re: Identification of HMC [Correspondence]

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June 15th, 1953
File:

Dr. C. S. Miller,
Sharp & Dohme, Ltd.,
West Point, Pennsylvania. USA.

Dear Dr. Miller:

I'm enclosing a copy of a paper which Seymour Cohen and I prepared for the Biochemical Journal. A propos of our reference to synthetic HMC and HMV on p. 5, I have received the following comment from the editors:

"It would be appreciated if Dr. Miller's intentions about publishing the syntheses of the two compounds prepared by him could be stated. *Ad hoc* publication in an Addendum to the present paper would be welcome, if no other publication is contemplated. We do not like references to new material without a full description of their manner of preparation being made available within a reasonable time."

So could you let me know, as soon as possible, what your intentions are about this? Either a reference to a paper in press or in preparation, or, if you care to take the B. J. up on their offer, a short manuscript which we could include as an addendum, under your name, to our paper.

The extinction data in table 1 are based on my own determinations, and you'll note I haven't given any coefficients for HMV, assuming you would eventually be publishing your values for these. If you want to take the course of submitting an addendum, perhaps you would to supply figures to complete this table.

I'd appreciate having the copy of our paper back when you're through with it. Best regards,

Sincerely,

GRW/m

G. R. Wyatt.

Sharp & Dohme
INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

JUNE 25, 1953

DR. G. R. WYATT
LABORATORY OF INSECT PATHOLOGY
DEPARTMENT OF AGRICULTURE
SAULT STE. MARIE
ONTARIO, CANADA

DEAR DR. WYATT:

WE VERY MUCH ENJOYED LOOKING OVER YOUR AND DR. COHEN'S PAPER FOR THE BIOCHEMICAL JOURNAL.

AT PRESENT WE ARE TRYING TO IMPROVE THE SYNTHESIS OF HMC AND HOPE TO EXHAUST THESE EFFORTS IN THE NEAR FUTURE. WE PLAN TO SUBMIT THE WORK TO THE JOURNAL OF THE AMERICAN CHEMICAL SOCIETY FOR PUBLICATION AT THAT TIME.

WE HOPE THIS STATEMENT OF INTENT WILL SATISFY THE EDITORS AND SPEED ACCEPTANCE OF YOUR PUBLICATION.

SINCERELY,

CHARLES S. MILLER, PH.D.
RESEARCH ASSOCIATE
ORGANIC CHEMISTRY

CSM/MWK

Sharp & Dohme

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

February 26, 1953

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

Dear Doctor Wyatt:

Thank you for both your letters on the analysis of the dried vaccinia virus. Naturally, our faces are "red" over the slip of the pencil in estimating the weight of the material, and I am glad there was sufficient material for your needs. Was the material suitable for electron microscopy?

I have circulated your findings to those of our group who were particularly interested in learning the results. We would be pleased to see these results included in your publication.

An acknowledgment of the preparation of the material by Dr. A. L. Brown of the Dept. of Virology, Research Division of Sharp and Dohme, Inc. would cover our small part in the program. My name need not be included.

Your visit to our laboratories was a pleasant and stimulating experience. I hope we shall see you at West Point again.

Sincerely,

Bettylee Hampil

Bettylee Hampil, Sc.D.
Director of Virus Research

BH/ej

February 21, 1953.

Dr. C.S. Miller,
Sharp and Dohme, Inc.,
Research Division,
West Point, Pennsylvania.

Dear Dr. Miller:

Thank you very much for the sample of 5-hydroxymethyluracil and I hope you will excuse my delay in acknowledging it. I have now compared it chromatographically with the deamination product of natural hydroxymethylcytosine, and get perfect correspondence in three very different solvents. There is also agreement of absorption maxima and minima at the three pH's within 1 mμ.

This seems to provide an elegant final confirmation of the identity of the natural base with 5-hydroxymethylcytosine, and I am very grateful to you for this sample.

Best regards,

Sincerely,

GRW/h

G. R. Wyatt.

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

JANUARY 22, 1953

DR. G. R. WYATT
LABORATORY OF INSECT PATHOLOGY
DEPARTMENT OF AGRICULTURE
SAULT STE. MARIE
ONTARIO, CANADA

DEAR DR. WYATT:

WE ARE SENDING YOU 5 MG. OF 5-HYDROXYMETHYLURACIL OBTAINED BY THE METHOD OF LITZINGER AND JOHNSON [(J. AM. CHEM. SOC., 58, 1936 (1936)] AND PURIFIED BY CHROMATOGRAPHING ON PAPER IN 85% ISOPROPANOL FOLLOWED BY RECRYSTALLIZATION FROM COLD METHANOL. ANALYSES AFTER DRYING IN VACUO OVER P_2O_5 AT ROOM TEMPERATURE: CALC. FOR $C_5H_6O_3N_2$: C, 42.26; H, 4.26; N, 19.71. FOUND: C, 42.19; H, 4.62%; N, 19.35. ASH, 0.19%.

pH	MAX (MU)	MIN.	E
6.1	260.5-261	231	8590
1.1	261	231	8510
12.3	285-286	245.5	7680

WE HAVE OBTAINED A SMALL SAMPLE OF MATERIAL BY TREATMENT OF 5-HYDROXYMETHYLCYTOSINE WITH NITROUS ACID AND PURIFYING IN THE SAME MANNER AS ABOVE. THIS SAMPLE, THOUGH INSUFFICIENT FOR CHEMICAL ANALYSES, GAVE THE FOLLOWING ULTRAVIOLET DATA:

pH	MAX.	MIN.	E
5.9	261	231	9540
1.1	261	231	9520
12.3	285-286	245-246	8570

THIS AND THE ABOVE SAMPLE GAVE ESSENTIALLY IDENTICAL INFRARED SPECTRA AND IDENTICAL SPOTS WHEN CHROMATOGRAMMED IN 85% ISOPROPANOL ON PAPER.

ANOTHER SAMPLE OF MATERIAL, INADEQUATE FOR CHEMICAL ANALYSES, WAS OBTAINED BY REDUCTION OF 5-CARBETHOXYURACIL WITH LITHIUM ALUMINUM HYDRIDE. IT WAS PURIFIED IN THE SAME MANNER AS THE ABOVE SAMPLES AND GAVE THE FOLLOWING U.V. DATA:

pH	Max.	Min.	E
6.0	261	231-232	7390
1.1	261	231.5-232	7280
12.3	286	246	6650

THIS LATTER SAMPLE BEHAVED AS DID THE OTHER TWO IN ISOPROPANOL WATER CHROMATOGRAMS BUT ITS INFRARED CURVES SHOWED DIFFERENCES WHICH WE HAVE BEEN UNABLE TO EXPLAIN.

WE HOPE THE SAMPLE WE ARE SENDING WILL BE ADEQUATE FOR YOUR COMPARISONS.

SINCERELY,

Charles S. Miller

CHARLES S. MILLER
RESEARCH ASSOCIATE
ORGANIC CHEMISTRY

MWK

January 31, 1953

Dr. Bettylee Hampil:
Research Division,
Sharp and Dohme Ltd.,
West Point, Penn., U.S.A.

Dear Dr. Hampil:

Here are some further results on your vaccinia preparation. Only very little nucleic acid was released from the protein by 36% urea (a method which is successful with some phages, but not with insect viruses) so I left the virus overnight in N-NaOH, and after deproteinization with chloroform got from 90 mg of virus a yield of 1.2 mg. of DNA. This was hydrolysed with formic acid and analysed by chromatography and gave the following base composition:

Adenine	29.5	moles per 100 moles total bases
Thymine	29.9	"
Guanine	20.6	"
Cytosine	20.0	"

Uracil and 5-methylcytosine, none detectable.
5-hydroxymethylcytosine, none detectable, maximum possible 0.6% of cytosine.

Total bases/DNA-P = 1.00

Because of the clean appearance of the chromatogram, and the good P recovery, I feel this can be regarded as a fairly reliable

quantitative analysis. It is notable that the molar quantities of adenine and thymine, also guanine and cytosine, are roughly equal, in accordance with what seems to be a quite general (and quite unexplained!) rule in DNA's.

Would you be in agreement with these base ratios being published, with acknowledgement to yourself and Dr. Brown (may I have his initials, please) for the material? I am not sure just yet what would be the best form of publication, but they could be grouped with results from other viruses (I am expecting preparations of T5 and meningopneumonitis), and if recorded they will eventually be useful to someone.

I'm afraid these analytical results are very dull compared with all that Seymour is doing on this problem!

Sincerely yours,

GRW/h

G. R. Wyatt.

January 23, 1953

Dr. Bettylee Hampil,
Sharp and Dohme Inc.,
Research Division,
West Point, Pennsylvania.

Dear Dr. Hampil:

Thank you very much indeed for the preparation of vaccinia virus, and please accept my aplogies for not acknowledging it sooner. Incidentally, although the package arrived in good condition, the bottle contained only about 160 mg. of powder, instead of 1.16 grams as labelled. Perhaps this was a slip of the pencil; in my case, 160 mg. is quite sufficient for our purposes.

The preparation has the following analysis:

P----------2.83%
DNA (by Dische)----2.06%
RNA (based on Dische value and uracil: thymine ratio)---approx. 0.6%

The nucleic acid bases found by chromatography of a hydrolysate of 50 mg. of whole virus were guanine, adenine, cytosine, uracil, and thymine. I have got no evidence for the presence of any hydroxymethylcytosine or other odd bases. The degradation products of the non-nucleic acid portion of the virus interfere on the chromatograms to such an extent, however, that the quantitative relations of the bases could not be reliably determined, nor could one set a definite maximum to the amount of hydroxymethylcytosine which could possibly be present. I am now using the remainder of the preparation in an attempt to isolate nucleic acid in order to get quantitative base ratios.

I do not think there is much point in your preparing any more

vaccinia for nucleic acid analysis. The essential question as to the presence of HMC or other unusual base appears to be answered in the negative, and this answer should be made definite with the remaining portion of this material. I am not especially interested in obtaining accurate quantitative DNA base ratios, since I suspect that the usefulness of this rather superficial approach is pretty well exhausted, and that further progress must come from the biological side along with a more fundamental attack on their structural chemistry.

5-hydroxymethylcytosine has not yet turned up anywhere outside of T2, T4, and T6, as far as I am aware.

Thank you once more for the material; you will hear from me again when the analysis is completed.

Sincerely yours,

GRW/h

G. R. Wyatt.

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

January 5, 1953

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Box 490, Sault Ste Marie
Ontario, Canada

Dear Doctor Wyatt:

At last we have come through with some vaccinia virus, and are shipping today 1.16 grams of dry powder.

This material was prepared by Dr. Brown from rabbit lymph by differential centrifugation according to the method of Hoagland, Smadel and Rivers (J. Expt. Med., 1940, 71, 737). Approximately 40 ml were lyophilized. The intradermal titer in rabbits (0.25 ml per dose) was 10^{-9}. (This order of activity is not as high as reported by Hoagland et al). Smears stained by the Paschen method for elementary bodies have given us the impression that there is considerably more material than is reflected in the infectious titer. The bacterial count was approximately 1 million organisms per ml or 40 million for the entire lot. Just prior to lyophilization the preparation was heated at 55°C. for 200 minutes so that it should not be highly infectious. I think, however, that it should still be suitable for electron microscopy.

I hope there is sufficient material for, at least, preliminary work. Now, that Dr. Brown has gone through the procedure, further preparations would be less time consuming.

Dr. Spizizen joins me in wishing you a happy and satisfying New Year.

Sincerely,

Bettylee Hampil

Bettylee Hampil, Sc.D.
Director of Virus Research

BH/ej

$LD_{50} = \frac{1.16}{40} \times 0.25^{-} \times 10^{-9} \times \frac{15}{100} = 1.1 \times 10^{-12}\ g\ N.$

October 23rd, 1952

Dr. J. Spizizen,
Research Division,
Sharpe & Dohme Inc.,
West Point, Pennsylvania. USA.

Dear John:

Thanks for the very generous preparation of T7. I am sorry I did not make clear the amount I actually use for analysis and saved you the trouble of making such a big batch. For a routine analysis for nucleic acid bases, I use 0.5 to 1.0 mg. of nucleic acid, and for a search for possible trace amounts of hydroxymethylcytosine 10 mg. NA is sufficient. So a quantity of virus containing some 20 mg. of NA is adequate for all contingencies. I mention this in the hope of saving some labour with the vaccinia.

The "T7, lot #1" contains 3.36% P and 4.0% DNA, and so must still contain considerable bacterial debris and non-nucleic acid P. It contains cytosine and no OC, so I think we are safe in concluding that T7 lacks this pyrimidine. What this might mean I haven't much idea. The DNA's of all phages must be synthesized after infection, and I don't see why it should be a matter of great importance whether the building blocks come from the host or the medium. After all, T2, which contains OC and no cytosine, derives some 20% of this from host DNA, and so must bring about interconversion of pyrimidines. It seems to be a matter of individual whim. I re-checked an insect virus recently and could find no trace of OC.

Thanks again for preparing this material.

Yours sincerely,

GRW/m

G. R. Wyatt.

Sharp & Dohme

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

October 10, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Box 490, Sault Ste. Marie
Ontario, Canada

Dear Jerry:

Thank you for your interesting letter of September 27. I wonder if the DNA's of all coliphages which are formed by conversion of host DNA have the normal base composition, whereas phages which contain DNA synthesized after infection would have the new pyrimidine?

I have sent you a new preparation of T_7 containing 2.5×10^{14} activity units per gram before lyophilization. We hope that this is a cleaner preparation in regard to bacterial debris. We are at present studying conditions for producing higher titers of T_7 so that pure T_7 can be made, since centrifugation of phage particles is so much easier when lysate titers attain 10^{12} phage units per ml or thereabouts.

Our production of vaccinia is in progress and, barring unforseen difficulties, should be ready soon. We will keep you informed.

Yours sincerely,

John

John Spizizen, Ph.D.
Associate in Virology

JS/ej

Sharp & Dohme
INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

October 7, 1952

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

Dear Dr. Wyatt:

Thank you for your letter of September 5. I am just getting around to catching up with some of my correspondence and I am writing to let you know of our status to prepare 5-hydroxymethyluracil. Dr. Miller in our laboratory has been attempting to prepare this in a manner analogous to the method he used for the preparation of 5-hydroxymethylcytosine; that is, by the lithium aluminum hydride reduction of ethyl uracil-5-carboxylate. Although the evidence is that the reduction occurred, the isolation of the 5-hydroxymethyluracil from the reaction mixture has presented a real problem. The product that Dr. Miller has been able to get out is heavily contaminated with lithium salts in addition to some of the uracil-5-carboxylic acid. He has also carried out the deamination on the synthetic 5-hydroxymethylcytosine as you did on C_X. The materials prepared in each of these methods behave similarly on paper chromatograms. With this information we are considering running through the synthesis of 5-hydroxymethyluracil as originally reported by Johnson and co-workers although there is some doubt in our minds that Johnson actually had 5-hydroxymethyluracil. Perhaps when our method for the synthesis of the cytosine derivative is better worked out, deamination procedure may be the easiest route to the uracil analog.

We shall keep you and Dr. Cohen informed of our progress.

Sincerely yours,

James M. Sprague, Ph.D.
Assistant Director of Research

JMS:mwk
cc - Dr. Cohen

September 27th, 1952
File:

AIRMAIL

Dr. John Spizizen,
Research Division,
Sharp & Dohme Inc.,
West Point, Pennsylvania. USA.

Dear John:

Thanks for your note of September 19th. From your infectivity data, the DNA of your T7 prep. must be pretty well all T7 DNA, and as I have repeated the quest for hydroxymethylcytosine (OC) in a sample of the unfractionated preparation with equally negative results, I am satisfied there is no OC in T7. Remarkable! While I am not anxious to run more chromatograms than I have to, we shall no doubt all be happier to have this result confirmed with a cleaner prep., so if you care to prepare some now, I shall see what I can find in it.

As for isolation of phage DNA, I simply used Cohen's method (Cold Spring Harbor Symposium 12, 38). This is not entirely satisfactory, as the product sometimes contains considerable protein, but it is easy to get a product which is water-soluble and suitable for hydrolysis. One thing I have found is that you should not leave the solution in urea longer than necessary, as this tends to depolymerize the NA and reduce the yield.

Sincerely yours,

GRW/m

G. R. Wyatt.

Sharp & Dohme

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

September 19, 1952

Dr. G. R. Wyatt
Department of Agriculture
Laboratory of Insect Pathology
Box 490, Sault Ste. Marie
Ontario, Canada

Dear Doctor Wyatt:

Your note was most interesting. As far as I can glean from my records this T_7 preparation should have contained at least 10^{15} active particles per gram. I am certain we can do better with our present facilities, and I hope to have some more material for you in a few weeks.

We are also beginning our preparation of the vaccinia virus and will keep you informed of its progress.

Also, I am interested in isolating some hydroxmethylcytosine nucleotide and nucleoside for microbiological study, and would like to know the technique you use for isolating DNA from T_6.

Thank you for keeping us informed of your activities.

Sincerely,

John Spizizen

John Spizizen, Ph.D.
Associate in Virology

JS/ej

September 11th, 1952

AIRMAIL

Dr. J. Spizizen,
Research Division,
Sharpe & Dohme, Inc.,
West Point, Pennsylvania. USA.

Dear Dr. Spizizen:

Thank you very much for the T7 preparation, and your letter of August 28th.

I have analysed the T7. It does not appear to be very clean: we find 4.4% P and 3.6% DNA (by Dische), which suggests it would not be more than about 10% virus. However, I isolated nucleic acid from it, getting 45 mg. of crude DNA from 1 g. of the preparation, and analysed this. It contains cytosine and apparently no hydroxymethylcytosine. The ultraviolet absorption of the appropriate region of a chromatogram indicates maximum possible hydroxymethylcytosine equivalent to 0.5% of the cytosine, and as this did not have the correct spectrum it may signify none at all.

Thus it seems that not all the T phages have hydroxymethylcytosine, and whatever function it has is not essential for phage production. I should be more confident of this result, however, if I had some data on how much T7 is actually in the preparation, and I wonder if you have anything on this, from activity titrations or what not?

Yours sincerely,

GRW/m

G. R. Wyatt.

September 5th, 1952

Dr. J. M. Sprague,
Research Division,
Sharp and Dohme, Inc.,
West Point, Pennsylvania. USA.

Dear Dr. Sprague:

Thank you very much for your letter of August 26th and enclosed cheque to cover my travelling expenses.

The minor changes you suggest have been incorporated in our manuscript, and it is being submitted to "Nature".

I am glad to have the information that the synthetic 5-hydroxymethylcytosine you sent is anhydrous. This being so, my estimate of the extinction coefficient at the maximum in 0.1 N HCl is 9700, in reasonable agreement with that found in your laboratory.

I have isolated some nucleic acid from the T7 phage which Dr. Spizizen sent, and find that the "cytosine" fraction consists very largely, if not entirely, of cytosine itself. There may be a trace of hydroxymethylcytosine, but is not more than 1% of the cytosine. I shall be writing Dr. Spizizen more fully about this when I have assayed a larger sample.

Yours sincerely,

GRW/m

G. R. Wyatt.

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

August 28, 1952

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

Dear Doctor Wyatt:

In Dr. Hampil's absence, I am replying to your inquiry concerning the possibility of obtaining some vaccinia virus for electron microscopy. I am quite certain that we can meet your requests when we have prepared the virus for you.

I hope that you received the T_7 preparation and that you found it satisfactory. Do not hesitate to let me know if you require any more material.

Yours sincerely,

John Spizizen

John Spizizen, Ph.D.
Associate in Virology

JS/ej

August 26, 1952

Dr. Seymour Cohen
Marine Biological Laboratory
Woods Hole, Massachusetts

Dear Dr. Cohen:

I am sorry I was delayed in replying to your note of August 18 regarding the attached manuscript. I was away from the laboratory attending the Gordon Research Conferences at New London, New Hampshire.

We have no important suggestions regarding the draft of your manuscript; only a few points come to mind. On page 2, last line, the Nitrogen value found was 29.33. Page 3, first line, I suggest that my name be deleted since Dr. Miller proposed the synthesis and carried it out. You may wish to correct the spelling of the Sharp in Sharp and Dohme by dropping the "e" on both page 2 and page 3. This is a very common misspelling that we encounter very frequently.

Thank you for the courtesy of submitting this draft to us and for the acknowledgment of the minor part that we played in this interesting development.

Sincerely yours,

James M. Sprague, Ph.D.
Assistant Director of Research

JMS:mwk

cc - Dr. Wyatt

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

August 26, 1952

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

Dear Dr. Wyatt:

Your letter of August 13 arrived while I was away at the Gordon Research Conferences in New Hampshire. I am attaching a check to cover your expenses for the trip to our laboratory on August 8. I am sorry that I was not here to take care of this more promptly. We certainly enjoyed your visit here and the work that we have been able to carry on with you and with Dr. Cohen.

Since your letter of August 13 crossed mine of the same date, I believe that I have answered your question regarding the Dumas Nitrogen values. In connection with the extinction coefficient obtained by you on our sample of 5-hydroxymethylcytosine, I can only say that the material we submitted to you as described in my letter of August 1 was the anhydrous material and therefore you should use the molecular weight of 141 in making your calculations. We have rerun the ultra-violet curve on our compound and still our extinction values are lower than you report, particularly in the 0.1 N hydrochloric acid solution. At the moment, we have no explanation for this difference.

Dr. Miller is finding that the isolation of the 5-hydroxymethyl uracil in pure form is much more difficult than the analogous cytosine derivative. He is having to resort to chromatographic techniques and we hope to be able to get enough material for characterization in this way. Thank you for the absorption maxima on the deaminated C_x. We shall keep you informed of our progress.

Dr. Miller has run parallel comparative ultra-violet and chromatographic studies on his synthetic material and upon C_x and he, too, can detect no difference between the two specimens. When this work is completed, we also will summarize our newer data for you.

Sincerely yours,

James M. Sprague, Ph.D.
Assistant Director of Research

JMS:mwk
Enclosure

August 15, 1952.

Dr. B. Hampil,
Research Division,
Sharpe and Dohme, Inc.,
West Point, Pennsylvania.

Dear Dr. Hampil:

When I mentioned to Dr. G.H. Bergold, of this laboratory, the other day, that I was expecting eventually to receive some purified vaccinia virus for analysis from you, he expressed great interest and asked if it might be possible for him to obtain a specimen for electron microscopy. Dr. Bergold has been studying the morphology of insect viruses and is anxious to compare how a vertebrate virus responds to some of his techniques. I wonder, therefore, if you would have any objection to some of your proposed vaccinia being used for electron microscopy, and if not, whether you could arrange to have a small sample taken from the preparation before drying and sent to us in liquid suspension. It would, incidentally benefit us to have the preparation examined on the electron microscope by providing an additional criterion of purity.

I have inquired of Dr. Cameron, the director of this lab., and find that any package labelled "Biological sample, of no commercial value" should come to us without interference. The virus in suspension we should, of course, treat with due precautions as infectious material.

Yours sincerely,

GRW/h

G.R. Wyatt.

August 13, 1952.

Dr. J.M. Sprague,
Research Division,
Sharpe and Dohme Inc.,
West Point, Pennsylvania.

Dear Dr. Sprague:

The synthetic 5-hydroxymethylcytosine was here when I got back, and I have had a look at it. It shows identical chromatographic movement with the natural material when run on the same papers. The wavelengths of absorption maxima and minima, read on our instrument, all agree within 0.5 mμ with those of my preparation. Taking these results together with the analytical data, there seems to be no doubt that the two specimens are identical.

Since my quantitative estimations are based on the ultraviolet extinction coefficient I re-determined this with some of your material, and got a value of 10,300 for the maximum in 0.1 N HCl--about 7% higher than reported by your lab. This is based on a molecular weight of 150.3, for the hydrate. Was the sameple you sent by any chance dried at 100°? I am getting N values on my solution as a further check.

I omitted to write down the Dumas N values for the natural hydroxymethylcytosine, and should be grateful if you'd send them to me. I take it you have no objection to your analytical figures being used for publication.

Our meeting the other day was, from my point of view, very worthwhile, and I am exceedingly grateful to you for making it

possible. My expenses for the trip were: return fare Sault Ste. Marie to Philadelphia, $95.72, hotel, $23.16, taxis, meals, etc., $16.22, making a total of $135.10.

I feel very fortunate to have had the assistance of you and Dr. Miller on this problem. I look forward to hearing of the synthesis of 5-hydroxymethyluracil. Incidentally, the absorption maxima of the deamination product of phage Cx, for comparison, are: 0.1 N HCl, 261 mμ; pH7, 261 mμ; 0.1 N NaOH, 285 mμ.

Yours sincerely,

GRW/h

G. R. Wyatt.

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

August 13, 1952

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

Dear Dr. Wyatt:

I want to thank you for your kindness in coming to our laboratories for the interesting discussions last Friday. I am sure that these discussions were helpful to us in orienting our thinking regarding the future work on the problem that you and Dr. Cohen have brought to our attention.

May I remind you again that we are expecting you to submit the expenses for your trip to us for reimbursement.

Although I told you last Friday of the Nitrogen determinations on C_X, I am recording the values here for your records. You will recall that these were done by the Dumas method by an outside laboratory.

Calc., 29.78%. Found, 29.33%.

Sincerely yours,

James M. Sprague, Ph.D.
Assistant Director of Research

JMS:mwk

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

August 1, 1952

Dr. G. R. Wyatt
Sault Ste. Marie
Ontario, Canada

Dear Dr. Wyatt:

We have analyzed the sample of pyrimidine C_X which we received from you and the carbon and hydrogen values agree with the calculated for the 1/2 hydrate of 5-hydroxymethylcytosine. We have dried to constant weight a sample of this material and are forwarding it to an outside laboratory for Dumas nitrogen determination. We shall forward these results to you as soon as received.

We believe that we have succeeded in synthesizing 5-hydroxymethylcytosine. The analytical data is correct and the R_f values and ultra-violet absorption spectra agree with the data supplied by you for C_X. It seems to us that at this stage of our examination, it is safe to conclude that the two samples are identical and that the natural material is, in fact, 5-hydroxymethylcytosine.

I am enclosing a copy of Dr. Miller's report on his synthetic specimen as well as on your C_X sample. Copies are being forwarded to Dr. Cohen also.

For your comparative examination we have forwarded to you 20 mg. of our anhydrous synthetic preparation designated as O.C. 1254-137-A. We have approximately 4.7 mg. of your sample remaining and with your permission, we may put it through some other tests when the analytical data is complete.

Dr. Miller is attempting to prepare the 5-hydroxymethylcytosine uracil by the same general procedure that he employed for 5-hydroxymethylcytosine; namely, the action of lithium aluminum hydride upon ethyl uracil-5-carboxylate. To date he has not been able to isolate a pure sample but we have every reason to believe that the preparation will be successful with a little further work.

We shall welcome your comments upon the information we have obtained thus far and any suggestions of further work we might do to assist you in this problem.

Sincerely yours,

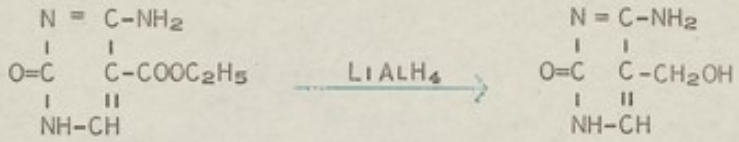
J. M. Sprague mwk.

James M. Sprague, Ph.D.
Assistant Director of Research

mwk
Enclosure
cc - Dr. Cohen

7/31/52

5-HYDROXYMETHYLCYTOSINE



Sample purified by recrystallization from alcohol-water and from water. Mp. chars slowly above 200°, no melt to 300°.

Analyses after drying in vacuo at room temperature: Calc. for $C_5H_7O_2N_3 \cdot 1/2\ H_2O$. C, 40.00; H, 5.37. Found: C, 40.07; H, 5.55.

Analyses after drying for 48 hours in vacuo over P_2O_5 at 100°: Calc. for $C_5H_7O_2N_3$. C, 42.55; H, 5.00; N, 29.78. Found: C, 42.61; H, 5.14; N, 29.72.

In 65% isopropanol-water, the R_F of 5-hydroxymethylcytosine is slightly less than cytosine. When the isopropanol content of the solvent is raised to 75%, the R_F of 5-hydroxymethylcytosine drops below that of cytosine.

Ultra-violet absorption data on rigidly dried material---

	Maxima	E	Minima	E
Distilled Water.	270	5975	250	4095
0.1N NaOH.	283.5	7630	253	1960
0.1N HCl	279.5	9630	241.5	1115

The curve from the 0.1N sodium hydroxide solution also exhibits a shoulder between 230 and 233 Mμ.

ANALYSES ON C_X FROM DR. WYATT

Analyses after drying in vacuo at room temperature: Calc. for $C_5H_7O_2N_3 \cdot 1/2\ H_2O$. C, 40.00; H, 5.37. Found: C, 40.23; H, 5.35.

A SAMPLE WAS DRIED TO CONSTANT WEIGHT IN VACUO AT 100° OVER P_2O_5 AND LOST 4.72% WEIGHT. THIS SAMPLE IS BEING SENT OUT FOR DUMAS NITROGEN DETERMINATION.

C. S. MILLER
7/31/52

MWK
CC - DR. COHEN

C_5	60.05
H_7	7.06
O_2	32.00
N_3	42.03
	141.14
$\frac{1}{2} H_2O$	9.00
	150.14

Fin.

Warnack Hotel

18 July, 1952.

Dr J.M. Sprague,
Research Division,
Sharpe & Dohme Inc.,
West Point, Pa.

Dear Dr. Sprague,

I am sending you 13 mg. of the supposed pyrimidine C_x, from T6r⁻ phage. This was isolated chromatographically, purified through the picrate, and three times crystallized from water at neutrality, and is, I think, not far from pure. It was dried in vacuo at room temperature.

Here are the ultraviolet absorptionspectral characteristics. Extinction coefficients are based on Kjeldahl nitrogen, and calculated assuming 3 atoms N per molecule.

	Maxima		Minima	
pH 1	279	10000	241	1220
pH 7.4	269.5	5920	251	4160
pH 13	283	7750	254	1940

The molar extinction coefficients are quite close to those of cytosine and 5-methyl cytosine (10,500 and 9800 respectively for maxima at pH 1).

On heating, the crystals decompose, turning progressively yellow, then brown, over the range 230° - 300°, and do not melt up to 320°. The substance is more labile than other natural pyrimidines, and is fairly rapidly destroyed, for example, by boiling in hydrochloric acid.

I presume C, H, and N analyses should be obtained first. If you have any of the sample left from that, please use it for whatever analyses or tests you think likely to be informative. I have another about equal amount of C_x in various stages of preparation, and should with luck be able to obtain another small crop of clean crystals.

Is there any information on the 5-methylol derivatives yet?

Yours sincerely,

G.R. Wyatt

absorption in the ultraviolet (Markham & Smith 1949); the latter technique has been found very convenient, since it is sensitive to less than 1 µg of purine base and provides a permanent photographic record of each chromatogram.

As a solvent for the separation of the free bases on paper chromatograms, aqueous isopropanol (65%)-HCl (2.0 N) (Wyatt, 1951) is very satisfactory, since the two purines and four pyrimidines commonly found in nucleic acids can all be resolved in a single one-dimensional run. The strong acid is helpful by increasing the solubility of guanine, of which only small amounts will migrate quantitatively in neutral or weakly acidic or basic solvents. This mixture resolves also the nucleosides and some, but not all, of the nucleotides.

The nucleic acid derivatives eluted from chromatograms are estimated by their ultraviolet absorption in the quartz spectrophotometer. The method is sensitive and precise, but depends on accurate knowledge of extinction coefficients, and there is some disagreement in the literature on these (Hotchkiss 1948; Vischer & Chargaff 1948a; Ploeser & Loring 1949; Smith & Markham 1949; Wyatt 1951b). Agreement on these coefficients, re-determined with substances of established purity, would enable better comparison of results from different laboratories.

The problem of obtaining quantitative yields of nucleic acid derivatives from hydrolysis has given much trouble, and many methods have been used. The purine bases are easily liberated with dilute mineral

July 7th, 1952
File:

Dr. J. M. Sprague,
Research Division,
Sharpe & Dohme Inc.,
West Point, Pennsylvania. USA.

Dear Dr. Sprague:

Thank you for your letter of July 1. I am glad to hear you will be able to undertake analyses on our unknown C_X. I have about 20 mg. of this material isolated from phage, but it is still obviously impure, probably with substances originating in the paper of the chromatograms. Just as soon as I am able to get it to a satisfactory degree of purity, I shall send it to you.

For the same reason, I have not obtained physical data on C_X, except the ultraviolet spectral characteristics which I presume Dr. Cohen has already transmitted to you.

I am glad to hear you are engaged on synthesis of 5-hydroxymethyl cytosine and 5-hydroxymeth uracil. If you have obtained any fractions which may contain a proportion of the desired product, even though not pure, I should be happy to examine them chromatographically to see if any substance similar to my C_X is present.

Yours sincerely,

GRW/m

G. R. Wyatt.

Sharp & Dohme

INCORPORATED

RESEARCH DIVISION, WEST POINT, PENNSYLVANIA

July 1, 1952

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

Dear Dr. Wyatt:

Dr. Seymour Cohen has inquired whether we would be willing to undertake the analyses on the crystalline preparation of C_x which you have been able to isolate from the coli phage. Needless to say, because of our great interest in this material and its identity, we will be only too happy to undertake this analysis when you have it available. We have a fairly complete microanalytical laboratory and should be able to carry out the complete analysis if there is adequate material. We also have access to outside consulting analytical facilities to supplement our own procedures. I am sure that through one of these arrangements we can take care of your specimen.

As Dr. Cohen has no doubt indicated to you, we are attempting to prepare a sample of 5-hydroxy-methyl cytosine as well as 5-hydroxy-methyl uracil. As soon as we have some definite information on these two preparations, I shall let you know our results. In the meantime, however, it will be helpful to us if we could know some of the physical constants on your C_x material if you have had an opportunity to collect such data.

Sincerely yours,

J. M. Sprague

James M. Sprague, Ph.D.
Assistant Director of Research

JMS:mwk