UV absorption spectrum:

Absorption maxima

	pH 1	pH 7	pH 13
Phage "C"	278	269	284, 231
Cytosine	274	267	279
5-methylcytosine	283	274	287
Cytidine	280	271	272
Desoxycytidine	280	272	272

The spectrum of the unknown is intermediate between those of cytosine and methylcytosine, having their characteristic shifts with change in pH, and is distinct from those of the ribosides, which have no shift in alkali. For the quantitative work, I used readings at 280 mu, and assumed E the same as cytosine (10,500 at pH 1). (b) Chromatographic properties: In 65% isopropanol "c" moves very slightly more slowly than cytosine regardless of pH - this suggests its ionizable groupings are identical with those of cytosine. On reducing the water content of the solvent, its RF drops below that of cytosine - this suggests a structural difference altering its partition coefficient in favor of the aqueous phase.

(c) "c" is largely unaltered by prolonged hydrolytic conditions (88% HCOOH at 175° for 2 hours). It gives no color reaction

for desoxypentose with cysteine. On treatment with HNO_2 it deami-nates to a substance having UV spectra very close to those of uracil but different chromatographic properties. I hadn't enough of this product to characterize it any further.

These properties seem to me to suggest a substance structurally homologous to cytosine but having some substitnant group which would cause the longer wavelengths of the UV maxima and alter the partition coefficients. I don't know how to reconcile these results with Weed's isolation of desoxycytidylic acid from phage DNA. In Smith's and my earlier work we probably had this substance but did not recognize it. Nor do I know how to proceed from here, short of isolation of enough of the stuff for elementary analysis, melting point, etc., which would take a lot of phage. Have you any suggestions?

As for the quantitative work, I can do some more analyses to get statistically more satisfactory figures, on the prep's you sent and others you may care to send, if not too many. It would be interesting to do some T-odds. I have some left of all of your last batch of DNA's, but none of the whole phages, sent earlier. I can also get Dische/P ratios (possibly this week), which should be instructive.

I can work only part-time on this project now, and have to bring it to some conclusion before too long because of other work. However, the chemistry of the "cytosine" seems too important to leave, and I am keen to bring this question to some sort of conclusion.

Incidentally, I have to apply very soon for permission to attend any U.S. scientific meetings, I may wish to go to this yeer, and I wonder if you know of anything of especial interest in the line of viruses-nucleic acids-proteins. We are a bit out of touch here, and I prefer smaller meetings than FASEB if possible.

Best regards,

Sincerely,

GRW/f

G. R. Wyatt.

I. Composition of DNA's

		Moles per 100 moles total nucleotide					
		A	T	G	"C"	MC	% P recovery
$HClO_4$ hydrolysis							
NaTN ①	(1)	28.1	28.0	21.6	21.1	1.1	103
	(2)	29.1	26.9	21.9	20.8	1.3	98
	$\bar{x}$	28.6	27.5	21.8	21.0	1.2	100
ERg ②		35.7	31.8	17.0	15.5	-	98
R. burneti (J.D. Smith)		29.5	26.0	22.5	22.0	<0.2	-
HCOOH hydrolysis							
NaTN ①	(1)	28.0	28.6	22.0	20.1	1.3	94
	(2)	28.0	28.8	21.9	20.0	1.2	98
	$\bar{x}$	28.0	28.7	22.0	20.0	1.25	96
ERg ②	(1)	35.2	33.9	15.8	15.1	<0.4	96
	(2)	35.0	34.5	15.7	14.7	<0.3	91
	$\bar{x}$	35.1	34.2	15.8	14.9		94
$T2r^+$ ③		33.5	36.4	21.1	9.0	-	82
$T2r^+$-1 ④	(1)	32.2	37.6	21.2	8.9	-	84
	(2)	33.5	36.3	19.3	10.9	-	87
	(3)	33.7	36.6	19.2	10.5	-	86
	(4)	33.8	37.6	21.2	7.4	-	82
	$\bar{x}$	33.3	37.0	20.2	9.4		85
$T4r^+$-2 ⑤		33.8	36.9	19.7	9.6		89
Whole phages:							
T4r-8		33.0	36.8	18.0	12.2		86
$T4r^+$-7		33.0	36.0	18.5	12.5		90
T6r-6		33.8	36.8	17.5	12.2		76 (?)
$T6r^+$-7		33.6	37.6	18.0	10.7		90

II. Molar proportions in DNA's

		A/T	G/C+MC	$\frac{\text{Purines}}{\text{pyrimidines}}$	A+T/G+C+MC
$HClO_4$:	NaTN	1.04	1.04	1.04	1.27
	ERg	1.12	1.10	1.10	2.08
	R. burneti (Smith)	1.13	1.02	1.08	1.25
HCOOH:	NaTN	0.98	1.03	1.00	1.31
	ERg	1.03	1.06	1.04	2.26
	$T2r^{+}$-1	0.90	2.15	1.15	2.38

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

February 14, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario
Canada

Dear Dr. Wyatt:

I have recently seen Smith's paper on the DNA of R. burneti in the Brit. J. Exp. Path. and am all eager to see how our R. prowazeki DNA compares. This is particularly interesting in view of the similarities of the chick embryo DNA to the R. burneti DNA that Smith reported.

And how are the others coming, if you don't mind my pressing?

I trust things seem to be settling down after your change of state. However, I can assure you that your new arrangement has started processes which will make your last two months seem like an absolute stasis in time and space.

With best regards I remain

Sincerely yours,

Seymour Cohen

Seymour Cohen

SC/go

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

December 19, 1951

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Sault Ste. Marie
Ontario, Canada

Dear Doctor Wyatt:

I have read your excellent paper. My only comment would point to the obvious interest of obtaining similar data as soon as possible on the phage DNA's, where the genetic and serological relationships are so much more clearly established. The other strains are coming through now.

I'd like to keep the manuscript, if you can spare it. I use data of this character in a course I teach in comparative biochemistry.

As for your leave and the temporary delay, it is said that marriage has a stabilizing effect and your productivity may well be multiplied thereby. However you may be running out of nucleic acid samples even at the present rate, and this may prove a problem in itself. In any case, my heartiest congratulations and best wishes for the future.

With best regards, I remain

Sincerely yours,

Seymour Cohen

Seymour Cohen

SC/GO

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

November 21, 1951

Dr. G. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Dr. Wyatt:

I am sending the following samples of sodium salts of DNA under separate cover:

1. My own DNA standard derived from thymus nucleohistone designated NaTN sol 4Δ, on which I would appreciate a base analysis to assist my work generally. It contains 8.6% P.

2. The DNA of epidemic rickettsiae, designated ERg C_h ↑2, whose isolation and properties are described in my paper in the J. Immunol. 65: 475 (1950). This is the last of this material. It contains 8.05% P.

3. The DNA of $T2r^+$, a preparation containing 9.3% P, whose isolation is described in my paper in the Cold Spring Harbor Symposium.

These are somewhat ancient preparations. Two preparations made this last week by the method described for (3) are

4. The DNA of $T2r^+$ - 1

5. The DNA of $T4r^+$ - 2

I do not know the P content of these preparations and hope they are comparable to (3). I will be making more of these

strains and our others in anticipation of your request for additional samples.

Sincerely yours,

Seymour Cohen

Seymour Cohen

SC:dk

December 11, 1951
File:

Dr. S. S. Cohen,
Research Department,
Children's Hospital of Philadelphia,
1746 Bainbridge Street,
Philadelphia 46, Pennsylvania. USA.

Dear Dr. Cohen:

I am sorry I have not had a chance to do anything with the NA samplse you sent, and am going away today for 4 weeks. I have been writing up the insect virus NA work, and have been pretty busy. Enclosed is a draft of the paper on this, which I hope you may find some interest in. I'd be glad to hear any cracks you may have about it. I should be grateful if you would return it when you're through with it.

With this out of the way, I should be able to concentrate on your stuff in January.

Best regards,

Sincerely,

GRW/m
Encl. 1

G. R. Wyatt.

November 27, 1951
File:

Dr. S. S. Cohen,
Research Department,
Children's Hosptial of Philadelphia,
1740 Bainbridge Street,
Philadelphia 46, Pennsylvania. USA.

Dear Dr. Cohen:

I have received the 5 DNA samples as described in your letter. I am going to be away from the laboratory for a month from December 11. However, we should be able to get started on them this week and next, and I shall let you know of any results before I go.

Sincerely,

GRW/m

G. R. Wyatt.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA
4
THE SCHOOL OF MEDICINE

Department of Pediatrics
1740 Bainbridge Street

Nov. 5, 1951

Dear Dr. Wyatt:-

I'm leaving the city for a week but when I return I'll begin sending some nucleic acids. Your P ~~fig~~ and N recoveries are good and we'll see what happens to phage DNA and the base, P, and N recovery.

Sincerely

Seymour Cohen

P.S. - or just base + P

S.S.C.

November 1, 1951
File:

AIRMAIL

Dr. S. S. Cohen,
Children's Hospital of Philadelphia,
1740 Bainbridge Street,
Philadelphia 46, Pennsylvania. USA.

Dear Dr. Cohen:

I was pleased to have your letter of October 24, and ought to have written you before now. I am glad to hear you are preparing DNA from the T-even phages.

Some difficulties in technique have been eliminated here, and P estimations are proceeding satisfactorily now. I have done a number of control experiments with purified thymus nucleic acid and am now getting regularly sufficient bases to account for 94 - 98% of DNA-P and about the same percentage of DNA-N. The recovery in terms of P is not significantly altered when the DNA is hydrolysed in the presence of protein mixed in proportions to simulate virus. With various insect viruses, hydrolysed whole, the recovery in terms of P has varied between 86 and 98%. It is probable that these viruses contain a small amount of non-nucleic acid P, so the recovery of DNA-P would be about the same as with purified DNA.

In recent analyses of your phages, I have got recoveries in terms of P varying between 70 and 80%. If the deficiency from 100% is due to loss in analysis, this is obviously quite unsatisfactory. Considering the good recoveries with other nucleic acids, however, I feel this is unlikely. Other causes might be the presence of non-nucleic acid P, or excess of P over bases in the nucleic acid.

Dr. S. S. Cohen November 1, 1951

I believe you have stated that phage DNA has the same N:P ratio as other DNA's. Because of its higher purine/pyrimidine ratio, however, it ought to have a higher N:P ratio, if there is still a 1:1 relationship of bases to P. Perhaps this is balanced by an excess of P over bases. A similar line of reasoning may apply to the relation between diphenylamine color and P.

However, there is no point in becoming too speculative if you have samples of phage DNA's for analysis. Provided these are reasonably free of protein, the question can be settled by comparing recovery of bases with both P and N.

The results from your phage preparations so far have been disappointing in that I have found no differences which I should be prepared to call significant between the preparations, except such as may be attributed to varying small amounts of "cytosine". I am not entirely happy that all this substance belongs to the phage --- what are the chances of adsorbed host DNA? I believe Putnam and Co. sometimes found this. If there were host DNA (or RNA) present, would it be carried along or eliminated by your method of isolating phage DNA?

I should like to undertake analysis of your preparations, if you are satisfied with what I report in this letter. I do not anticipate any further hitches here, and if the preparations are pure, the analyses should go very quickly. I should like to know your opinion, however, on the amounts of host DNA, host RNA, and protein which could be present.

Sincerely yours,

GRW/m G. R. Wyatt.

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

October 24, 1951

Dr. G. Wyatt
Laboratory of Insect Pathology
Department of Agriculture
Sault Ste. Marie, Ontario
Canada

Dear Dr. Wyatt:

As we agreed, I have undertaken the preparation of various phages and phage nucleic acids and anticipate having the sodium salts of T2r$^+$ and T2r, T4r$^+$ and T4r, T6r$^+$ and T6r in approximately two weeks. We have worked out a procedure for obtaining satisfactory amounts of virus from quite small amounts of lysate thereby minimizing our centrifuging problems. This means that the difficulties of preparation of numerous samples can be reduced as a chore at our end.

Have you resolved your difficulties in P analysis? And do you wish to undertake the analysis of these substances, checking base recovery with P content of the sample? In my estimation, the series provides a unique opportunity in comparing small discrete genetic changes and relationships with chemical composition.

Sincerely yours,

Seymour Cohen

Seymour S. Cohen

SSC/drm

Marine Biological Laboratory
WOODS HOLE. MASSACHUSETTS

August 9, 1951

Dear Dr. Wyatt: —

Your letter was forwarded to me here. I do not intend to be at Brookhaven or the half dozen other meetings around N.Y., but if you have a chance to visit me in Philadelphia after September 5, I'd be very pleased to discuss things with you.

I was very much interested in your results but unfortunately in the absence of P values I consider them meaningless. Both Chargaff and Marshak were unable to find cytosine in my preparations of T2. However the absolutely critical point is that their base recovery to P was only 0.70. In fact I consider Marshak's paper fraudulent precisely because he did not report his low and inadequate base recovery.

In view of your unknown recoveries I am opposed to any mention of the phage data since they could only be taken by uncritical observers to constitute a confirmation of Marshak's paper. Only such results should be presented in which the base recovery is in excess of 90% of the nucleic acid P. Both T2 and T4 DNA which I have isolated had molar N:P ratios of about 3.75, indicating that there is the usual base:P ratio.

Also, you and Smith did report significant amounts of cytosine in T2. And more convincingly to me, Weed isolated desoxycytidylic acid from T6r+ and T6r-. This substance was unequivocally characterized and can not be confused with cytidylic acid. I can only conclude that your present procedure, like Chargaff's and Marshak's, is unsatisfactory for these phages. At least until you report satisfactory base: Precoories.

If you feel you wish to try to straighten this out I will supply you with phages and their isolated DNA's this fall. I trust you will not think me unduly difficult however, if I place the base recovery as a condition for my continuing supply, in so far as there is an effort involved in preparing the phages. Of course this is no more than you wish anyway.

With best regards I remain

Sincerely yours

Seymour S. Cohen

Airmail

2 August, 1951.

Dr Seymour S. Cohen,

Children's Hospital of Philadelphia,

Philadelphia 46, Pa.

Dear Dr Cohen,

Your phage preparations have been very interesting, but have proven a bit more complex than I had anticipated. I at first hydrolysed whole untreated phage, following the procedure I have been using with insect viruses, and got pictures like the enclosed print (1). ~~There were weak~~ There was practically no detectable uracil, and weak spots in the ~~spots in the~~ cytosine position, which were almost negligible in the T6 preparations but bigger in T4, ~~and practically no detectable uracil.~~ I therefore assumed that RNA was absent, and did a number of analyses, but got rather erratic results, especially with respect to "cytosine", and so checked the identity of this spot. It proved to be not cytosine, but has an absorption ~~xxxxx~~ spectrum closer to that of cytidine (which has the same RF), and in another solvent (butanol-NH_3) has an RF equal to that of cytidine. In butanol-NH_3 cytidine happens to fall on top of guanine, which explains the anomalous absorption curve for guanine from phage which Marshak reported.

I am not yet certain that this substance is cytidine, since the ribosides are ~~xxx~~ usually hydrolysed as well as the desoxyribosides by the $HClO_4$ treatment. Also, if it represents RNA, one would expect some uracil or uridine, which I cannot find. However, it does seem to represent an impurity, since the amount of it varies from one preparation to another more than do the other bases. Also, it can be greatly reduced by NaOH treatment (Schmidt & Thannhauser) or by ribonuclease, suggesting RNA. The effect of RNase, which I only just tested a couple of days ago, looks promising--print (2) shows the results of this experiment. I am trying it on some of the other preps.

The figures on the prints are calculated as xxx molar ratios, taking total bases equal to 4.00. They would be better as moles per mole P, but unfortunately we have been having trouble with our P estimations, as we use a sensitive but tricky nephelometric method. This should be ironed out soon, and I shall be able to put the results on a sounder basis.

So it looks as if Marshak is right that phage DNA lacks cytosine, in spite of Smith's and my previous results. However, some more work is needed to identify the "cytidine" and a couple of other minor substances which you can see on print (2), which xx are probably artefacts but may be important, and I do not yet feel happy that any of my results so far represent the true composition of phage DNA. The results so far do not show significant differences between the different strains, except for "cytidine", but I still hope that with purified material and standardized technique some differences may show up.

So I am afraid my original estimated of the amount of material required were a bit optimistic. I was thinking in terms of our insect viruses, which we are lucky to have remarkably pure. Fractionation with NaOH does not seem very satisfactory for removing the "cytidine"; I shall do more tests with RNase, but I am getting near the end of the material you sent. I am very much interested in this problem, and should like to get it cleared up, and should welcome your views on it. Certainly, analyses of isolated polymeric phage DNA, as you suggested, would be worthwhile. Also, with slightly larger amounts of material, I should be able to isolate and, with luck, identify the unknowns.

If the further analyses of RNase-treated phage this week and next give good results, I shall let you know. After this, I shall be away from the lab until September.

Meanwhile, I am giving a paper on the composition of nucleic acids at a conference on the cell nucleus being held at the Brookhaven National Lab., Upton, L.I., on the 15th of this month, and wonder if you would have any objection to my mentioning these results, with acknowledgements. The proceedings are being published. I should be very cautious about quantitative results, and should probably not give any, but I should like to be able to point out that the DNA of some phages consists at least principally of three kinds of nucleotide, as this fits in well with the general picture of ~~increasing complexity of DNA in different types of organism as one ascends the evolutionary~~ parallelism between complexity of DNA composition and evolutionary development of the organism.

Incidentally, shall I be seeing you at either the Brookhaven meeting or the Gordon Research Conference on proteins and nucleic acids being held at New Hampton, which I hope also to attend?

With best regards,

Yours sincerely,

G.R.Wyatt

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

June 6, 1951

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault Ste. Marie, Ontario
Canada

Dear Dr. Wyatt:

I am sending under separate cover up to 10 mg amounts of 7 phage preparations including T6r, T6r^+, T4r, and T4r^+ strains. The precautions which you indicate you will take are quite imposing and should tell us whether the material in its present state is satisfactory for analysis.

I have informed Marshak of the inadvisability of statements concerning T2 in the absence of a decent base recovery.

With best regards, I remain

Sincerely yours,

Seymour Cohen

Seymour Cohen

SSC/dk

KINGSLEY 5-2051 FOUNDED 1855 KINGSLEY 5-2089

THE CHILDREN'S HOSPITAL OF PHILADELPHIA
1740 BAINBRIDGE STREET
PHILADELPHIA 46, PA.

RESEARCH DEPARTMENT

May 15, 1951

Dr. G. R. Wyatt
Laboratory of Insect Pathology
Sault St. Marie, Ontario

Dear Dr. Wyatt:

Thank you very much for your letter of May 7. I have the following virus preparations dialysed, lyophilized and dried in vacuo

$T4r^+ - 7$	32 mg
$T4r^+ - 6$	6 mg
$T4r^+ - 7$	17 mg
$T4r^+ - 8$	31 mg
$T6r^+ - 6$	3 mg
$T6r^+ - 7$	56 mg
$T6r^+ - 6$	80 mg

I would be pleased to send you these preparations, each of which contains about 40 per cent DNA. Let me apprise you of the following however: they have not been analysed to determine the relation of total P to DNA-P, nor have tests been made with antiserum to E. coli to determine the extent of contamination with host debris. This contamination is the major source of extraneous P (RNA-P) which sometimes may be found in these preparations. It strikes me that an estimation of uracil, which has not been found in other T6 preparations similarly collected, would be a check on possible RNA contamination.

May I ask, therefore, if you would be in a position to 1) assess possible RNA contamination by this or other methods, and 2) present base composition as a function of the P content of the preparation, with reasonable assurance of a recovery of base corresponding to virus DNA-P in excess of 90 per cent. It seems to me that only in such a context would analyses be significant and I would not wish you to waste your time.

If you are willing to proceed with the analyses on this basis, I shall certainly dispatch the preparations post haste. If the results are interesting and you wish to continue in the Fall I can extend the list

to T2r$^+$ and T2r, with as many more of the preparations of the others as you deem desirable. Furthermore since it is possible to isolate the polymeric DNA from the virus in high yield it may prove useful to check further the analyses of the virus by analyses of the DNA. It may be necessary in any case to approach the problem from the isolated DNA, if host debris contamination proves to be a serious factor in analyses of intact virus.

In any case, whether you indicate that you wish to proceed with this or not, I shall be indebted to you for details of your methods.

Sincerely yours,

Seymour Cohen

Seymour S. Cohen

SSC/dk

14 June 1951

Dr. Seymour S. Cohen,
Research Department,
Children's Hospital of Philadelphia,
Philadelphia 46, Pa.

Dear Dr. Cohen,

Thank you for the phage preparations which arrived in good shape. I shall try to get on to them very soon and shall let you hear of results.

I see that Marshak has already published in the Proceedings of the National Academy of Sciences, on the lack of cytosine in T2. I cannot understand this difference in our results, but anyway it is incentive for me to get busy.

Best regards.

Sincerely yours,

A.D.H.

May 22, 1951

Dr. Seymour S. Cohen,
Research Department
Children's Hospital of Philadelphia,
Philadelphia 46, Pa.

Dear Dr. Cohen,

Thank you for your letter of May 15. Of course the possible presence of RNA of host origin in phage preparations would complicate estimation of the DNA bases, but there seem to me at least three feasible methods of checking this:

(1) Estimation of uracil on the chromatograms. This is easily done as long as there is enough of it, but where there is very little uracil compared with the other bases (much under 5%), as we expect there will be, the figure might not be very reliable.

(2) Comparison of DNA-P estimated by diphenylamine with total P. We could do this if necessary. Knowledge of the purine/pyrimidine ratio in phage DNA should help in interpreting results from the Dische reaction. Incidentally, I note in one of your papers that DNA-P was sometimes greater than total P, which probably results from the different purine/pyrimidine ratio in phage DNA and in the standard.

(3) Schmidt and Thannhauser fractionation. One would have to beware of deamination of cytosine by this, but I have done some tests which indicate that there is very little deamination even at 37°, and by using 30°, there should be virtually none.

Tests of this sort, perhaps supplemented by analyses of isolated phage DNA, should enable us to get significant results in spite of some contaminant RNA, and if there is no RNA present, we are of course saved trouble. I can assure you that the estimated DNA bases will account for better than 90% of DNA-P. I quite agree that we cannot expect significant results without every precaution against error, for the differences in DNA composition between two viruses so closely related as T4 and T6 will probably be pretty small.

If you agree that the precautions I have suggested are adequate, I should like to try some analyses on the material you have available. A single analysis, comprising duplicate chromatogram runs big enough to show uracil, and P estimations, would take about 3 mg of phage. It is obviously desirable to do replicate analyses, either on the same or on different preparations, but I do not see that we should have any need for more than 10 mg of any one preparation, unless it were a case of isolating the virus DNA. However, if you care to send larger amounts, I can return the unused excess.

I take it from your letter that you will not be in a position to make any other preparations until the fall. Actually, I am pretty busy with other work too. However, we can judge from the first results what else is worth doing. Within a month or so I shall be writing a paper on the nucleic acids of insect viruses, which will describe the method in detail, and you shall have a typescript copy of this.

Yours sincerely,

GRS

Dr Seymour S Cohen.

Dear Dr Cohen,

Enclosed is a reprint of a note reporting 5-methyl-cytosine in some nucleic acids, which may be of some interest to you. When they come, I shall send copies of the two papers at present in press in the Biochemical Journal, which give more useful information on methods and results from animal DNA's. A third paper with J D Smith, giving analyses of some bacterial and virus DNA's will probably not be out for a few months, so for your interest, here are mean results on two phages and their host:-

	A	G	C	T
T_2	1.33	0.73	0.505	1.44
T_5	1.35	0.83	0.42	1.40
E. coli B/r	0.92	0.96	1.02	1.10

These are molar ratios, calculated arbitrarily to total 4.00 (this is not intended to imply tetranucleotides!), and accounting for about 92 % of virus P. The standard error of an analysis is about ± 2 %, so the differences in guanine and cytosine between the two phages are significant.

The technique which I am at present using for mixed viruses involves hydrolysis of whole virus in a very

small volume of 72% $HClO_4$ at 100° for 2 hr., followed by chromatography on paper in the solvent described in the reprint. This enables an analysis to be done on about 0.5 γ DNA, and owing to technical improvements, I am getting rather better reproducibility and recovery than in the analyses above.

The four species of insect virus that I have done so far show surprisingly wide differences in DNA composition, but it is hard to interpret these results since we have as yet no other criteria of the relationships of these viruses. The T series of phages, whose biological relationships are so well worked out, seem to be an ideal system for getting some clue as to what the DNA composition means, and I should like to see the whole series analyzed in this way.

If you start doing these analyses, I should be glad to let you know any further details of the method that may be helpful. On the other hand, we have here the apparatus assembled and the procedure in regular use, and I should be pleased to analyze any samples you care to send. This is entirely up to you. I have plenty else to keep busy with, but it seems a pity not to make the maximum use of our analytical set-up, which we believe is more satisfactory than those being used for NA micro-analysis in other labs, and certainly the T series should be analyzed by someone.

Yours sincerely,

A.H.C.