Dr. Kiehl Labor- und Forschungs-GmbH

Laboratory and research for Molecular Medicine/Biology
Naturwissenschaftlich-technische Gutachten und Forschung

Dr. Kiehl · Labor- und Forschung · Amselweg 12 · 93437 Furth i. Wald

Herrn Prof. Philipp Christen
Editorial Office, Eur.J.Biochem.
Apollostr. 2
Postfach A 152

CH-8032 Zürich
Switzerland

Dipl. Ing. (FH), Dr. rer. nat Reinhold Kiehl
Dipl. Chemiker, Biochemiker
Molekular-Mediziner

Amselweg 12
93437 Furth i. Wald

Telefon 09973/5139
Telefax 09973/2934

Ihre Zeichen und Nachricht vom	Geschäftszeichen (bitte bei Antwort angeben)	Telefon 09973/5139	Datum
			10.04.1996

Manuscript: Transport and ATP synthesis in mitochondria
(Ref.no.: 95-0810, 0811, 0812, 0813)

Dear Prof. Christen,

I like to submit the original manuscript with the topic

"Transport and ATP synthesis in mitochondria (part I to IV)"

for publication in Eur.J.Biochem.

The four parts of the topic belong together and should be reviewed under this aspect. I submitted these parts already one year ago (see ref.no. above), you may therefore treat this (these) manuscript(s) as a new submission.

The manuscript(s) has (have) been rewriten and corrected under consideration of all the comments abtained by your reviewer and the help of Mrs. C. Cavanna and her husband (Canadian/english lecturer at the University of Regensburg).

I hope the manuscript fullfills now the requirements of Eur.J.Biochem. and remain

yours sincerely

Reinhold Kiehl

Encl.: Original manuscript plus 3 copies

European Journal of Biochemistry

Zürich, 27/06/96

Dr. Reinhold Kiehl
Laboratory and Research for
Molelular Medicine/Biology
Amselweg 12
D-93437 FURTH IM WALD
Germany

Reference no.: 96-0560, 96-0561, 96-0562, 96-0563

Transport and ATP synthesis in mitochondria.
I. Evidence for mitochondrial 2,4-dinitrophenol accumulation across the Pi/H+-symport system
II. Glutathione: an endogenous regulatory factor for mitochondrial phosphate/proton-symport
III. Mitochondrial ATP synthesis in the phosphate/proton-symport system with oxidized glutathione as a catalyst
IV. K+-transport : evidence for mitochondrial FOF1-ATPase being a K+-pump

by
Kiehl Reinhold

Editor: Pettersson

Dear Dr. Kiehl,

Thank you for submitting your manuscript.

I regret to inform you that the Editor responsible for the manuscript has advised me that it cannot be accepted for publication in the Journal. The referees' reports are enclosed for your information.

The top copy of your manuscript will be returned to you by separate printed-matter mail.

Yours sincerely,

pp. Philipp Christen

Chairman of the
Editorial Board

Encl.: 2 reports
Copy : Editor

Editorial Office: From 1.2.96:
Kleinstrasse 6, Postfach Phone: +41 1 268 1150
CH-8032 Zürich, Switzerland Fax: +41 1 268 1151

EJB MS No. 96-0560-P Report No.: 1

Referee: please complete if appropriate

- ☐ Table(s) .....can be eliminated
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- ☐ Fig(s) .....can be eliminated
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This paper deals essentially with the effect of an SH-reagent NSPM. The results are complex and difficult to interpret. NSPM appears to be different from NEM. The compound has been used earlier by the author in his thesis work. The source of the compound is not mentioned.

The paper contains a few measurements, such as on the ^{14}C-DNP uptake into mitochondria and its influence of NSPM. The explanation that DNP is taken up by the phosphate carrier is doubtful. Why are not other SH-reagents, such as mercurials etc. used to make this point more clear. Most of the results shown in Fig. 2 A and B are presented in an unclear manner. The confusion is greatest in Fig. 4 where a very particular interpretation of these results is discussed invoking SS bridges in the membrane as part of the phosphate translocator, although according to the literature no SS bridge exists in the phosphate carrier.

This paper is unsuited for publication.

EJB MS No. 96-0560-P Report No.: 2

This manuscript title indicates that it is an investigation of 2,4-dinitrophenol transport through the Pi / H^+ -symport system. The involvement of the Pi / H^+ -symport system in DNP transport is inferred from differential NSPM inhibition kinetics on a variety of mitochondrial phenomena including Pi and Ca^{2+} accumulation into mitochondria, mitochondrial swelling, NSPM-dependent inhibition of DNP accumulation and its competitive displacement of a DNP analog, [^{3}H]NPA. Unfortunately the style of this revised manuscript remains so awkward that I am unable to judge he merits of Dr. Kiehl's arguments. It appears to me that NSPM is an inhibitor of all membrane proteins and that it is problematic to define the role of a single transporter through the action of a broad spectrum inhibitor. This would require careful experiments comparing the inhibitory potency of NSPM for Pi accumulation, Pi dependent H^+ transport and DNP uptake. This manuscript does not provide this data but instead bases specificity on relative potency measured in several complex systems and apparent competition between a component of NPA binding inhibited by NSPM. I could not understand Dr. Kiehls component analysis as no evidence was provided to define the interaction between NPA and DNP binding sites, but not withstanding this must question whether the inhibitory phenomena is related to DNP transport via the Pi / H^+ transport.

If this were an original submission I would recommend that Dr. Kiehl carefully revise this manuscript to exclude all extraneous material, clarify his explanations of experimental design and succinctly state the mechanistic interpretations permitted in this complex system. Unfortunately, this revision remains problematic in its awkward prose, inadequate documentation and confusing presentation.

I would recommend rejection of this manuscript without opportunity for a 3rd revision.

EJB MS No. 96.0561P Report No.: 1

Referee: please complete if appropriate

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In the second paper it is still much more difficult to appreciate what has actually been achieved. Nonylthiouracil has a glutathione trapping range. No proof to the effect of nonylthiouracil with glutathine has actually been produced. The author claims that this compound mainly reacts with the membrane-bound glutathione. As a result, he concludes that most of the glutathine is bound to the mitochondrial membrane and there is no free glutathione in mitochondria, in contrast to what is well-known in the literature by other authors. Besides, the SH-reagent cadmium is introduced and its influence on what the author claims to be measurements of phosphate and Ca^{+}-transport. Further, the cross-linking reagent diamide is used and picryl acetate for oxidizing SH-groups. Very few data are presented and these are experimentally not sound. For example the results illustrated in Fig. 1 are completely unsatisfactory. The interpretation of these results is far out of touch with the reality of the poor experimental data.

This paper is unsuited for publication.

EJB MS No. 96.0561P Report No.: 2

This manuscript reports an investigation to establish that glutathione is an endogenous regulatory factor for mitochondrial phosphate/proton-symport. It appears to be based on 3 observations; (1) phosphate modulates the bound and free components of mitochondrial glutathione following [^{35}S] n-nonylthiouracil (NTU) inhibition of the mitochondrial state 4-state 3 transitions and 2.4-dinitrophenol-dependent uncoupling. (2) Cd^{2+} inhibits Pi and Ca^{2+} accumulation. (3) There is competition between a high affinity binding component of NPTU and [^{3}H] NPA. Dr. Kiehl proposes a mechanism whereby glutathione is an endogenous regulator of various membrane associate transporters including the Pi/H^{+} symporter, transhydrogenase and H^{+}-pumping ATPase.

This manuscript, like the previous manuscript (96-0560) fails to succinctly present a convincing body of experimental evidence in support of the authors hypothesis. The failure is complex and includes an inability to define essential observations, consideration of largely irrelevant data, inadequate or inappropriate documentation and a jargon laden, awkward presentation style. Together these limitations obscure the authors experimental arguments and limit the suitability of this work for publication.

I would recommend rejection of this revised manuscript without the opportunity for a 3rd revision.

EJB MS No. 96.0562 R Report No.: 1

Referee: please complete if appropriate

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☐ Fig(s) .....can be eliminated
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This is a purely speculative paper which is out of touch with what is well-known about ATP synthesis in mitochondria. A new type of ATP synthesis by the phosphate transporter is postulated. F_1F_o ATPase is claimed to be a K^+ pump as to be shown by the experimental work in paper No. IV:

This paper is unsuited for publication.

EJB MS No. 96.0562 R Report No.: 2

This manuscript presents the hypothesis that a 30 kDa, mitochondrial phosphate/proton symport system functions in mitochondrial ATP synthesis. The evidence for this hypothesis is that ; (1) both thiol reactive agents and picrylate abolish the respiratory control ratio in mitochondria. This is attributed to an identical site of action of these inhibitors as well as DNP within a membrane-associated, 30 kDa membrane protein (i.e Pi / H^+ symporter). (2) DNP binds (high affinity site seen in [^{3}H]NPA data) to the mitochondria in a Pi-dependent manner. (3) NSPM inhibits Pi accumulation. This suggests that nucleotide binding sites are present on the 30 kDa membrane protein. (4) ATP synthesis has never been demonstrated in various preparations of defined mitochondrial energy transduction complexes.

The presentation of a provocative hypothesis requires careful documentation of experimental evidence and thoughtful analysis of competing hypothesis. Unfortunately, a significant portion of the experimental basis of this hypothesis is provided in manuscripts 96-0560 and 96-0561, neither of which are presently acceptable for publication. Furthermore a thorough referencing of the literature would be needed to substantiate the authors claim that ATP synthesis can not be demonstrated in mitochondrial transduction complexes.

Without these publications there is inadequate experimental basis to consider the publication of this hypothesis.

EJB MS No. 96.0563P Report No.: 1

Referee: please complete if appropriate

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- ☐ Table(s) .....can be shortened
- ☐ Fig(s) .....can be eliminated
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This last paper contains 14 figures out of which 12 present a confusing array of experimental results. Again, the interaction of the SH-reagent NSPM with transport reactions in mitochondria are studied as well as the influence of a number of uncouplers. Several of these reagents are claimed to stimulate the K+-pump, whereas ATP synthesis is delegated to the phosphate transporter. The referee does not feel the necessity to refer in detail to all the different types of experiments performed. All what can be said is that most experiments are based on a phenomenology of osmotic swelling of mitochondria and on dyes indicating proton, cation movements and the membrane potential.

Most of these experiments lack the elementary controls. This may be one reason why the author comes to a number of strange conclusions. The paper apparently contains fragments of experiments which could not be completed and which may date back to many years before and had been left unpublished. The conclusions are out of touch with the present day evidence on the mechanism of oxidative phosphorylation and the role of the various transporters.

This paper is unsuited for publication.

EJB MS No. 96.0563P Report No.: 2

This manuscript provides evidence that mitochondria contain Mg^{2+}-sensitive transporters including a K^+/H^+-antiporter and a K^+-pump (F_0F_1) which share a variety of inhibitor sensitivities. It is speculated that these transporters and the Pi / H^+ transporters are "linked" and are controlled by cytoplasmic levels of O_2, Ca^{2+} and Mg^{2+}. It is also speculated that there is a "direct connection" between the nervous system and mitochondria that mediates events such as thermoregulation, substrate oxidation and O_2-uptake that function in a variety of disease states.

This manuscript seems to be a catalogue of experiments that never leads to a conclusion. The speculation is unsubstantiated and inappropriate for publication in European Journal of Biochemistry.

GhK

Universität
Gesamthochschule
Kassel

Fachbereich 19
Biologie/Chemie

Prof. H. Follmann
Biochemie

Universität Gesamthochschule Kassel FB 19 · D-34109 Kassel

Lieferanschrift Heinrich-Plett-Straße 40
D-34132 Kassel

Telefon 0561 804 4511
Sekretariat 0561 804 4161
Telefax 0561 804 4466

Dr. Reinhold Kiehl
Labor und Forschung
Saliterweg 1

93437 Furth im Wald

Datum 26.11.1996

Sehr geehrter Herr Dr. Kiehl,

Besten Dank für Ihr Interesse an der Frühjahrstagung der GDCh-Biochemie. Wir würden uns freuen, wenn Sie teilnehmen. Leider sind -weil das Themenspektrum umfänglich ist - schon alle Kurzvortragstermine besetzt. Eine Posterpräsentation (z.B. über Glutathion) ist selbstverständlich willkommen.

Mit freundlichem Gruß

Prof. Dr. Hartmut Follmann

Telefon (0561) 804-0
Telefax (0561) 804-2330
Btx * 920135 #
Telex 99572 ghkks d

MAGISTRAT DER STADT WIEN

AKH – 1090 Wien, Währinger Gürtel 18–20

ALLGEMEINES KRANKENHAUS
1090 Wien, Währinger Gürtel 18–20
Telefon 40400-

Universitätsklinik für Kinder- und Jugendheilkunde
Vorstand: o. Univ.-Prof. Dr. R. Urbanek

Dr.R.Kiehl
Furth

Betrifft: ☎ Nebenstelle Datum 1997

Dear Dr.Kiehl:

The referees are of the opinion that
your proposed review grtble is confusing and
the conclusions drawn are not holding water.
Your many citations of your name are not published as
peer reviewed papers.
If you are able to present a lecture i.e. review on
glutathion in Greece this year I and a few colleagues involved
in metabolism would listen to it and may be giving you
advice, hints and instructions for the resubmission
of a potential review for our journal.
If you accept this proposal, contact Prof.M.Kyriakides,

0030-31-99 6193 fax and phone. ?

This is not an invitation waiving the registration fee or so.

Mit besten Grüssen

Ihr

Gert Lubec

PCCP
Physical Chemistry Chemical Physics

6 March 2000

Dr R Kiehl
Laboratory and Research for Mol. Medicine/Biology
Furth im Wald-93437
Germany

Dear

Paper ref: B001809K
Title: Review. The Essential Factor for Mitochondrial Energy-Linked Functions

I acknowledge receipt of the above paper. Please quote the reference number assigned to it in all future correspondence.

Please supply your FAX number if you have not already done so. This may be used for future correspondence.

To maintain and improve publication times, you can help us by:

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Yours sincerely

JWindsor

Jen Windsor
Editorial Secretary
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PCCP
Physical Chemistry Chemical Physics

RW/DJH

E: 22 April 2000

13 April 2000

The Royal Society of Chemistry
Thomas Graham House
Science Park
Milton Road
Cambridge
CB4 0WF
United Kingdom

Telephone
+44 (0)1223 420066

Fax
+44 (0)1223 420247

E-mail
pccp@rsc.org

World Wide Web
http://www.rsc.org

Dr R Kiehl
Laboratory and Research for Mol Medicine/Biology
Furth im Wald
93437
GERMANY

Dear Dr Kiehl

Paper Ref: B001809K
Title: Review. The Essential Factor for Mitochondrial Energy-Linked Functions

Thank you for submitting your manuscript to PCCP for the Special Issue on 'Interaction of Biomolecules with Model Membranes and Monolayers'. However, apart from some invited lectures, we are only publishing regular research papers in the Special Issue and not reviews. I enclose a copy of the call for papers. I regret, therefore, that we are unable to consider your review for publication in the Special Issue.

Yours sincerely

Rosemary Whitelock

Rosemary Whitelock
Managing Editor
PCCP

VAT Registration Number
GB 342 1764 71

Registered Charity Number
207890

→ Haut

BIOLOGICAL CHEMISTRY

Internet: www.deGruyter.de

Editorial Office
Walter de Gruyter GmbH & Co. KG
Genthiner Str. 13
D-10785 Berlin
Germany
Tel.: +49-30-260 05-176
Fax: +49-30-260 05-298
e-mail: biol.chem.editorial@deGruyter.de

Dr. R. Kiehl
Laboratory and Research for Mol. Medicine/Biology
Saliterweg 1
93437 Furth im Wald

11.08.2000

Re: **R. Kiehl**
Glutathione: The essential factor for mitochondrial energy-linked functions

Dear Dr. Kiehl,

Thank you for submitting your above manuscript. I regret to inform you your manuscript is not suitable for publication in this journal. The present submission is virtually identical to a version previously submitted to *Biological Chemistry*; both reviewers of that version did not recommend publication of the review. We therefore suggest to first extensively re-write und update the article before another round of peer-reviewing can be initiated.

Thank you very much for your interest in *Biological Chemistry*.

ab. 21.08.
10[30] - 11[00]
Beispiel
neueste Heft
14-14[30]

Sincerely yours,
Torsten Krüger
Dr. Torsten Krüger
Editorial Office

lit. ab 1990/1991 - Rev. - Wenpert Junge

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1, Avenue du Pavé Neuf
F-93160 NOISY-le-GRAND
Tel: (33-1) 45 92 37 19
Fax: (33-1) 43 04 20 30

Herrn
Dr. R. Kiehl
Saliterweg 1
93437 Furth im Wald

Jena, 29.11.2000

Sehr geehrter Herr Kollege Kiehl,

ich habe mir Ihr Manuskript aus dem Jahre 1995 näher angeschaut. Grundsätzlich halte ich die von Ihnen vorgelegte Substanz für außerordentlich interessant und auch im wesentlichen für publikationsfähig, jedoch unter Berücksichtigung folgender Aspekte:

1. In einem Review müssen die rezentesten Resultate auf dem jeweiligen Gebiet, das überstrichen wird, sorgsam berücksichtigt und ausdiskutiert werden. Sie kündigen selbst an, daß zwischenzeitlich auch von Ihnen neue Ergebnisse gewonnen worden sind. Diese müssen natürlich hinzugefügt werden.
2. 4 ausführliche Arbeiten avisieren Sie bei Herrn Michel und Herrn Huber und betonen, daß die Versuchsbedingungen zu Ihren Kapiteln 1-4 sich dort bei diesen Kollegen befinden sollen. Mit diesem Hinweis kann ich leider wenig anfangen, da Sie die Kapitel ohnehin nicht nummeriert haben und ich infolgedessen keine Zuordnung nachempfinden kann. Sie sollten daher diese Arbeiten aufnehmen und sich mit denen in ausreichender Form auseinandersetzen. Im übrigen scheint es mir nicht unwesentlich, obwohl dies für Reviews nicht zwingend ist, die Versuchsbedingungen, d. h. die experimentellen Voraussetzungen, die Sie gewählt haben, herauszukristallisieren und an Methodologien anderer Gruppen zu messen.
3. Rein formal entsprichen der Manuskriptaufbau sowie die äußere Form nicht den Instructions of Authors der Zeitschrift CMB. Das Manuskript müßte also inhaltlich erweitert und zum zweiten formal umgeändert werden.

Wenn dies soweit gediehen ist, bin ich gern bereit, das abgeänderte Manuskript zum peer reviewing an kompetente Kollegen weiterzuleiten. Ich darf Sie daher bitten, mir geeignete Reviewer zu nennen, die sinnvollerweise infrage kommen.

Mit freundlichen Grüßen

Prof. Dr. K.-J. Halbhuber

Anlage
Instructions