European Journal of Biochemistry

Zürich, 15/07/92

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Reference no.: 92-0998

Transport and ATP synthesis in mitochondria 1
I. Evidence for mitochondrial 2,4-dinitrophenol accumulation across the Pi/H+-symport system

by

Kiehl Reinhold,

Editor: Böck

Dear Dr. Kiehl,

Thank you for submitting your manuscript.

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Please sign the enclosed copyright form and complete the computer questionnaire, then return both to the Editorial Office.

You will be advised of the Editor's decision in due course.

Yours sincerely,

Dr. John W. Aitken
Editorial Manager

Encl.: Copyright form
Computer questionnaire

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European Journal of Biochemistry

Zürich, 12/08/92

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Reference no.: 92-0998/92-0999AB

Transport and ATP synthesis in mitochondria 1
I. Evidence for mitochondrial 2,4-dinitrophenol accumulation across the Pi/H+-symport system

II. Glutathione and endogenous regulatory factor for mitochondrial phosphate/proton symport

by

Kiehl Reinhold

Editor: Böck

Dear Dr. Kiehl,

Thank you for submitting your manuscripts.

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

The top copy of your manuscripts are being returned to you by separate printed-matter mail.

Yours sincerely,

Philipp Christen

Chairman of the
Editorial Board

Encl.: 4 reports
Copy: Editor

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30.10.

Re.: MS No. EJB 92.0998, EJB 92.0999

Report 1

thank you for your FAX from Sept. 14. It is admittedly always disappointing to realize the rejection of a paper, and thus the author clearly argues against this decision. However, the authors arguments in this particular case are not solid both with respect to scientific reasons and to the style of his argumentation. In the following I will answer to the main arguments of the author criticizing the rejection of the two papers.

It is in fact obvious that it is not clear to anybody how transport and ATP synthesis in mitochondria works (at least on a molecular level). That is a good reason not to spoil the field with another unproven hypothesis. The general disappointment of the author about the current state of bioenergetics is to some extend justified, however, this is not the point to discuss here, and it will - in the opinion of the reviewer - not be improved by publishing these two papers.

Furthermore, it is in fact the general procedure in reviewing a scientific paper to "pick out one data of a set of connected data", as the author complains, if that particular result seems to be not correctly obtained and methodically doubtful. In his reply (MS 92.0998), the author gives some explanation to criticized point No 2, but no answer at all to point 1, 3 and 4. In his reply to the criticism of MS 92.0999 the author obviously overlooks that a paper to be published in a Journal of the level of EJB should include a sound presentation of experimental facts, on which only then a hypothesis may be constructed in the discussion part. This order is clearly turned round here: very limited amount of results and a lot of hypotheses. This fact is only expressed by the number of corresponding pages and the page numbers are of course not the basic reason for the rejection.

A last word to the English which was criticized by another reviewer. The author should not emphasize the point too much that the other reviewers have not criticized the poor English. In general, papers which are rejected on the basis of severe lacks in methodical aspects or regarding content are not criticized in addition with respect to poor English, since that is estimated as a comparatively minor point. Also in this case, absence of evidence does not mean evidene for absence.

... the two papers cannot be

30,10.

EUB n° 920998/ 0999/ 92 1073

Report 2

The refered manuscripts E.J.B. n° 92/0998, 0999 and 92/1073 ??
are very poor, and sometimes non sense.

The paper "Transport and ATP synthesis in Mitochondria. I. Evidence for mitiochondrial 2,4-dinitropehnol ..." by R.Kiehl cannot be accepted for publication in EJB.

1. There is not even circumstantial evidence that DNP is in fact transported by the phosphate carrier. The inhibiting agents are unspecific and have a lot of other effects on mitochondria besides inhibition of the phosphate carrier.

2. I do not understand what the aouther means by the term "inhibition" of phosphate transport in Fig. 1. Phosphate was added before the inhibitor, how can NSPM inhibit transport under these circumstances.

3. Uncoupling by Triton clearly changes the membrane, it may thus also change the binding properties of the amphiphilic compounds like DNP. Addition of Triton is no proof at all for DNP to be transported to the matrix space instead of being equilibrated by diffusion of even being only bound to the membrane.

4. The context of the NPA-data is completely unclear and not explained in the paper.

EJB MS No. 92.0998AB Report No.: 1

This paper is concerned with the problem of the mechanism of action of 2,4 dinitrophenol. The experiments have focused on the role of Pi/H^+ symport system in this mechanism, and it is concluded that the Pi/H^+ carrier is involved in the entry of 2,4 DNP into mitochondria. The experimental basis leading to this demonstration is poor and not convincing. It is partly based on the old fashioned swelling approach which is not really appropriate in the present work, due to uncontrolled artifacts. The experiments carried out with nonyl sulfamoylphenyl maleimide are not either convincing. Maleimide derivatives react with a number of protein components in mitochondria and the conclusion that maleimide derivative tested acts on the 2,4 dinitrophenol binding is not founded.

EJB MS No. 92.0999AB Report No.: 1

The paper "Transport and ATP synthesis in Mitochondria. II. Glutathione and endogeneous regulatory factor ..." by R.Kiehl and I.Gruia cannot be accepted for publication in EJB.

The amount of experimental data presented (less than one page of results) is negligible in view of the body of hypotheses constructed (more than four pages of discussion): Furthermore, the few data are only in circumstantial connection to the speculations in the Discussion part.

EJB MS No. 92.0999AB Report No.: 2

This paper is concerned with the formation of a complex between the mitochondrial glutathione and nonylthiouracil by incubation of mitochondria with nonylthiouracil. The complex is further cleaved to give a sulfenic acid derivative of glutathione. This is essentially a paper of methodology with limited interest in Biochemistry. Furthermore the Discussion Section is much too long and quite verbose (6 pages of Discussion for 1 page of Results).

European Journal of Biochemistry

Zürich, 26/08/92

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Reference no.: 92-1073

Transport and ATP synthesis in mitochondria 1
III. Hypothesis: mitochondrial ATP synthesis of the phosphate/proton symport system with oxidized glutathione as catalyst

by

Kiehl Reinhold

Editor: Böck

Dear Dr. Kiehl,

Thank you for submitting your manuscript.

I regret to inform you that the Editor responsible for your 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 is being returned to you by separate printed-matter mail.

Yours sincerely,

Philipp Christen

Chairman of the
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/EJB MS No. 92-1073AB Report No.: 1

Rep. 1

This paper describes a mechanism for ATP synthesis in mitochondria which depends on glutathione as regulatory factor and involves the Pi/H^+ symport as a key component in this system. There is no serious experimental evidence for such a mechanism.

Report 2

Re: MS No. 92-1073 AB

Rep. 2

I just have read the manuscript by R. Kiehl entitled: "Transport and ATP synthesis in mitochondria. III. hypothesis: Mitochondrial ATP synthesis on the phosphate/proton symport system with oxidized glutathione as catalyst".

The paper presenting no experimental data but referring to rather old (and in part non-reviewed) publications or to submitted manuscripts was hard to read - in part due to the really poor English. The author postulates a new mechanism for mitochondrial ATP synthesis occuring on the phosphate/proton symporter. I hesitate to refuse this manuscript as pure speculation not supported by any experimental evidence but rather suggest you to consult a real expert on the field of bioenergetics e.g. Prof. Klingenberg (Munich) or Prof. Kröger (Frankfurt).
Sorry for this. I return the MS by seperate mail. Nevertheless, I would appreciate if you could keep me informed on the final evaluation of the MS.

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SWITZERLAND

Unser Zeichen	Ihre Nachricht vom	Ihr Zeichen	Datum
			September 9th, 1992

Reference no.: 92-0998 / 92-0999 AB and 92-1073

Dear Prof. Christen,

I just got my manuscripts, reference no. 92-0998 / 92-0999 AB and 92-1073, together with the reviewers comments back from you (12/8 and 26/8/92). The editors responsible for the manuscripts came to the conclusion that the manuscripts cannot be accepted for publication in your Journal. The came to their conclusion because of the reviewers comments.

Before discussing these comments I give you some background informations to the manuscripts:

Until today, I am working almost 20 years in bioenergetics, especially on transport and ATP synthesis, and I was involved into almost all the discussions going on in this field of research during that time. Today, it seems clear to almost everyone how transport and ATP synthesis in mitochondria were performed - but not at all it really is. Therefore I may give you 3 examples out of the biochemistry book of L. Stryer (German, 1991):

1. The experiments of W. Stoeckenius and E. Racker (p. 427) are not clear at all.

2. The essential components of the mitochondrial ATP synthesizing complex (p. 430) are not known either.
3. The protons pumped by the respiratory chain are not appearing in the bulk phase and it is not clear how their energy is transformed into the γ -phosphat of ATP (p. 433).

A lot of real good work done in bioenergetics during the last 20 to 30 years has been neglected. See for instance the papers of Lardy et al, Painter and Hunter, Wieland and Bäuerlein, Hatefi and Hanstein. There are a lot more papers which have to be mentioned.
Also, a lot of logical manuscripts were teared up by reviewers looking in just one direction. I discussed with Hatefi more than 10 years ago the situation in bioenergetics. My conclusions:

1. Bioenergetics was already at this time in a dead end street.
2. Molecular biology is not able to resolve any problem in bioenergetics without the correct informations of biochemists (bioenergists).

<u>To the reviewers comments</u>

Some of the comments would not have been raised if the same reviewer would have reviewed all 3 manuscripts and if the different reviewers would have <u>read</u> the papers and their headings. Some of the comments were standard comments (referring to old publications, poor english, speculations, etc.) normally used to neglect papers for publication.

MS No. 92.0998 AB, Report 1:

Point 1. The reviewer did <u>not</u> read this MS. He should read for further informations also MS No. 92.0999 AB and 92.1073 AB.

Point 2. This point is explained in the 3 papers: NSPM reacts with the p_i/H^+ activated disulfides. The difference in the two inhibition curves (Fig. 1) is due to phosphate cycling and above all the preformed activated respectively residing/normal disulfide(s): NSPM inhibits much easier in the presence of phosphate, in contrast NSPM inhibition takes some time if phosphate has to activate first the disulfide(s). The reviewer should look also at the short reaction times!

→ MS I. Fig. 3

Pi-transport and ATP-synth.

This system remembers somehow on the well-known glutathione-S-transferases.

Point 3. The reviewer should discuss all the data given and not just one data picked out of a set of connected data. See also point 1. The concentrations of Triton, NSPM and DNP applied have to be related to the concentrations bound to the membrane, to the concentrations free extern and free intern: equilibrium by diffusion is with the data presented not at all possible!
I like to consider: mitochondria contain only a few percentage of phospholipides in contrast to phospholipide vesicles and data obtained on vesicles

are therefore not to compare with data obtained in mitochondria.

Point 4. See point 1: The reference for performance of the NPA experiments as well as the legend to the figure is given. I don't think, that it is necessary to describe well-known literature experiments again.

MS No. 92.0998 AB, Report 2:

For this reviewer applies the same as for reviewer 1: He should have read this manuscript as well as the other manuscripts. One more point has to be stressed: He did not even read the manuscript moreover he must have read another manuscript. I am not aware in using an "old fashioned swelling approach"?

MS No. 92.0999 AB, Report 1:

I did not know, that manuscripts have to be discussed on page numbers rather than essential new facts? The MS connects well-known literature data in a mechanism obtained out of new presented data and gives than more insight into the effects of a lot of compounds used in bioenergetics for some decades.

MS No. 92.0999 AB, Report 2:

For this report is essentially the same valid as for report 1. Glutathione and nonylthiouracil from no complex but a new chemically reactive compound. The method is essential for the MS and of interest and also of importance for "real" biochemists.

MS No. 92.1073 AB, Report 1:

The reviewer should read heading and content of the MS to see

that his comment does not fit: I don't need experiments for a hypothesis!

MS No. 92.1073 AB, Report 2:

My above mentioned comments fit for this reviewer as well. Are results not valid because they have reached a certain age? I wrote a hypothesis partly on neglected real good data and this hypothesis can be proven in future experiments!
This reviewer is the first out of the six reviewers criticising my English! I wrote the paragraph about the K-transport in the obviously criticised English with the intention to focuse the reader on this subject. I think "bad" English is by no means a reason to reject a paper as long as the reader is able to understand the messages. I remember that journals are normally taking care of "real poor" English.

I hope my comments are able to convince you and I send the manuscripts therefore back to you for reconsideration by your journal.

Yours sincerely

Dr. R. Kiehl

Enclosure:
6 Reports
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