

BIELEFELDER
ÖKOLOGISCHE
BEITRÄGE

Beiträge zur Ökologie und Geobotanik
und zu
Natur- und Umweltschutz

BAND 6 (1992)

**Beiträge aus dem workshop
“Stress on all levels”
(from the molecular to the
ecosystems level)**

**Abteilung Ökologie/Department Ecology
Universität Bielefeld**

Herausgegeben von Breckle, S.-W.

Bielefelder Ökologische Beiträge (BÖB) Band 6 (1992)

Nachdruck 2018

**Herausgegeben von der
Abteilung Ökologie
Fakultät für Biologie
Universität Bielefeld
Postfach 8640
D-4800 Bielefeld 1**

**Alle Rechte vorbehalten/Copyright
ISSN 0178-0698**

BIELEFELDER
ÖKOLOGISCHE
BEITRÄGE

Beiträge zur Ökologie und Geobotanik
und zu
Natur- und Umweltschutz

BAND 6 (1992)

**Beiträge aus dem workshop
“Stress on all levels”
(from the molecular to the
ecosystems level)**

Referat-Beiträge im Rahmen der Deutsch-Israelischen Begegnung
zwischen der George Wise Life Science Faculty / Tel Aviv und der Fakultät
für Biologie / Univ. Bielefeld im Rahmen der Universitäts-Partnerschaft am
2. und 3. Juli 1992 im ZiF in Bielefeld.

Contributions from the workshop held July 2nd and 3rd, 1992 at ZiF /
University of Bielefeld within the German-Israeli-Cooperation between the
Universities of Tel Aviv and Bielefeld

**Abteilung Ökologie/Department Ecology
Universität Bielefeld**

Herausgegeben von Breckle, S.-W.

ISSN 0178-0698

Content / Inhaltsübersicht

Beiträge aus dem workshop "Stress – auf allen Ebenen. Stress-adaptations, from the molecular to the ecosystems level" (Hg.: Breckle, S.-W.) 118pp.
Contributions from the workshop „Stress on all Levels“, ZiF/Bielefeld 2.-3. July 1992

PreliminaryRemarks/Vorwort	1
OETKER, Maja: Welcome Address	3
RON, Eliora: Adaptation of bacteria to elevated temperatures . .	7
 AHARANOVITZ, Yair et al.: Low molecular weight thiols and a broad range disulfide reductase system in <i>Streptomyces</i> .	11
HAGEMeyer, Jürgen: Stress of toxic trace elements and effects on tree growth	15
ESHEL, Amram: Response of plant roots to water and oxygen stress 23	
 WAISEL, Yoav: How to cope with a low CO ₂ stress ? A new interpretation of an old observation	33
BRECKLE, Siegmund-W.: Salinity-stress and salt excretion in plants	39
SHKOLNIK, Amiram: The black Bedouin goat (Being productive in extreme environments)	53
 AR, Amos: Physiological adaptations to underground life: Reptilian embryos, tunnel nesting birds and fossorial mammals as examples	61
TEUCHERT-NOODT, Gertrud: Stress-induced alterations in both brain functioning and brain structure: Stress and the mesocorticolimbic dopamine system	69
 DAWIRS, Ralph R.: Stress and synaptic rearrangement: a mutual principle of adaptation to normal and aberrant stimuli	71
ZIMMERMANN, Elke & Christiane STOCK: Stress adaptations to moderate altitude conditions. I. Sympathetic activity and metabolic responses	81
 STOCK, Christiane & Elke ZIMMERMANN: Stress adaptation to moderate altitude conditions. II. Effect on blood lymphocytes and β -adrenergic receptors	87
 WÄGELE, Johann W.: Cold-water stress, evolution and adaptation of polar isopod crustaceans	93
TRILLMICH, Fritz: El Niño: Effects of environmental stress on pinnipeds	103
HENDRICHS, Hubert: On social stress in mammals	105
BRECKLE, S.-W., R. MANNESMANN & Y. WAISEL: Conclusions - what is stress?	111
WAISEL, Yoav: Epilogue and Future	115
Kurzbericht....	117

Preliminary Remarks / Vorwort

The "Bielefelder Ökologische Beiträge" is a series, which is published somewhat irregularly, but especially focussing on distinct subjects. The workshop on "stress - on all levels (from the molecular level to the ecosystems level)" which was held in beginning of July in the ZiF (center for interdisciplinary research) in Bielefeld as the first joint seminar within the partnership of the Faculty of Biology from the Bielefeld University and the George Wise Life Sciences Faculty from Tel Aviv University was an excellent opportunity to meet and to discuss this extremely complex subject, as it turned out during the workshop. Thus, it is very well suited to publish the papers altogether in that volume.

It is obvious that the scientific language and the applied methods for research differ strikingly between somebody looking for heat-stress in bacteria and synthesis of heatshock-proteins, and somebody else looking for mammals under social stress or even for human beings under altitude stress.

Stress is a very common phenomenon. It influences apparently all organisms. It is some kind of motor for evolution. It is also some kind of adaptive force during ontogenesis. LEVITT (1980) has given a comprehensive monograph on stress in plants, but he has only taken into account **abiotic factors** from the environment. This is a good base for a general discussion. In this respect every organism has to suffer or to adapt always to various different stress-factors. The normal temperature range is comfortable. Each deviation from normal - too cold (cold-stress, frost-stress) or too hot (heat-stress) causes stress. The same is true for water availability (drought-stress, flooding stress), for radiation, for mechanical or chemical factors.

The **biotic stress factors** are more complex. They include competitors, predators, parasites, bacterial infections, induction of flowers and fruiting by external factors etc.

Apparently in almost all cases in multicellular organisms stress causes some kind of change of the hormonal equilibrium. This seems to be the case in plants and in animals, despite the fact that the hormonal systems differ totally.

It was a great pleasure for all participants to have this meeting. We all feel that it was very successful. May this volume (BÖB 6) help to widen not only the interdisciplinary views, but also strengthen friendships and future cooperation on many levels, in an optimal way under **moderate stress**.

(LEVITT, J. 1980 Responses of plants to environmental stresses, vol. I,II, 497, 606pp.; Acad. Press NY, London)



**Empfang für die Teilnehmer des gemeinsamen workshops
im Rahmen der Begegnung zwischen
der Fakultät für Biologie der Universität Bielefeld
und der George Wise Life Sciences Faculty
der Tel Aviv University**

Freitag, 3. Juli 1992, 19.00 Uhr
Altes Rathaus, Kleiner Sitzungssaal

Frau Bürgermeisterin Maja OETKER :

Sehr geehrter Herr Prof. Breckle, meine sehr geehrten Damen und Herren, liebe Gäste aus Israel,

als Bürgermeisterin der Stadt Bielefeld heiße ich Sie sehr herzlich in unserer Stadt willkommen!

Ich weiß, daß Sie schon arbeitsreiche Tage hinter sich haben, in deren Verlauf Sie sich mit dem Thema "Stress" auf den verschiedensten Ebenen beschäftigt haben. Daneben sind aber, so hoffe ich, die privaten Kontakte und freundschaftlichen Beziehungen zwischen Ihnen nicht zu kurz gekommen. Ich weiß aber auch, daß morgen das wissenschaftliche Thema Ihrer Begegnung in den Hintergrund tritt und eine Stadtbesichtigung mit Einkaufsbummel auf dem Programm steht. So möchte ich denn diese Gelegenheit, sozusagen als Einstimmung, dazu nutzen, Ihnen die gastgebende und - wie ich hoffe - gastfreundliche Stadt Bielefeld ein wenig vorzustellen.

Bielefeld ist mit seinen rund 320.000 Einwohnern die Metropole der Region Ostwestfalen-Lippe, in der nahezu 2 Millionen Menschen leben. Mit einer Fläche von rund 260 Quadratkilometern ist sie darüberhinaus eine der größten Flächenstädte der Bundesrepublik Deutschland. Nach der deutschen Einheit ist Bielefeld aber auch geographisch in den Schnittpunkt der neuen Bundesrepublik gerückt.

Bekanntgeworden ist Bielefeld vor allem durch seine wirtschaftliche Entwicklung, die eng mit dem Leinen verbunden ist. Noch heute spricht man manchmal von "Bielefeld der Leinenstadt". So zieht denn auch durch die Jahrhunderte unserer Wirtschaftsgeschichte der sprichwörtlich gewordenen Leinenfaden. Erinnerungen daran sind im Leineweber-Brunnen in unserer Altstadt - Sie werden ihn sicherlich morgen sehen - und in unserem Bauernhausmuseum dokumentiert. Zu Beginn des vorigen Jahrhunderts entwickelten sich dann parallel zur industriellen Leinenindustrie das textilverarbeitende Gewerbe, der Maschinenbau, die Druck- und Papierindustrie sowie die Nahrungsmittelindustrie.

So sichert heute eine mittelständische breitgefächerte Wirtschaftsstruktur aus Industrie und Handwerk der Bielefelder Bevölkerung eine über dem Landesdurchschnitt liegende Beschäftigungsrate. In Bielefeld und in der umliegenden Region sind darüberhinaus Weltunternehmen, wie die Firmen Granini, Ostmann-Gewürze, Nixdorf, Miele, Oetker und Bertelsmann, um nur einige aufzuzählen, beheimatet.

Bielefeld ist aber auch, wie es ein hiesiger Redakteur einmal formulierte, "ein starkes Stück Kultur". So haben sich die Kunsthalle mit ihren Ausstellungen und die Städtischen Bühnen mit ihren Inszenierungen national und international einen guten Namen erworben.

Ganz charakteristisch für unsere Stadt ist der Teutoburger Wald, der sich mitten durch das Stadtgebiet zieht und damit Bielefeld auch die Umschreibung "Stadt im Grünen" gebracht hat. "Stadt der Bäder" oder "Stadt der Schulen" sind weitere gern genutzte Definitionen, wenn von Bielefeld die Rede ist.

Bielefeld ist auch eine weltoffene Stadt und pflegt Partnerschaften mit sechs europäischen Städten: Rochdale in England, Concarneau in Frankreich, Enniskillen in Nordirland, Novgorod in Rußland, Rzeszow in Polen und letztlich Naharya in Israel.

Darüberhinaus sind es aber vor allem auch die vielen, unzähligen Kontakte und Freundschaften von Vereinen, Verbänden und Institutionen, die oft internationale Gäste nach Bielefeld führen. Ihre Begegnung ist dafür das beste Beispiel.

Letztlich möchte ich Sie auch noch mit den Von-Bodelschwingh'schen Anstalten in Bethel bekannt machen. Denn eine Stadt ist nicht nur geprägt von Kultur, Freizeit und Arbeit, sondern auch von der sozialen Infrastruktur, von dem Umgang der Menschen miteinander. Die Von-Bodelschwingh'schen Anstalten sind eine Einrichtung für Behinderte und nicht-seßhafte Menschen. Bethel ist ein eigener ganzer Stadtteil für sich, mit Kliniken, Langzeiteinrichtungen, Schulen, Werkstätten, Geschäften, Privathäusern und Freizeitangeboten. Tausende von Behinderten und Nichtbehinderten leben und arbeiten gemeinsam hier.

Meine Damen und Herren, vieles könnte ich Ihnen noch über unsere Stadt erzählen. Ich denke aber, daß es jetzt an der Zeit ist, ein wenig für unser leibliches Wohl zu sorgen und ein Glas auf den Erfolg Ihrer Begegnung, die ich auch als Teil der guten und vielfältigen deutsch-israelischen Beziehungen werte, zu trinken.

In diesem Sinne wünsche ich den israelischen Gästen einen angenehmen Aufenthalt in Bielefeld und würde mich freuen, wenn Sie Ihren Besuch bei der Universität und in dieser Stadt in angenehmer Erinnerung behalten.

Zum Wohl!

Ladies and Gentlemen, Dear Guests from Israel!

As the mayor of Bielefeld, I would like to welcome you in our town!

I know that the last days were quite busy for you and you were engaged in the subject of "stress" in various ways. But I hope that private contacts and friendly relationships have not been neglected.

I also hope that tomorrow the scientific subject will be dismissed into the background, and your program schedules sightseeing and shopping. I would like to take this opportunity, then, to introduce the hosting and - as I hope - hospitable city of Bielefeld.

With about 320 thousand citizens, Bielefeld is the metropolis of the Eastern Westfalia-Lippe region where nearly 2 million people live. The area of 260 squarekilometres make it one of the widest expanded cities in the federal republic of Germany. After the German unity, Bielefeld also moved into the intersection of te new federal republic.

Economic development which is closely connected to the "linen", made Bielefeld famous. Even today, you sometimes talk about Bielefeld as the "city of linen". There is no surprise that through the centuries the economic history the linen yarn is literally drawn. Reminders are the "Leineweber-Brunnen" (linenweaver-fountain) in our medieval center - I'm sure you will see it tomorrow - and the "Bauernhausmuseum" (museum of medieval farmhouses). At the beginning of the last century, he parallel development to the industrial linenweaving was the textile industry, mechanical engineering, print and paper industries, as well as the food industry. A wide-spread middle-class economic structure of industry and trade is the security of Bielefeld's citizens to have an employment rate which is above the country's average. Bielefeld and the neighbouring regions are home to world-wide companies like Granini-fruit-juices, Ostmann spices, Nixdorf computers, Miele, Oetker, and Bertelsmann.

But Bielefeld is also, as a local editor once said, " a strong part of culture". The "Kunsthalle" (hall of art) with its exhibitions and the local theaters with their plays have a national and international good reputation.

The characteristic of our city is the "Teutoburger Wald", a mountain ridge which draws straight through the center of the urban area and gives Bielefeld also the name: "City in the Green". "City of Baths" and "City of Schools" are further definitions when people refer to Bielefeld.

Bielefeld is also a worldly city and has partnerships with six European cities: Rochdale in England, Concarneau in France, Enniskillen in Northern Ireland, Novgorod in Russia, Rzeszow in Poland, and finally Nahariya in Israel.

Beyond this, many uncountable contacts and friendships of clubs, federations, and institutions bring international guests to Bielefeld. Your meeting is the best example.

Finally I would like to introduce the "Von Bodelschwingh'sche Institutions" in Bethel, because a city is not only coined by culture, recreation-facilities and work, but also by the social infrastructure and the relations between the people. The "Von Bodelschwingh'sche Institutions" are taking care of disabled and homeless people. Bethel is a seperate part of the city with clinics, long-term institutions, schools, workshops, shops, private homes, and offers recreation-grounds for spare-time. Thousands of healthy and disabled people live and work there together.

Ladies and gentlemen, there is still a lot more I could tell you about our city. But I think it's time to care about the physical well-being, and to raise a glass on the accomplishments of your meeting which I would rate as part of the various and good German-Israeli relationship.

In this sense, I wish the Israeli guests a pleasant stay in Bielefeld, and would be glad if you pleasantly remember this visit at the University and in this city.

Cheers! - Lechaim!

(transl. by R.Breckle)

Adaptation of Bacteria to Elevated Temperatures

Eliora Z. RON*

Department of Microbiology, The George S. Wise Faculty of Life Sciences,
Tel-Aviv University, Tel-Aviv, Israel

*This paper is based on experiments performed by Sari ALAJEM, Dvora BIRAN
and Nili GROSSMAN

Bacteria can be divided into three groups on the basis of their temperature of growth - one group grows in moderate temperature and two groups grow at extreme conditions. The three groups are:

Thermophilic bacteria - these grow at high temperatures.

Their growth optimum is higher than 40°C (usually around 45°C) and they tolerate very high temperatures. Bacteria of this group can be found in hot springs with temperatures as high as 80°C.

Psychrophilic bacteria - grow at very low temperature and can

be found in ice and frozen soil. These are responsible for many problems with storage of food.

Mesophilic bacteria - grow with a temperature optimum of 30°C

- 37°C. These constitute most of the bacteria that are around us - including the bacteria that are associated, in one way or another, with mammalian hosts.

In order to survive and multiply at a given temperature, the bacteria are composed of enzymes that can function in the range of temperatures which constitute the natural habitat. For example - thermophilic bacteria contain enzymes that are extremely heat stable. Examples of these are the proteases and lipases that are used in laundry, as bio-detergents, and are active above 40°C. Another example is the TAC-polymerase that is used to replicate DNA in for PCR (polynucleotide chain reaction).

For each group of bacteria there is a range of about 20°C around the optimal growth temperature in which the bacteria can multiply. For mesophilic bacteria this range is largely between 10°C and 45°C. If we take *Escherichia coli* as an example, it is killed at temperatures higher than 48°C, and can multiply at a reasonably fast rate between 18°C and 43°C. Within this range of temperatures, a shift in temperature requires an adaptation process. This adaptation of microorganisms to temperature shifts is a complex regulatory process. It involves a fine and immediate adjustment of all the cellular networks to the new rates of activities of the various enzymes.

When the shift is to a higher temperature the adaptation of mesophilic bacteria - such as *Escherichia coli* - involves at least two distinct control mechanisms. One mechanism is designed to take care of the change in temperature, and is the "heat shock response", the other control mechanism is a maintenance control - it is active as long as the bacteria are exposed to the

higher temperature. I will discuss these two control mechanisms, and also show that there is a molecular - biochemical link between them.

The heat shock response occurs when cells are shifted to a higher growth temperature. It is a response to the change in temperature, and therefore occurs even if the shift is to a relatively low temperature (18 to 32°C, for example). In *E. coli*, for example, the heat shock response involves the induction of a large number of genes - about 17. This response is not specific to temperature, and is actually a stress response, as it is induced by environmental factors such as exposure to ethanol, to peroxides or to heavy metals (cadmium). All of these factors induce the whole group of heat shock proteins (HSP) with very few variations (for review see ref. 6 and 7).

There are several interesting features in this global control system called "heat shock response", and I would like to stress several of them:

1. The heat shock response is universal - its existence has been demonstrated in all the living organisms that have been examined, plants, animals and microorganisms.
2. The induction is brought up by similar environmental factors in all the organisms.
3. The factors that induce heat shock response denature proteins.
4. The heat shock response is transient - the rate of synthesis of the induced proteins decreases after a short time period (several minutes in bacteria). Following a shift in temperature, the cellular concentration of the HSP is stabilized at a value typical of the new temperature (3,12).
5. In all the systems similar kinds of proteins are induced.
6. The HSP include proteins that are important for correct folding of other proteins - "chaperons" (2).
7. Several of the HSP, mainly the chaperons, show an extremely high degree of homology in many organisms. For example - DnaK of *E. coli* shows is homologous to the eukaryotic family of HSP70 proteins.
8. The increase in the synthesis of HSPs is controlled at the level of transcription, in prokaryotes and eukaryotes.

How is the stress response operating? How do the various stress conditions activate the same group of proteins? The analysis of the stress response at the transcriptional level was easier in prokaryotes, where in *E. coli* the following principles were established:

- a. All the genes coding for HSPs contain in their upstream regions specific sequences that act as heat shock promoters (1).
- b. These heat shock promoters are recognized by a specific heat shock sigma factor - sigma-32 (molecular weight 32 kd) - that binds to core RNA polymerase and transcribes the heat shock genes. The cellular concentration and activity of sigma-32 increase at higher temperatures, resulting in its preferential binding to core RNA polymerase under these conditions (5,13).

The eukaryotic equivalents of sigma-32 have recently been discovered. These are called Heat Shock Factors whose DNA binding sites are contiguous arrays of the sequence nGAAn arranged in alternating orientations (11).

The heat shock response is presumably essential for acquiring resistance to environmental stress. It has been shown to be important for thermotolerance.

In mesophilic bacteria there is another control mechanism that is important at high temperatures - this is a control mechanism that insures the maintenance of balanced growth when the temperature is elevated.

The existence of this second control mechanism was revealed when we studied growth of wild

type strains of *E. coli* as a function of temperature. When cells growing exponentially at 37°C in minimal (salt-glucose) medium, are shifted to higher temperatures (up to 44°C), the growth rate immediately assumes a new, lower value, characteristic of that temperature. This change in growth rate does not occur in medium containing amino acids, suggesting that there is a compound which is essential for growth, and its synthesis is temperature dependent - above 35°C its availability is limiting the growth of *E. coli*. From the fact that the growth was not limited in the rich medium could deduce that this compound was present in nutrient broth.

I would like to stress the point that we discuss here wild type *E. coli* and not a temperature-sensitive mutant, this is just any *E. coli*.

At this point it took a few steps to determine what this compound is, and it turned out to be the amino acid methionine. The synthesis of methionine is temperature dependent due to the extreme temperature-sensitivity of the first enzyme in the pathway - homoserine transsuccinylase (HTS) (4,9,10). Our conclusion was that cells regulate the growth rate at elevated temperatures by controlling methionine biosynthesis. This is probably an adaptation process designed to fit it with the overall metabolic balance that is changing as the temperature is elevated.

Regulating growth rate by the availability of methionine should be very effective, as methionine is a key amino acid - in its absence protein synthesis stops at the level of initiation, DNA replication stops at initiation and RNA synthesis stops by stringent control, which also inhibits cell wall formation. In addition, limitation in the availability of methionine will affect the transfer of C1 fragments and all the biological reactions involving S-adenosylmethionine, such as the biosynthesis of spermidine and spermine.

If it is important to regulate the growth rate as a function of temperature, and if methionine biosynthesis is a good way to do it, we would expect that *E. coli* is not the only bacterium that knows the secret. Indeed, the control of growth rate by the temperature sensitivity of HTS is a general property of enteric bacteria (8).

As mentioned earlier, the heat shock response in *E. coli* involves the induction of 17 new proteins - the heat shock proteins. The need to synthesize a large number of new proteins in response to a shift to high temperatures may present a problem, since under these conditions the cells are severely limited for methionine. One way to ease the limitation for methionine would be to increase the cellular levels of HTS as a function of temperature. The higher enzyme concentration could, then, compensate for the reduced activity.

Indeed, when we measured the level of HTS after a shift to 42°C we learned that there was an increase of about 5 folds. This increase could also be seen after the addition of ethanol, another inducer of the heat shock response and did not occur in *htpR* mutants that are defective in sigma-32. The induction was specific to HTS, and did not increase the level of another enzyme in the methionine biosynthetic pathway - beta cystathionase.

From these results it is clear that the synthesis of HTS is regulated in coordination with the synthesis of HSP itself, and is dependent on the activity of sigma-32. Further experiments, involving direct *in vitro* transcription of the *metA* gene by sigma-32 are required in order to find out whether HTS is a heat shock protein, or whether its activity is heavily dependent on the induction of other HSPs or chaperons.

To summarize, we have studied mechanisms by which *E. coli* - and probably other mesophilic bacteria - control growth at elevated temperatures. One mechanism is the heat shock response that is a transient response, induced by the shift in temperature and confers thermotolerance. The other mechanism is designed to maintain a balanced growth as long as the bacteria stay in the new temperature. The two mechanisms are connected by the first enzyme in the biosynthesis of methionine. This enzyme is probably the most temperature-sensitive biosynthetic enzyme in mesophilic bacteria, and it is induced concurrently with the heat shock proteins.

References

1. COWING, D.W., BARDWELL, J.C.A., CRAIG, E.A., WOLLFORD, C., HENDRIX, R.W. and GROSS, C.A. 1985. Consensus sequence for *Escherichia coli* heat shock promoters. Proc. Natl. Acad. Sci. USA 82:697-2683.
2. CREIGHTON, T.E. 1988. Toward a better understanding of protein folding pathways. Proc. Natl. Acad. Sci. USA 85:5082-5086.
3. LEMAUX, P.G., HERENDEEN, P.I., BLOCH, P.L. and NEIDHARDT, F.C. 1978. Transient rates of synthesis of individual peptides in *Escherichia coli* temperature shifts. Cell 13:427-434.
4. MICHAELI, S., MEVARECH, M. and RON, E.Z. 1984. Regulatory region of the *metA* gene of *Escherichia coli* K-12. J. Bacteriol. 160:1158-1162.
5. NEIDHARDT, F.C. and VANBOGELEN, R.A. 1981. Positive regulatory gene for temperature- controlled patterns in *Escherichia coli*. Biochem. Biophys. Res. Commun. 100: 894-900.
6. NEIDHARDT, F.C. and R.A. VANBOGELEN. 1987. Heat Shock Response. In: F.C. NEIDHARDT, J.I. INGRAHAM, K.B. LOW, B. MAGASANICK, M. SCHAECHTER and H.E. UMBARGER. (eds.) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. Vol. 2. p. 1334-1343. American Society of Microbiology, Washington, D.C.
7. NEIDHARDT, F.C., VANBOGELEN, R., and VAUGHN, V. 1984. The genetics and regulation of heat shock proteins. Ann. Rev. Genet. 18:295-329.
8. RON, E.Z. 1975. Growth rate of *Enterobacteriaceae* at elevated temperatures: limitation by methionine. J. Bacteriol. 124:243-246.
9. RON, E.Z. and DAVIS, B.D. 1971. Growth rate of *Escherichia coli* at elevated temperatures: limitation by methionine. J. Bacteriol. 107:391-396.
10. RON, E.Z. and SHANI, M. 1971. Growth rate of *Escherichia coli* at elevated temperatures: reversible inhibition of homoserine trans-succinylase. J. Bacteriol. 107: 397-400.
11. SORGER, P.K. 1991. Heat shock factor and the heat response. Cell 65:363-366.
12. YAMAMORI, T., ITO, R., NAKAMURA, Y. and YURA, T. 1978. Transient regulation of protein synthesis in *Escherichia coli* upon shift-up of growth temperature. J. Bacteriol. 134:1133-1140.
13. YURA, T., TOBE, T., ITO, K. and OSAWA, T. 1984. Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperatures but is dispensable at low temperature. Proc. Natl. Acad. Sci. U.S.A. 81:6803-6807.

Low Molecular Weight Thiols and a Broad Range Disulfide Reductase System in *Streptomyces*

Y. AHARONOWITZ¹, Y. AV-GAY¹, R. SCHREIBER¹, A. ARGAMAN¹, R. C. FAHEY²,
G. L. NEWTON², and G. COHEN¹

¹ Tel Aviv University, the George S. Wise Faculty of Life Sciences, Department of Molecular Microbiology and Biotechnology, Tel Aviv, 69978, Israel.

² University of California San Diego, Department of Chemistry, La Jolla, California 92014, USA

Regulation of cellular processes is frequently associated with chemical modification of macromolecules. A major class of such reactions are the reversible covalent modifications of proteins such as phosphorylation-dephosphorylation and adenylation-deadenylation reactions (1). Little is known about potential regulatory mechanisms involving thiol-disulfide interchanges, but numerous examples have been documented showing that the biological activity of a protein can be modulated through formation of a mixed disulfide of a protein thiol group and a low molecular weight thiol (2). The extent of thiol-disulfide interchange reactions need not be limited to individual proteins, but could in fact encompass multifunctional biochemical processes such as cell differentiation and sporulation (3). In general, these reactions are coupled to the glutathione-glutathione disulfide status of the cell which, in turn, is governed by enzymatic oxidoreductase systems.

The most prevalent intracellular thiol is the tripeptide glutathione (L- γ -glutamyl-L-cysteinyl-glycine). Glutathione is known to function directly or indirectly in many important biological processes, including reductive reactions such as those involved in the conversion of ribonucleotides to deoxyribonucleotide precursors of DNA, the reversible reduction of disulfide bonds of proteins and other low molecular weight compounds and the destruction of peroxides and free radicals (4). These reactions lead to the formation of oxidized glutathione (disulfide), GSSG, which is converted by glutathione reductase back to reduced glutathione, GSH. Under normal physiological conditions glutathione reductase maintains the GSH/GSSG ratio at a value near to or in excess of 100:1 and ensures an environment in which the essential sulfhydryl groups of key enzymes and coenzymes are protected (3).

The development of new techniques by R. C. FAHEY and his coworkers (Fig. 1) for the analysis of low molecular weight thiols (5) has led to a systematic study of these compounds in a wide variety of organisms. Glutathione was found to be present in all fungal and animal tissues examined. Many bacteria, however, including some strict aerobes, lack GSH and contain other low molecular weight thiols. In some of these bacteria (such as *Bacillus megaterium*),

Coenzyme A appears to function as the main thiol (6). Indeed, in analogy to glutathione reductase activity, Coenzyme A disulfide reductase activity is found in extracts of *Bacillus* species (7). Among those microorganisms that lack glutathione an isolate of *Streptomyces griseus*, a gram-positive soil actinomycete, was identified (8).

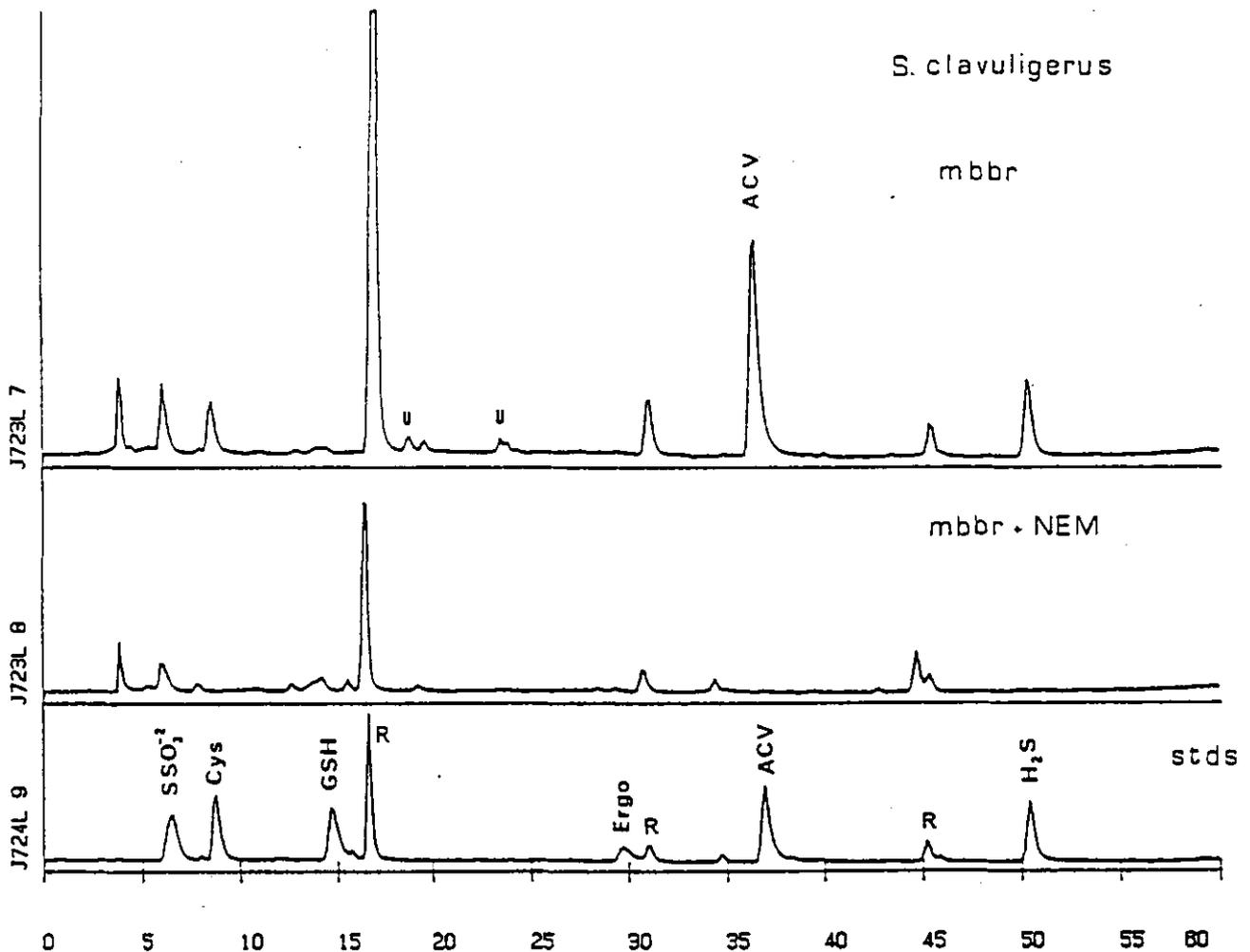


Figure 1. HPLC chromatograms obtained using the method developed by FAHEY and NEWTON (5). Cell samples were prepared from 19 hr cultures of *S. clavuligerus*: Upper chromatogram, cell extracts derivatized directly with mBBr to convert all thiols fluorescent thiol-mb derivatives; middle chromatogram, cell extracts reacted with N-ethylmaleimide (NEM) to block thiols prior to reaction with mBBr in order to identify fluorescent nonthiol components; lower chromatogram, a known mixture of thiol-mb standards. Abbreviations are as follows: ACV: δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine; Cys: cysteine; Ergo: ergothionine; GSH: glutathione; R: mBBr derived reagent peak; U: unknown thiol.

The principal thiol found in this organism did not correspond to any of the known thiols thus far characterized. We have recently analyzed the intracellular low molecular weight thiols in four Gram-positive *Streptomyces* and one related *Nocardia* species, by HPLC after fluorescent-labeling with monobromobimane (Table 1).

Table 1. Thiols in different streptomycetes and in *E.coli* (μ mole per g residual dry weight)

Organism	A650	Cys	ACV	GSH	CoA
<i>S.clavuligerus</i>	4.5	0.69	0.95	<0.01	0.65
<i>S.jumonjinensis</i>	2.0	0.71	0.70	<0.09	0.70
<i>S.lividans</i>	3.4	0.58	<0.016	<0.06	1.00
<i>E.coli</i> B	--	0.10	n.d.	24.00	n.d.

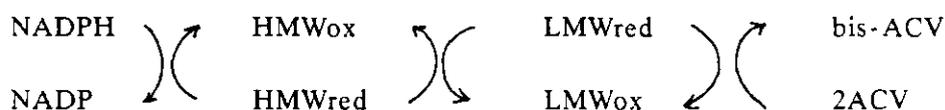
Bacteria were chosen to include penicillin and cephalosporin β -lactam producers and nonproducers. No significant amounts of glutathione (GSH) were found in any of the streptomycetes. The major intracellular thiols in all strains were an unidentified cysteine containing thiol U17, cysteine, coenzyme A, sulfide, and thiosulfate. Significantly, U17 occurred at millimolar levels. Those streptomycetes that produce β -lactam antibiotics make significant amounts of δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine (ACV) a key intermediate in their biosynthesis. A high level of ergothioneine was also found in *Nocardia lactamdurans* and several unidentified thiols were detected in this and other streptomycetes.

Our results raise several intriguing questions concerning the nature and the role of low molecular weight thiols different from glutathione. Since glutathione is absent in cells of gram-positive bacteria then what is the major thiol compounds occurring in these bacteria? and what biological function do they specify, and what is the nature of the oxidoreductase systems that determines their redox state? In the following we summarize recent studies which address some of these questions. The bacteria used in this study is a β -lactam producing strain, *Streptomyces clavuligerus*.

We have search for and identified a disulfide reductase system in *S. clavuligerus* that able to reduce the oxidized, disulfide, form of ACV. We reasoned that since oxidized bis-ACV is not a substrate for isopenicillin N synthase (IPNS), *S. clavuligerus* may possess a disulfide reductase that maintains ACV in its reduced state. We tested glutathione reductases from several different sources for their ability to reduce bis-ACV. None of the commercially available enzymes recognize ACV as a substrate, even though GSSG is reduced very effectively. Crude mycelial extracts of *S. clavuligerus* possess significant amounts of a bis-ACV and GSSG reductase activity. Significantly, its activity was lost after dialysis or after fractionation by gel chromatography. Attempts to recover reducing activity revealed that full activity could be restored only by combining a high molecular weight (HMW) fraction (>30 kd) with a low molecular weight (LMW) fraction of about 12-14 kd. Our results show that *S. clavuligerus* contains a potent thiol-reducing activity that is quite different from any of the known eucaryotic and gram-negative bacterial glutathione reductases. Whereas the *S. clavuligerus* reductase is able to efficiently reduce GSSG, structurally it is unlike that of the glutathione reductases. The latter are usually dimers with a molecular weight of about 150 kd. They possess a disulfide bridge in each subunit which is alternately oxidized and reduced as part of its catalytic mechanism without any further requirement for a low molecular weight protein (9).

Furthermore, in contrast to glutathione reductase the *S. clavuligerus* ACV disulfide reductase has a broad substrate specificity and is a potent reductant of disulfides in proteins as well as low molecular weight disulfides.

The structural and functional properties of the *S. clavuligerus* ACV reducing system bear a strong resemblance to the thioredoxin-thioredoxin reductase system. Thioredoxin and glutathione serve as alternate sources of electrons for a variety of enzymes (4). Thioredoxin is a small, ubiquitous, protein which contains in its active center a disulfide/thiol moiety used for oxidation-reduction. The protein exists either in the reduced form $-(SH)_2$ or in the oxidized form $-S_2$. Thioredoxin participates in numerous redox reactions through the reversible oxidation of its active center and catalyzes dithiol-disulfide exchange reactions. Thioredoxin- S_2 is generally reduced by NADPH and a flavoprotein thioredoxin reductase (a dimer protein of molecular weight 70kd). The dithiol protein, thioredoxin, and the associated enzyme thioredoxin reductase, have been isolated from many procaryotic and eucaryotic sources (10). Thioredoxin is, for example, the hydrogen donor for a variety of reductive enzymes, a photosynthesis regulation factor, a general protein and peptide disulfide oxidoreductase, a subunit of phage T7 DNA polymerase and an essential component for assembly of filamentous phages (10). According to the above ideas, a plausible mechanism for bis-ACV reduction by the *S. clavuligerus*, disulfide reductase would be:



Further interest in the disulfide reductase system concerns its potential roles in key metabolic processes in cell growth and differentiation and in secondary metabolite production. We also wish to determine whether this reductase is prevalent among other gram-positive bacteria and if it is equivalent to the thioredoxin family of oxidoreductases.

References

1. KREBS, E.G., BEAVO, J.A., (1979). *Ann. Rev. Biochem.* 48: 923-959.
2. KOSOWER, N.S., KOSOWER, E.M., (1976). in *Glutathione: Metabolism and Function.* (ARIAS, I.M., JAKOBY, W.B., eds) pp.159-172, Raven Press, New York.
3. KOSOWER, N.S., KOSOWER, E.M., (1978). *Int. Rev. Cytol.* 54: 109-160.
4. HOLMGREN, A., (1985). *Ann. Rev. Biochem.* 54: 237-271.
5. FAHEY, R.C., NEWTON, G.L., (1987) *Methods Enzymol.* 143: 85-96.
6. SETLOW, B., SETLOW, P., (1977). *J. Bacteriol.* 132: 444-452.
7. SWERDLOW, R.D., SETLOW, P., (1983). *J. Bacteriol.* 153: 475-484.
8. FAHEY, R.C., BROWN, W.C., ADAMS, W.B., WORSHAM, M.B., (1978). *J. Bacteriol.* 133: 1126-1129.
9. WILLIAMS, C.H., Jr., (1976). in *The Enzymes* (BOYER, P.W., ed.) 13: 89-173, Acad. Press. London.
10. HOLMGREN, A., (1986). in: *Thioredoxin and Glutaredoxin Systems: Structure and Function.* (HOLMGREN, A., et al. eds), Raven Press, NY.
11. PERHAM, R.N., (1983) in *Frontiers in Biochemical and Biophysical Studies of Proteins and Membranes.* (LIU, T.Y., et al. eds), pp. 249-258, Elsevier, NY.
12. SUMMERS, A.O., (1986) *Ann. Rev. Microbiol.* 40: 607-634.

Stress of toxic trace elements and effects on tree growth

Jürgen Hagemeyer

Bielefeld University, Faculty of Biology, Department of Ecology,
P.O.Box 100131, D-4800 Bielefeld 1, Germany

Summary: Trace elements, like Cd, Pb or Zn, can cause stress in plants. This has been repeatedly shown for a large variety of plant species. The presented work focusses attention particularly on the life-form 'tree'. Such long-lived organisms are especially put at risk by steadily increasing concentrations of potentially toxic trace elements in their environment.

Trees and trace metals - some remarks

Before entering into a more detailed discussion of trace element stress, it seems useful to give some introductory remarks.

In the first place, it should be considered what we are dealing with. What is a tree? This seems to be a rather simple question. However, by extending our view beyond Europe into areas with a different climate, for instance into desert environments or arctic tundras, the term 'tree' takes on a much broader meaning. In our context, let us consider the tree from an ecological point of view, as a life-form. In the terminology of RAUNKIAER (1934) a tree is a woody plant with buds more than 25 cm above ground. Thus, it is a perennial plant that can survive above snow cover. Obviously, this definition refers to habitats with cold winters. Such a plant has the ability to grow very old and its life-span can cover ages. The discovery of more than 4600-year-old, live bristlecone pines in North America demonstrated the inherent potential for longevity of trees most impressively (FERGUSON 1968).

The strategy clearly offers some advantages, but has also notable disadvantages. Otherwise, trees would occupy almost all habitats and niches in the ecosystems.

The apparent advantages of the life strategy of a tree are: (a) a tall shoot ensures a good position in the competition for light, (b) a large root system to exploit extensive volumes of soil for water and nutrients, (c) large storage capacity to sustain periods of unfavourable conditions, (d) reproduction is necessary only with long intervals (K-strategy) and (e) longevity allows permanent occupation of a habitat.

On the other hand, the disadvantages of being a tree are: (a) the dependence on favourable environmental conditions during a long, unpredictable period of time, (b) Immobility for a long lifetime and, by that, the inability to migrate and avoid unfavourable conditions, (c) the possible exposure to hazardous situations for long periods (dose = concentration x time !) and (d) the accumulation of toxic substances, since there is no frequent and complete renewal of the plant body.

The following discussion will show, that these points have a particular significance for our topic: Trace element stress and tree growth. The term 'stress' has been used with different meanings. The broadest definition was proposed by LEVITT (1980): Stress is any environmental factor that is potentially unfavourable to the living organism.

In this sense, we are considering a special variety of stress factors. We may call them 'potentially toxic trace elements' (instead of the widely used, though rather unfortunate term 'heavy metals'). Such substances are characterized as follows: They are elements, that cause stress in organisms and usually occur in low concentrations (ppm or less). Adverse effects depend on the dose, i.e. the concentration and time of exposure. Examples are Cd, Pb, Hg, Zn, Cu, Al, As or Se. The list demonstrates the heterogeneity of this class of elements. We find included metals as well as non-metallic elements. From the physiological point of view the group contains essential plant nutrients as well as highly toxic elements without any known purpose in the plant. Therefore, we always have to consider the nature of the respective element, when we are dealing with effects on organisms.

What we want to find out is, whether trees in polluted habitats suffer from stress caused by certain potentially toxic trace elements. In a first step to approach this problem, the trace element concentrations in trees were determined, in order to get some information on the trace element burden of the plants.

Distribution of trace metals in tree stems

Trace element concentrations in stem wood of trees are used as an indicator for the long term exposure of the organism. The values are more stable during prolonged periods of time, than for example contents of leaves or bark. Such data can provide information about the average pollution level of a certain environment. In a number of studies, element concentrations in wood of different annual rings, i.e. of different age, were even used to obtain time sequences of trace element pollution in the past. However, interpretations of such analytical data in terms of exact pollution chronologies are questionable (HAGEMEYER & BRECKLE 1986, HAGEMEYER et al. 1992, HAGEMEYER, in press).

In the presented study, wood samples were obtained from mature, live beech and spruce trees with an increment borer. The cores were taken at breast height. Such radial cores were dissected into portions covering 5 or 10 annual growth rings. The material was dried and wet ashed under pressure with concentrated nitric acid. Element concentrations were measured with atomic absorption spectrophotometry (AAS).

The first example shows the radial distribution of Pb in a beech tree growing in a rural area near Bielefeld (Fig. 1). A distinct distribution pattern is displayed with lowest concentrations in the outermost and, thus, youngest annual rings. Peak concentrations are found in the stem center of about 1.5 ppm Pb ($= 7.2 \mu\text{mol kg}^{-1}$). Such values are in the natural range of Pb concentrations in beech wood. The site near Bielefeld is only slightly affected by trace element pollution.

As a second example, Pb concentrations in wood of a beech tree from a highly contaminated site are presented (Fig. 2). The tree grows in Stolberg, at the northern end of the Eifel, near the western border of Germany. This region is notorious for its severe trace element pollution. The wood concentrations of Pb are much higher than in the tree in Bielefeld, with peak levels up to 10 ppm ($= 48 \mu\text{mol kg}^{-1}$). Highest concentrations are found at the transition between sapwood and heartwood. Obviously, this physiological border has an effect on the element distribution in the tree trunk. The Pb contents show seasonal variations with highest levels in June during the season of vigorous growth. Such variations have also been observed in other trees and with a variety of different elements (HAGEMEYER et al. 1989, GLAVAC et al. 1990, HAGEMEYER et al. 1992).

Similar results were obtained with the element Zn. The radial distribution pattern of Zn concentrations in the wood of a beech tree from Bielefeld shows an even distribution in the outer part of the stem (Fig. 3). Towards the stem center Zn levels tend to increase. In this tree from a site with only low contamination, highest values are about 10 ppm Zn ($= 150 \mu\text{mol kg}^{-1}$).

Zinc concentrations in wood of a tree from a polluted site (Fig. 4) are considerably higher and reach values above 40 ppm ($= 610 \mu\text{mol kg}^{-1}$). Lowest contents are found in the region of the transition between sapwood and heartwood. Some seasonal variations are obvious, although they are less prominent than in the case of Pb.

These are merely some selected examples of trace element distributions in stem wood of trees. Differences in element contents of the wood depending on the site of growth are apparent. Therefore, the next step was an investigation of the differences in soil concentrations of the various forest sites.

Trace metals in soil and wood

In order to investigate the relation between concentrations of Pb and Cd in stem wood and in the forest floor, soil samples were collected near the sampled trees. The soil was taken from the mineral layer in 10-20 cm depth. In this horizon many of the fine roots are growing. Trace element concentrations were determined in ammonium acetate extracts. This method gives a better indication of the plant available fraction of trace elements in the soil than the total contents.

A close correlation between concentrations of Pb in soil and in wood was found in the

than the total contents.

A close correlation between concentrations of Pb in soil and in wood was found in the data of 3 different sites in Northrhine-Westphalia (Fig. 5). In Stolberg, abandoned ore mines and metal processing industrial complexes cause severe trace element pollution. The levels of Pb in the soil are exceedingly high. This is well reflected by high Pb contents in the wood of the investigated spruce trees. In Schwaney pollution is only light to moderate and soil levels are in the natural range. Lowest Pb contents are observed in an uncontaminated, remote control site in Glindfeld.

Similar results were found with Cd in the same places. Again, the soil in Stolberg shows highest values with 4.2 ppm (= 37 $\mu\text{mol kg}^{-1}$) in an ammonium acetate extract. The total Cd content of the analyzed samples of the Stolberg soil is 34.5 ppm.

The described relations are observed in trees under field conditions. The question is: Do the trees suffer from trace element stress?

What are the lower limits of trace element concentrations causing stress in trees?

- An experimental approach

This problem required an experiment under controlled conditions. Young spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees were planted in soil with different levels of Cd and Zn (BRECKLE & HAGEMeyer 1992, HAGEMeyer et al. in press, HEPPEL et al. in prep.). The applied concentrations ranged from an unpolluted control up to very high, toxic levels. After 2 seasons of growth the widths of annual xylem rings in the stems produced in the first and in the second year of treatment were measured.

In the first year, the young spruce trees formed significantly narrower growth rings in a Cd concentration of 90 $\mu\text{mol kg}^{-1}$ soil dry weight (ammonium acetate extract), i.e. 10 ppm Cd (Fig. 7). A comparable effect was observed with about 1000 $\mu\text{mol Zn kg}^{-1}$, i.e. 65 ppm, in the substrate.

In the second year of the experiment, the effects of the trace elements on stem growth were even stronger (Fig. 8). Annual stem diameter increments were significantly narrower in 50 $\mu\text{mol Cd kg}^{-1}$, i.e. ca 6 ppm Cd in the soil.

Thus, some 6 ppm Cd in the soil caused significant growth reductions in young spruce. Such concentration levels were almost reached in a contaminated site in Stolberg, where 4.2 ppm Cd were actually determined (Fig. 6).

These comparisons between experimental data and field investigations show, that in some polluted places trace element levels can be high enough to reduce tree growth. Under such conditions trees probably suffer from trace element stress.

Conclusions and final comments

The outlined observations have an evident relation to the particular problems that a tree faces during its life: The tree cannot escape from an environment which becomes increasingly contaminated with toxic substances. Such an extremely long-lived organism is exposed to trace elements for a long time. The dose will increase with time, even if current concentration levels are still below the threshold of acute toxicity. Furthermore, toxic elements tend to accumulate in the soil as well as in the tree itself.

The presented results suggest, that levels of chronic toxicity will soon be reached in certain forest ecosystems (see also NEITE et al. 1992). The stress caused by some potentially toxic trace elements is already strong enough to cause growth reductions in trees.

This is, however, not an inevitable prospect for the future. We can do something to prevent it. We must (1) reduce emissions of potentially toxic trace elements and (2) reduce the emissions of gases causing acidification that results in mobilization of toxic minerals. This will reduce the stress of toxic trace elements.

Acknowledgements - The investigations were funded by the Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, Düsseldorf.

References

- BRECKLE, S.-W. & HAGEMEYER, J. 1992. Abschlußbericht des Forschungsvorhabens "Untersuchungen des Einflusses von Schwermetallen (insbes. Cd, Zn) auf die Kambiumaktivität mitteleuropäischer Waldbäume (Buche, Fichte)". - Forschungsberichte zum Forschungsprogramm des Landes NRW "Luftverunreinigungen und Waldschäden" Nr.19: 1-143.
- FERGUSON, C.W. 1968. Bristlecone pine: Science and esthetics.- Science 159: 839-846.
- GLAVAC, V., KOENIES, H. & EBBEN, U. 1990. Seasonal variations in mineral concentrations in the trunk xylem sap of beech (*Fagus sylvatica* L.) in a 42-year-old beech forest stand. - New Phytol. 116: 47-54.
- HAGEMEYER, J. Monitoring trace metal pollution with tree rings: a critical reassessment. - in: B. MARKERT (ed.) Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment. VCH-Publisher, Weinheim. in press.
- HAGEMEYER, J. & BRECKLE, S.-W. 1986. Cadmium in den Jahrringen von Eichen: Untersuchungen zur Aufstellung einer Chronologie der Immissionen. - Angewandte Botanik 60: 161-174.
- HAGEMEYER, J., KAMRADT, B., SCHÄFER, H., SCHLAGINTWEIT, K., VERLAGE, L. & BRECKLE, S.-W. 1989. Saisonale Schwankungen der Elementgehalte und Histologie des Kambiums von Buchenholz in Nordrhein-Westfalen. - AFZ 29-30: 769-771.
- HAGEMEYER, J., LÜLFSMANN, A., PERK, M. & BRECKLE, S.-W. 1992. Are there seasonal variations of trace element concentrations (Cd, Pb, Zn) in wood of *Fagus* trees in Germany? - Vegetatio 101: 55-63.
- HAGEMEYER, J., LOHRMANN, D. & BRECKLE, S.W. Development of annual xylem rings and shoot growth of young beech (*Fagus sylvatica* L.) grown in soil with various Cd and Zn levels. - Water Air Soil Pollut., in press.
- HEPPEL, T. 1991. Untersuchung des Holzzuwachses bei schwermetallbelasteten Jungfichten (*Picea abies* (L.) Karst.) im Topfversuch. - Dipl.-Thesis, Bielefeld University, 71pp.
- HEPPEL, T., HAGEMEYER, J. & BRECKLE, S.-W. Effects of Cd and Zn on the development of annual xylem rings of young Norway spruce (*Picea abies*) saplings. - in preparation.
- LEVITT, J. 1980. Responses of Plants to Environmental Stresses.- Academic Press, New York.
- NEITE, H., KAZDA, M. & PAULISSEN, D. 1992. Schwermetallgehalte in Waldböden Nordrhein-Westfalens - Klassifizierung und kartographische Auswertung. - Z. Pflanzenernähr. Bodenk. 155: 217-222.
- RAUNKIAER, C. 1934. The Life Forms of Plants and Statistical Plant Geography. - Oxford, Clarendon Press.
- SAHRHAGE, E. 1992. Schwermetall- (Pb, Cd) und Nährelementverteilung (Mg, K) im Stammholz der Fichte (*Picea abies* [L.] KARST.) und deren Bestimmung mit Hilfe der AAS. - Dipl.-Thesis, Bielefeld University, 105pp.

Fig. 1. Radial distribution of Pb in stem wood of a beech tree (*Fagus sylvatica*) growing near Bielefeld. The samples were taken at ca 1.5 m height in April 1989. Means \pm SD of 4 samples. From HAGEMEYER et al. (1992).

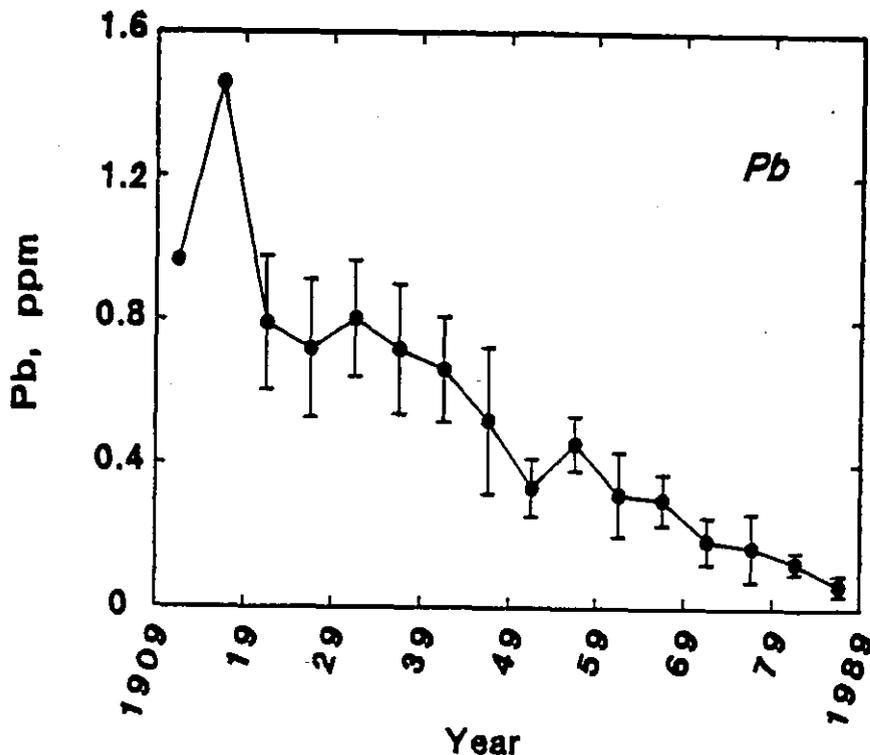


Fig. 2. Radial distribution of Pb in stem wood of a beech tree growing in Stolberg, Eifel. Samples were taken from the same tree in different months between April 1988 and January 1989. Sampling height was 1.5 m. The broken vertical lines denote the transition zone between sapwood and heartwood. After HAGEMEYER et al. (1992).

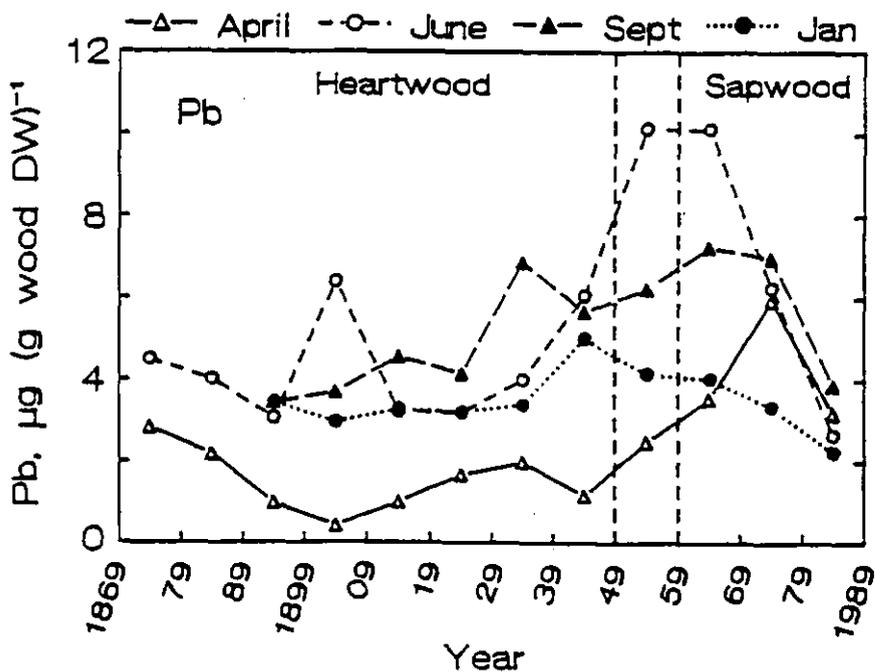


Fig. 3. Radial distribution of Zn in stem wood of a beech tree growing near Bielefeld (same tree as in Fig. 1). The samples were taken at ca 1.5 m height in April 1989. Means \pm SD of 4 samples. From HAGEMEYER et al. (1992).

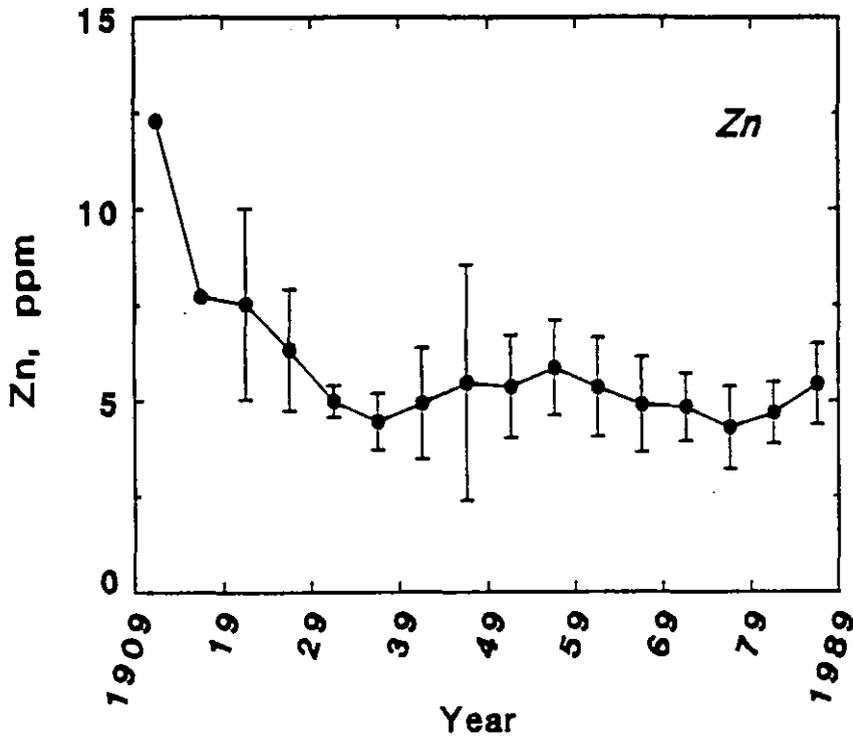


Fig. 4. Radial distribution of Zn in stem wood of a beech tree growing in Stolberg, Eifel (same tree as in Fig. 2). Samples were taken from the same tree in different months between April 1988 and January 1989. Sampling height was ca 1.5 m. The broken vertical lines denote the transition zone between sapwood and heartwood. After HAGEMEYER et al. (1992).

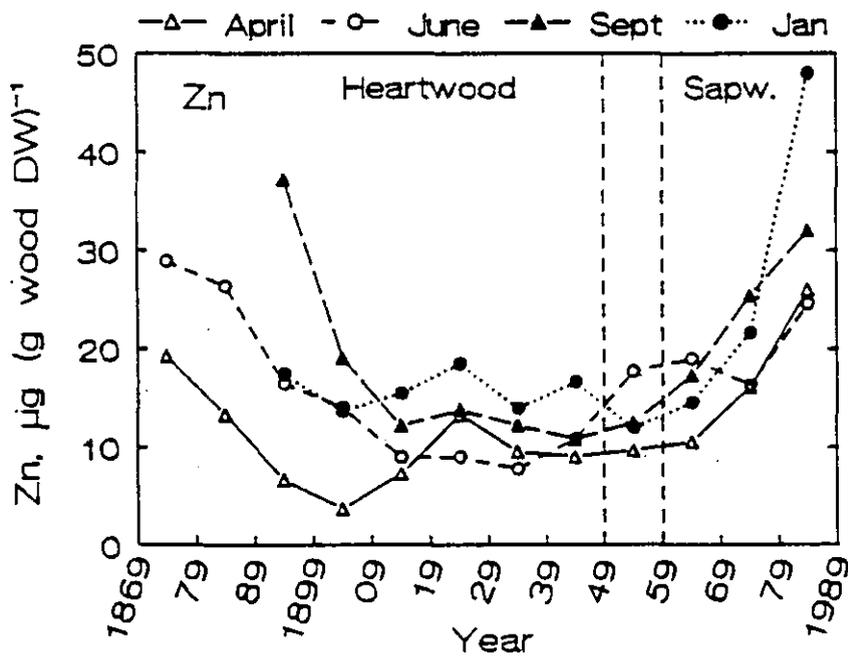


Fig. 5. Concentrations of Pb in soil and stem wood of spruce trees (*Picea abies*) growing on 3 different sites in Northrhine-Westphalia, Germany. Soil samples were taken from 10-20 cm depth (mineral layer) and extracted with 1 M ammonium acetate. Wood concentrations are means of samples taken from different stem heights and directions. Adapted from SAHRHAGE (1992) and BRECKLE & HAGEMeyer (1992).

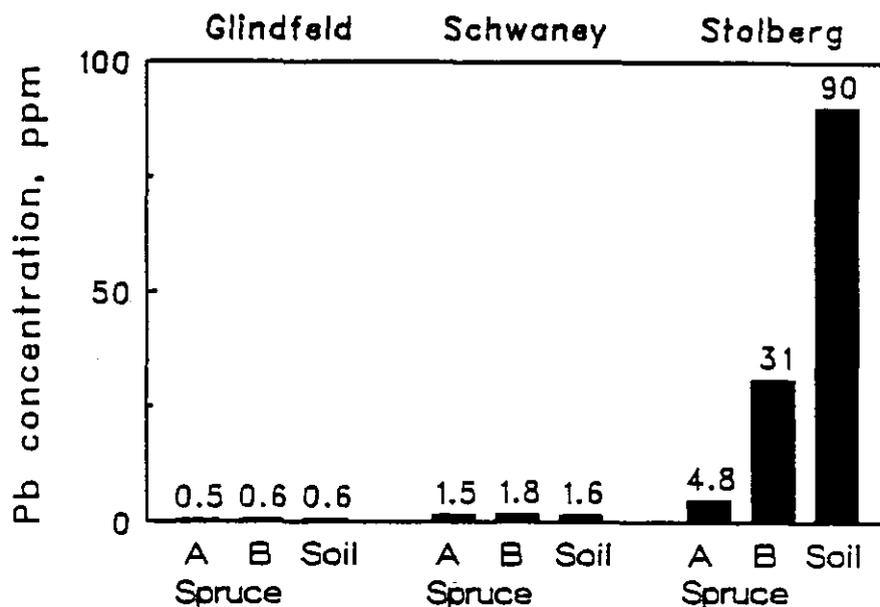


Fig. 6. Concentrations of Cd in soil and stem wood of spruce trees growing on 3 different sites in Northrhine-Westphalia, Germany (same trees as in Fig. 5). For further details see Fig. 5. Adapted from SAHRHAGE (1992) and BRECKLE & HAGEMeyer (1992).

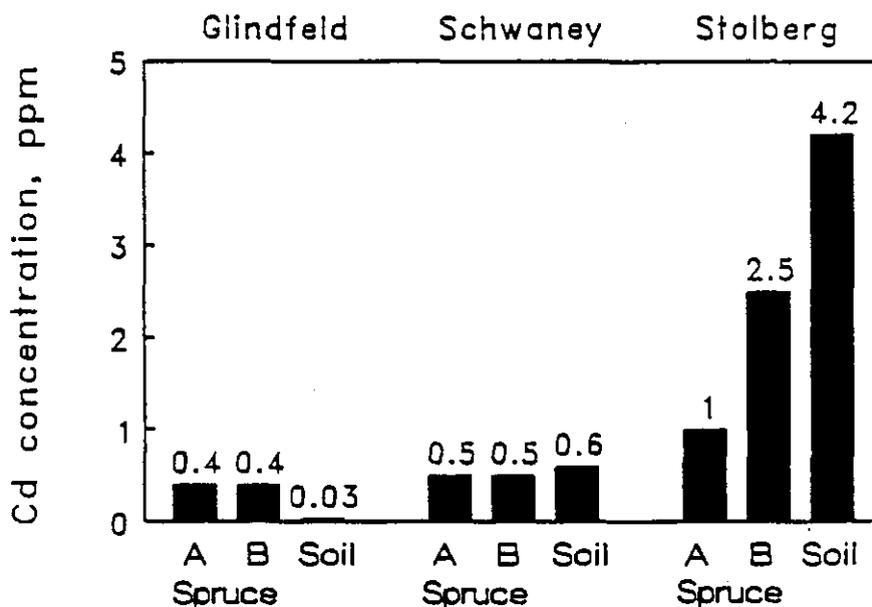


Fig. 7. Relative annual xylem diameter increments in stems of 4-year-old spruce trees produced in the first year of treatment with different concentrations of Cd and Zn in the soil. Diameter growth increments are given relative to the widths of the last xylem rings formed before the treatment. Means \pm SD of 13-46 plants. Levels of significance for difference to the control: - not significant, * $p < 0.05$, ** $p < 0.01$. After HEPPEL (1991) and BRECKLE & HAGEMEYER (1992).

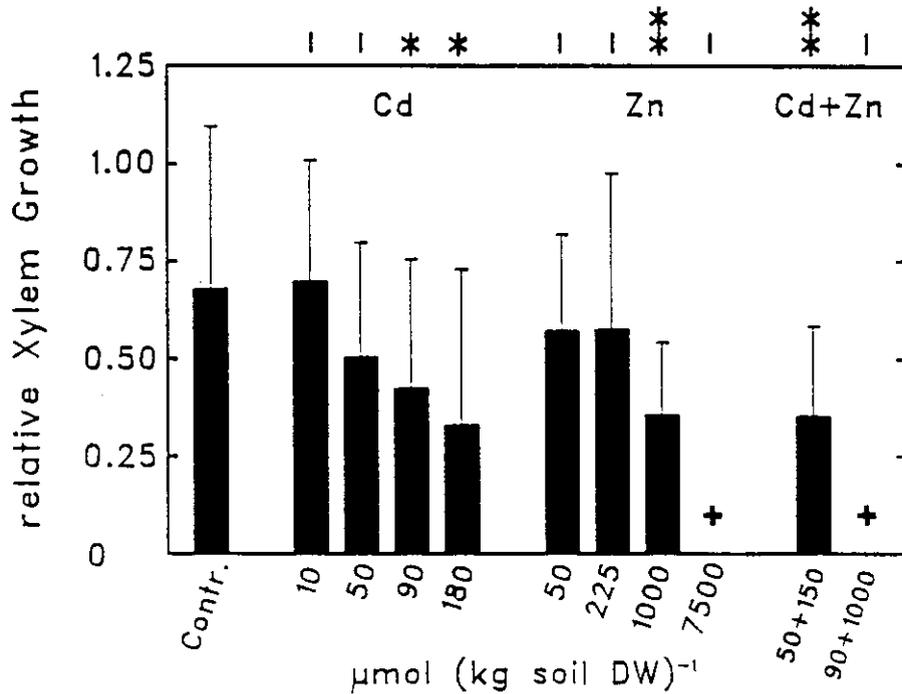
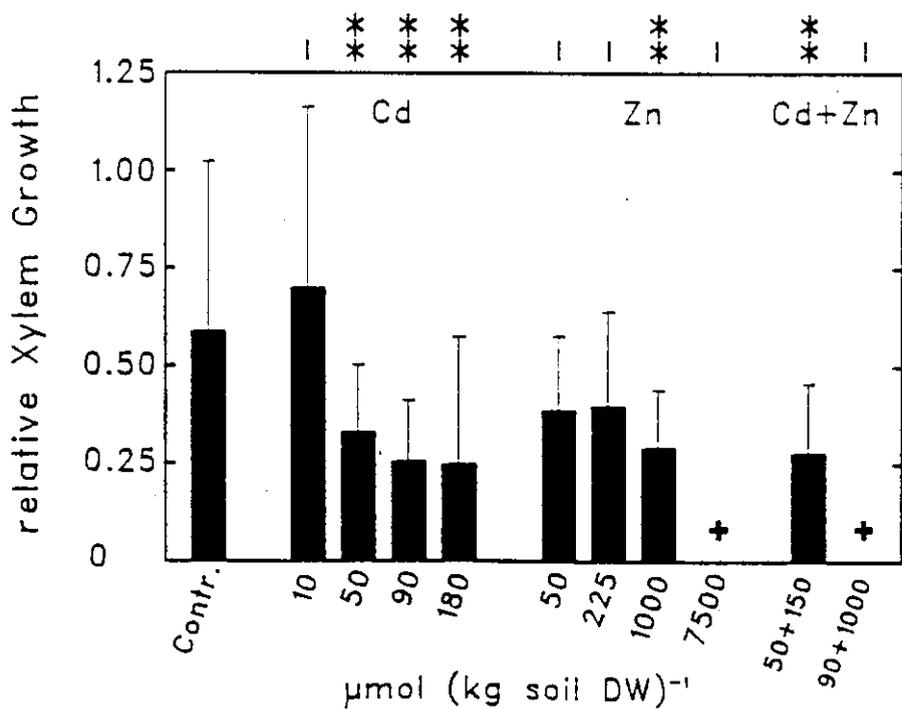


Fig. 8. Relative annual xylem diameter increments in stems of 5-year-old spruce trees produced in the second year of treatment with different concentrations of Cd and Zn in the soil. For further details see Fig. 7. After HEPPEL (1991) and BRECKLE & HAGEMEYER (1992).



Response of Plant Roots to Water and Oxygen Stresses.¹⁾

A. ESHEL

Dept. of Botany, George S. Wise Faculty of Life Sciences,
Tel Aviv University, Tel Aviv 69978, Israel.

Introduction

Field studies which compared irrigated with non-irrigated crops found that the non-irrigated ones developed more roots. This was shown for soybeans (MAYAKI et al., 1976; Mason et al., 1982; GARAY and WILHELM, 1983; HUCK et al., 1983; 1986; HOOGENBOOM et al. 1987b), for *Dactylis* (MOLYNEUX and DAVIES, 1983) and for oak seedlings (OSONUBI and Davies, 1981). Hurd (1968) concluded that wheat roots penetrated dry soil more quickly than wet soil. HEMSATH and MAZURAK (1974) went as far as suggesting that the greater amount of water mechanically prevented the sorghum roots from extending into the soil. Molyneux and DAVIES (1983) found the same effect on *Phleum* and *Lolium*.

These results made HUCK & HILLEL (1983) and SCHULZE et al. (1983) to reach the conclusion that plants developed an efficient form of response to environmental conditions. According to this theory, plants grow only as much roots as they need. Under luxurious conditions when water supply is sufficient, only few roots are grown, and under drought stress conditions root growth is enhanced to enable better water absorption from the soil. This is a most appealing idea which combines theories of evolution and selection with those of economic and engineering efficiencies.

However, not all experimental results are unanimous in that respect. HURD (1968) found in the study quoted earlier that wheat produced more roots in wet than in dry soil. MAYAKI et al. (1976) found that corn and grain sorghum had larger root systems under the irrigated conditions. GREGORY (1987) summed much of the research in a review concluding that in general, well watered crops have larger root systems than crops that are droughted, and that root density of field crops is several times greater than the amount required for water supply of the shoots.¹⁾

This poses before us several questions: Is the maximal efficiency theory supported by the experimental data or not? What could be the reason for deviation from such a general principle?

One difficulty in interpreting root response to stress from field studies, results from the effect of water regime on soil aeration. Since soil wetting is always associated with reduced gas filled volume and reduced oxygen diffusion, it is impossible to differentiate between the effects of water and oxygen stresses on root development.

The objective of the experiments reported here was to differentiate between these effects by the use of controlled irrigation in combination with forced gas purging through the rooting volume.

¹⁾ This research was performed when the author was on sabbatical with Prof. A.J.M. SMUCKER, Dept. of Crop & Soil Sciences, Michigan State University, East Lansing Michigan.

Materials and Methods

Bean (*Phaseolus vulgaris* cv. Seafarer) were grown in 7.5 cm diameter 80 cm long plastic columns filled with coarse silica sand in the greenhouse. All plants were irrigated with half-strength modified Hoagland's nutrient solution at a rate of 6 ml min⁻¹ for 15 min four times a day during three weeks establishment period after planting. Excess water was drained through a drain at the base of the column. At the end of that period the plants were randomly assigned different treatments. A factorial experimental design of three levels of water supply by two gas treatments in a complete block balanced arrangement with 10 replicates was used. The three levels of water supply were: (a) Flooded: A tube was connected to the drainage outlet of these columns and brought up so it served as a stand-pipe maintaining a constant water table at 30 cm depth while allowing excess solution to flow through. (b) Drained: The same as during the establishment period. (c) Drought: These plants were subjected to five day treatment cycles during which they were irrigated for two days and then irrigation was withheld for three days.

All the columns were fitted with gas inlets at 40 and 80 cm depths in the sand, to impose two levels of oxygen supply through the root volume. Each column was purged with one of two gases, air or nitrogen. Air was streamed through the columns continuously. Nitrogen was streamed through the columns for 8 h a day only. The nitrogen treated columns did not receive any gas during the other 16 h but free air could diffuse through the top resulting in a deep-soil anoxia thought to be similar to that which may be found in flooded fields. Preliminary experiments have shown that continuous nitrogen streaming was too severe, and was killing the plants.

The treatments were applied for 25 days after which the plants were harvested. At harvest plants were in the podfilling stage. The sand was extracted from the columns with the roots intact and divided into 10 cm segments. The sand was washed off the roots in the hydropneumatic elutriation system (SMUCKER et al., 1982). Leaf area was measured with a Li-Cor 3050A area meter. Leaves, stems, pods and roots were dried at 70°C for 48 h and weighed.

Results

The experimental design allowed for differentiation between the effects of the two factors - water supply and oxygen supply to the roots - on growth of the various plant parts (Fig. 1). The treatment period bridged the vegetative stage through flowering until mid-podfill. It was observed that water supply level determined plant growth under forced soil aeration. Sizes of all parts of the shoot decreased with decreasing water supply but pods proved to be more sensitive to drought than leaves or stems. This may be in part the result of early shoot development during the pre-treatment establishment period.

Under intermittent nitrogen purging, plant growth was severely limited by the reduced oxygen supply to the roots. Under drought and nitrogen gas treatments an additive effect of the two factors was observed. Specific leaf area averaged 300 cm² g⁻¹ with no significant differences among the six treatments. Hence the leaf area histogram (Fig. 2A) is similar to that of leaf weights.

Response of the total amount of roots per plant to the different treatments (Fig. 2B) was similar to that of the shoots. Decreasing water supply resulted in smaller root systems. The intermittent anoxia and flooded treatment reduced root growth by about 60% as compared to the corresponding aerated treatments.

There was no significant effect of soil aeration level on root/shoot or root/leaves weight ratios (Fig. 2C & D). Drought, however, increased these ratios significantly as compared with the higher water supply levels. An allometric analysis of dry weights of root vs. leaves

following the method of HUNT and NOBEL (1987a) is presented in Fig. 3. Plants from all treatments seem to follow the same regression line which explained half of the variance ($r = 0.7$). The exponent of 0.55 indicates that root/leaves ratio will decrease as plant size increases.

Root profiles presented in Fig. 4 show that soil aeration had a larger effect on root distribution in the soil column than water supply. The effects of the various treatments on root weight densities down to 40 cm depth were similar to their effects on total root size. A reduction in root weight density with decreasing water supply under aerated treatments were observed to depths of 40 cm. Nitrogen purging resulted in very small root growth below 40 cm. With air purging, there were only small differences among the root weight densities below 40 cm at the three water supply levels.

Examination of the fraction of the root system at every depth (Fig. 5) reveals clearly that nitrogen purged profiles had larger proportions of the root system in the top layer. Only under normal irrigation and well drained conditions did the roots penetrate beyond 40 cm when the column was purged with nitrogen.

Discussion

The results presented here indicate that as long as aeration is not reduced, root development will increase with increasing water supply. There is no support for the idea brought forward by HUCK and HILLEL (1983) and by SCHULZE et al. (1983) that carbohydrate partitioning in plants is governed by optimization rules which limit root growth as long as there is ample water supply to the shoot. The same conclusion can also be drawn from the prolific root development in hydroponic cultures. CALDWELL (1976) already noted that plants are not following the optimal strategy outlined by the physical models. The response time of the root extension-water supply processes is relatively long. If root growth is withheld until onset of water stress, the annual plant may run out of reserves before enough root growth can occur to alleviate this stress. This may be the main difference between this process and that of stomatal control which served as a basis for the theory developed by SCHULZE et al. (1983). Additionally, HOOGENBOOM et al. (1987a) reported that the simulation model of plant growth based on these principles was unrealistically sensitive to moisture stress.

The increase in root/shoot ratios of plants which were subjected to drought was a result of the maintenance of the allometric relationships between root and shoot sizes (Hunt, 1975; RICHARDS et al., 1979). HUNT and NOBEL (1987a) who analyzed root/shoot relationships in that form, pointed out that an exponent lower than unity will indicate the increase of root/shoot ratio as plant size decreases. TURNER and BEGG (1981) suggested that this change in root/shoot ratio is an adaptive mechanism of many crop and pasture species to water stress conditions.

ITOH and KUMURA (1986) found such a response of soybeans to prolonged drought. An increase in root/shoot ratio was also found as a response to mineral nitrogen deficiency (ROBINSON, 1986; JOHNSON, 1985; VESSEY and Layzell, 1987), to potassium deficiency (COALE and GROVE, 1986), and to deviation of root temperature from the optimum (DAVIDSON, 1969; COOPER and THORNBY, 1976; MACDUFF et al., 1987). The physiological mechanisms underlying these responses determine that shoot growth is more sensitive to adverse conditions than that of the roots. GULMON and TURNER (1978) noted that differences in total root growth among varieties subjected to different soil conditions tended to parallel differences in shoot growth. KLEPPER et al. (1984) and BELFORD et al. (1987) described the close coordination which exists between phenological development of the shoot and that of the root system of wheat. Similar relationships were reported by HOPPE et al. (1986) for corn.

In the experiments reported here it was found that reduced water supply decreased root growth under high and low oxygen levels, and improved aeration increased it under all irrigation treatments. Hence we propose that the absolute increase in root size, which were reported for nonirrigated situations in the field, is a direct response to improved soil aeration. In many cases it was reported that root distribution and rooting depth and not the total root weight were the parameters influenced most. KLEPPER et al. (1973), ALLMARAS et al. (1975), and Burch (1978) found that root distribution of field crops was affected by prolonged drought in a way which resulted in larger fraction of the roots growing in deep parts of the profile. BLUM and RITCHIE (1984) and LIEFFERS and ROTHWELL (1987) observed the same effect by changes in the level of the water table. Anoxia does not occur only in fine textured soils or under flooding conditions. Impaired soil aeration was found to limit pea root growth even in coarse textured soil, if kept at high water potential by EAVIS (1972), who coined the term "Aeration Deficiency Index." The same effect was found in sand for pea, corn and *Lolium* (WARNAAR and EAVIS, 1972) and for soybean (BALIGAR et al., 1980). The later scientists found that the reduced root growth at high water potential was correlated with decrease in soil redox potential due to reduced aeration.

It is thus concluded that plants follow the safe, if not always efficient, strategy of developing new roots continuously trying to increase rooting depth as fast as possible in order to avoid possible stress. The partitioning of carbohydrate reserves is governed by the allometric relationships between roots and shoots which increase the rooting effort relatively more than shoot growth whenever plant size is limited by adverse edaphic conditions (HUNT and NICHOLLS, 1986). Actual root distribution in the profile will be determined by the balance between contrasting effects of soil water on aeration and desiccation at various soil layers (CROSSETT et al., 1975; TAYLOR and KLEPPER, 1978; WHITE, 1987). This type of life strategy must have been favoured by natural selection in stressful situations. The efficient stress-prone forms have probably been eradicated by drastic changes and catastrophes in the past. Relieving plants from these constraints and developing more efficient crops for intensive agricultural systems, in which stress is avoided due to human intervention, is an important challenge to plant physiologists and crop breeders.

References

- ALLMARAS, R.R., W.W. NELSON, and W.B. VOORHEES. 1975. Soybean and corn rooting in southwestern Minnesota: I. Water-uptake sink. *Soil Sci. Soc. Amer. Proc.* 39:764-777.
- BALIGAR, V.C., F.D. WHISLER, and V.E. NASH. 1980. Soybean seedling root growth as influenced by soil texture, matric suction and bulk density. *Commun. in Soil Science and Plant Analysis* 11:903-915.
- BELFORD, R.K., B. KLEPPER, and R.W. RICKMAN. 1987. Studies of intact shoot-root systems of field-grown winter wheat. II. Root and shoot developmental patterns as related to nitrogen fertilizer. *Agron. J.* 79:310-319
- BLUM, A., and J.T. RITCHIE. 1984. Effect of soil surface water content on sorghum root distribution in the soil. *Field Crops Res.* 8:169-176.
- BURCH, G.J., R.C.G. SMITH, and W.K. MASON. 1978. Agronomic and physiological responses of soybean and sorghum crops to water deficits. II. Crop evaporation, soil water depletion and root distribution. *Aust. J. Plant Physiol.* 5:169-177.
- CALDWELL, M.M. 1976. Root extension and water absorption. p. 63-85. In: O.L. Lange et al. (eds.) *Water and plant life: Problems and modern approaches*. Springer-Verlag, Berlin.
- COALE, F.J., and J.H. GROVE. 1986. Effect of soil potassium availability on soybean root and shoot growth under unrestrained rooting conditions. *J. Plant Nutrition* 9:1565-1584.

- COOPER, A.J., and J.H.M. THORNLEY. 1976. Response of dry matter partitioning, growth, and carbon and nitrogen levels in the tomato plant to changes in root temperature: Experiment and theory. *Ann. Bot.* 40:1139-1152.
- CROSSETT, R.N., D.J. CAMPBELL, and H.E. STEWART. 1975. Compensatory growth in cereal root systems. *Plant and Soil* 42:673-683.
- DAVIDSON, R.L. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. *Ann. Bot.* 33:561-569.
- EAVIS, B.W. 1972. Soil physical conditions affecting seedling root growth: I. Mechanical impedance, aeration and moisture availability as influenced by bulk density and moisture levels in a sandy loam soil. *Plant and Soil* 36:613-622.
- GARAY, A.F., and W.W. WILHELM. 1983. Root system characteristics of two soybean isolines undergoing water stress conditions. *Agron. J.* 75:973-977.
- GREGORY, P.J. 1987. Development and growth of root systems in plant communities. p. 147-166. In: P.J. GREGORY et al. (eds.) *Root development and function*. Soc. Exptl. Biol. Seminar ser:30. Cambridge Univ. Press, Cambridge.
- GULMON, S.L., and N.C. TURNER. 1978. Differences in root and shoot development of tomato (*Lycopersicon esculentum* L.) varieties across contrasting soil environments. *Plant and Soil* 49:127-136.
- HEMSATH, D.L., and A.P. MAZURAK. 1974. Seedling growth of sorghum in clay-sand mixtures at various compactions and water contents. *Soil Sci. Soc. Amer. Proc.* 38:387-390.
- HOOGENBOOM, G., M.G. HUCK, and D. HILLEL. 1987a. Modification and testing of a model simulating root and shoot growth as related to soil water dynamics. *Adv. Irr.* 4:331-387.
- HOOGENBOOM, G., M.G. HUCK, and C.M. PETERSON. 1987b. Root growth rate of soybean as affected by drought stress. *Agron. J.* 79:607-614
- HOPPE, D.C., M.E. MCCULLY, and C.L. WENZEL. 1986. The nodal roots of *Zea*: their development in relation to structural features of the stem. *Can. J. Bot.* 64:2524-2537.
- HUCK, M.G., K. ISHIHARA, C.M. PETERSON, and T. USHJIMA. 1983. Soybean adaptation to water stress at selected stages of growth. *Plant Physiol.* 73:422-427.
- HUCK, M.G., AND D. HILLEL. 1983. A model of root growth and water uptake accounting for photosynthesis, respiration, transpiration, and soil hydraulics. *Adv. Irr.* 2:273-331.
- HUCK, M.G., C.M. PETERSON, G. HOOGENBOOM, and C.D. BUSCH. 1986. Distribution of dry matter between shoots and roots of irrigated and nonirrigated determinate soybeans. *Agron. J.* 78:807-813.
- HUNT, R. 1975. Further observations on root-shoot equilibria in perennial ryegrass (*Lolium perenne* L.). *Ann. Bot.* 39:745-755.
- HUNT, R., and A.O. NICHOLLS. 1986. Stress and the coarse control of growth and root-shoot partitioning in herbaceous plants. *Oikos* 47:149-158.
- HUNT, E.R., Jr., and P.S. NOBEL. 1987a. Allometric root/shoot relationships and predicted water uptake for desert succulents. *Ann. Bot.* 59:571-577.
- HUNT, E.R., Jr., and P.S. NOBEL. 1987b. A two-dimensional model for water uptake by desert succulents: implications of root distribution. *Ann. Bot.* 59:559-569.

- HURD, E.A. 1968. Growth of roots of seven varieties of spring wheat at high and low moisture levels. *Agron. J.* 60:201-205.
- ITOH, R. and A. KUMURA. 1986. Acclimation of soybean plants to water deficit. I. Effects of prolonged water deficit on the production and partition of dry matter. *Japan. J. Crop Sci.* 55:367-373.
- JOHNSON, I.R. 1985. A model of the partitioning of growth between the shoots and roots of vegetative plants. *Ann. Bot.* 55:421-431.
- KLEPPER, B., H.M. TAYLOR, M.G. HUCK, and E.L. FISCUS. 1973. Water relations and growth of cotton in drying soil. *Agron. J.* 65:307-310.
- KLEPPER, B., R.K. BELFORD, and R.W. RICKMAN. 1984. Root and shoot development in winter wheat. *Agron. J.* 76:117-122.
- LIEFFERS, V.J., and R.L. ROTHWELL. 1987. Rooting of peatland black spruce and tamarack in relation to depth of water table. *Can. J. Bot.* 65:817-821.
- MACDUFF, J.H., M.J. HOPPER, and A. WILD. 1987. The effect of root temperature on growth and uptake of ammonium and nitrate by *Brassica napus* L. in flowing solution culture. *J. Exptl. Bot.* 38:42-52.
- MASON, W.K., H.R. ROWSE, A.T.P. BENNIE, T.C. KASPAR, and H.M. TAYLOR. 1982. Responses of soybeans to two row spacings and two soil water levels. II. Water use, root growth and plant water status. *Field Crops Res.* 5:15-29.
- MAYAKI, W.C., L.R. STONE, and I.D. TEARE. 1976. Irrigated and nonirrigated soybean, corn, and grain sorghum root systems. *Agron. J.* 68:532-534.
- MOLYNEUX, D.E., and W.J. DAVIES. 1983. Rooting pattern and water relations of three pasture grasses growing in drying soil. *Oecologia* 58:220-224.
- OSONUBI, O., and W.J. DAVIES. 1981. Root growth and water relations of oak and birch seedlings. *Oecologia* 51:343-350.
- RICHARDS, D., F.H. GOUBRAN, and K.E. COLLINS. 1979. Root-shoot equilibria in fruiting tomato plants. *Ann. Bot.* 43:401-404.
- ROBINSON, D. 1986. Compensatory changes in the partitioning of dry matter in relation to nitrogen uptake and optimal variations in growth. *Ann. Bot.* 58:841-848.
- SCHULZE, E.-D., K. SCHILLING, and S. NAGARAJAH. 1983. Carbohydrate partitioning in relation to whole plant production and water use of *Vigna unguiculata* (L.) Walp. *Oecologia* 58:169-177.
- SMUCKER, A.J.M., S.L. MCBURNEY, and A.K. SRIVASTAVA. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agron. J.* 74:500-503.
- TAYLOR, H.M., and B. KLEPPER. 1978. The role of rooting characteristics in the supply of water to plants. *Adv. Agron.* 30:99-128.
- TURNER, N.C., and J.E. BEGG. 1981. Plant-water relations and adaptation to stress. *Plant and Soil* 58:97-131.
- VESSEY, J.K., and D.B. LAYZELL. 1987. Regulation of assimilate partitioning in soybean: Initial effects following change in nitrate supply. *Plant Physiol.* 83:341-348.

WARNAARS, B.C., and B.W. EAVIS. 1972. Soil physical conditions affecting seedling root growth. II. Mechanical impedance, aeration and moisture availability as influenced by grain-size distribution and moisture content in silica sands. *Plant and Soil* 36:623-634.

WHITE, E.M. 1986. Spring wheat growth at high and low soil water with constricted upper roots. *Soil Sci.* 143:44-48.

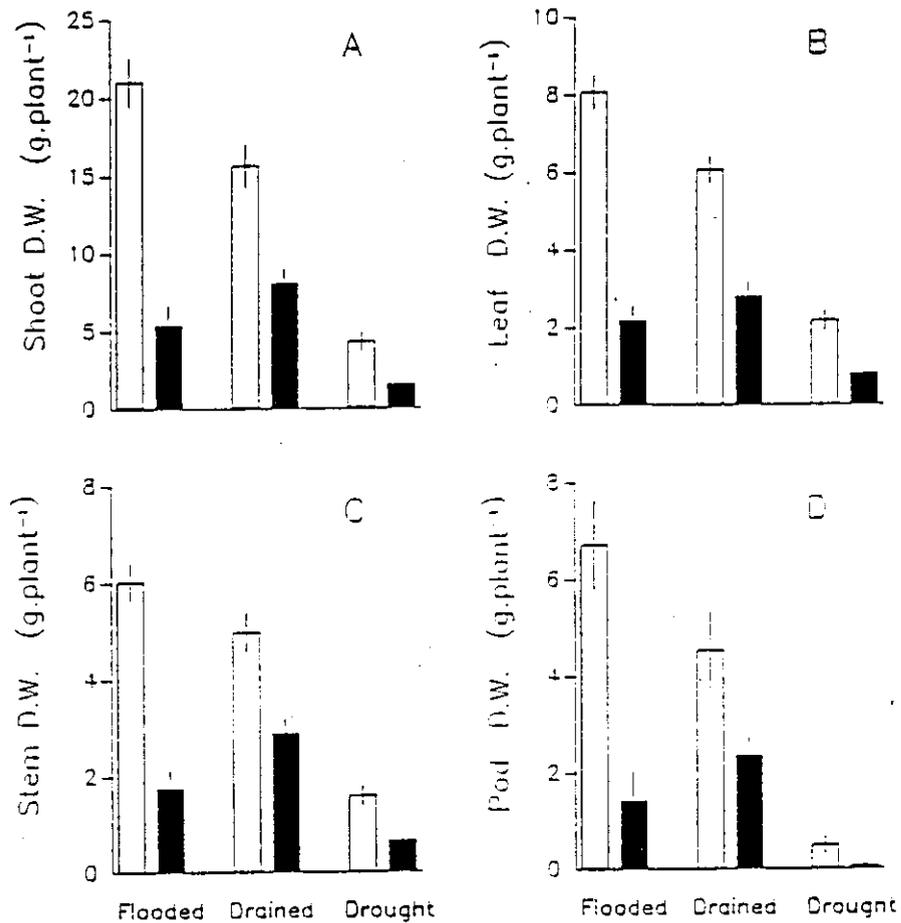


Fig.1 • Dry weights of the shoot (A) and its components (B-D) of beans grown under air (open columns) or nitrogen gas (closed columns) purging through the root volume at three water supply levels. Means \pm S.E.

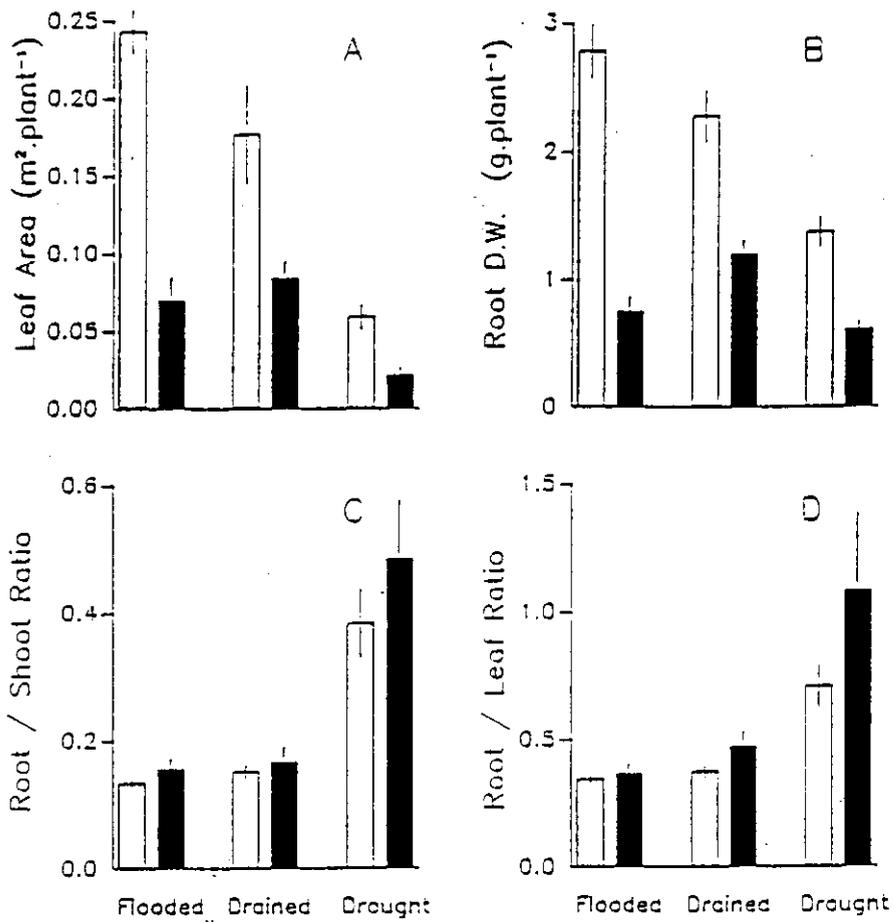


Fig.2 - Leaf area (A), dry weight of roots (B), and root to shoot (C), and root to leaf (D) weight ratios of beans grown under air (open columns) or nitrogen gas (closed columns) purging through the root volume at three water supply levels. Means \pm S.E.

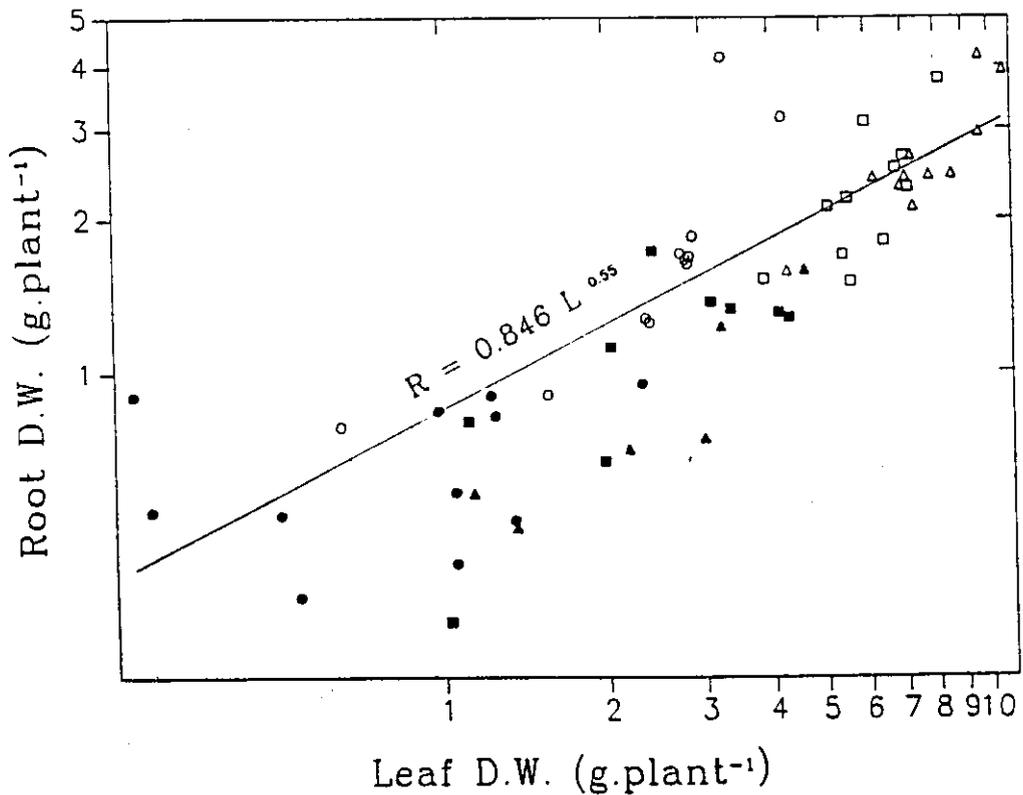


Fig.3 - Allometric relationships between dry weights of roots and shoots of beans grown under air (open symbols) or nitrogen gas (closed symbols) purging through the root volume at three water supply levels, flooded (triangles) drained (squares) and drought (circles). Means \pm S.E.

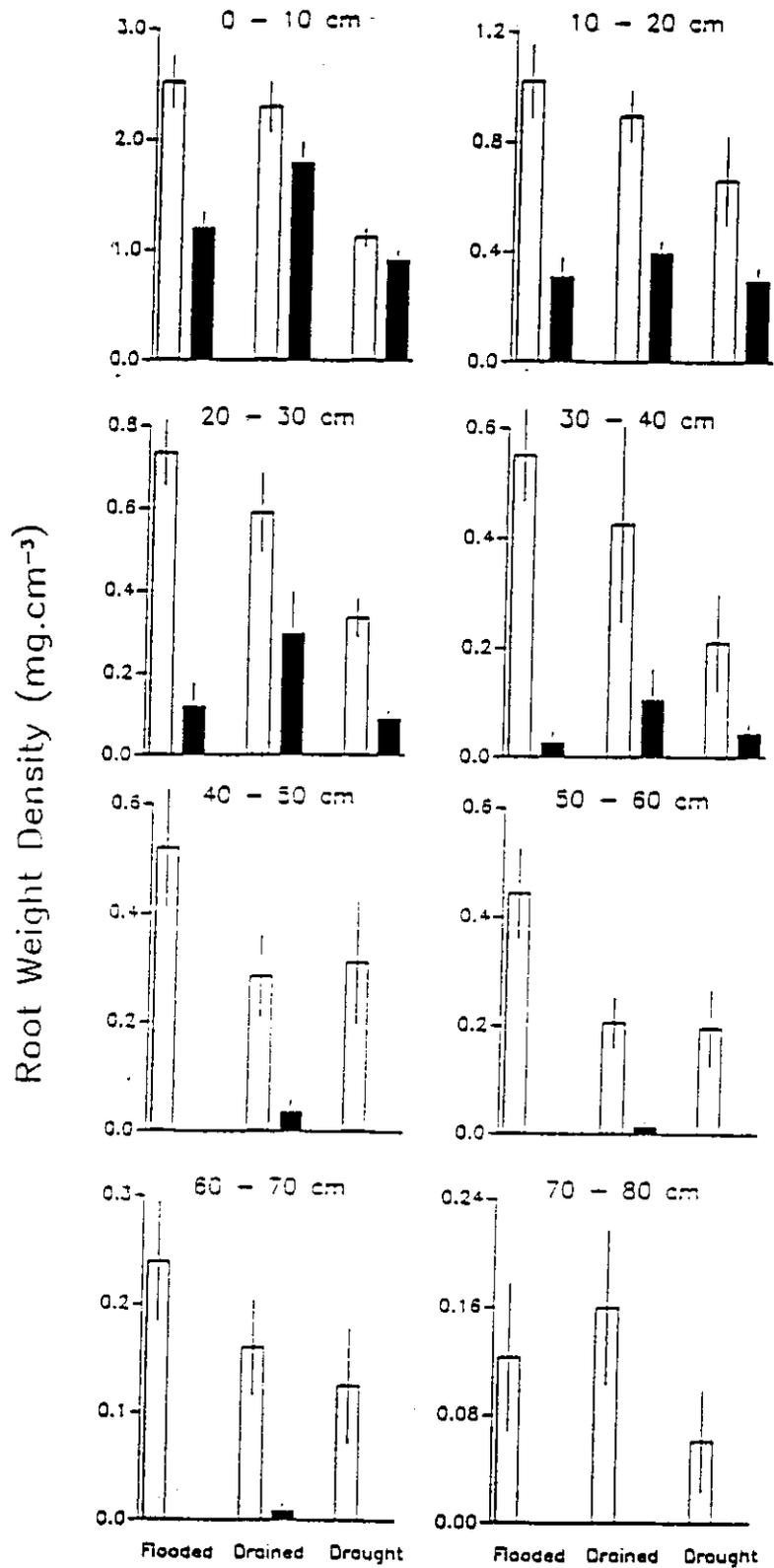


Fig.4 - Depth distribution of dry weight of roots of beans grown under air (open columns) or nitrogen gas (closed columns) purging through the root volume at three water supply levels. Means \pm S.E. calculated per soil volume

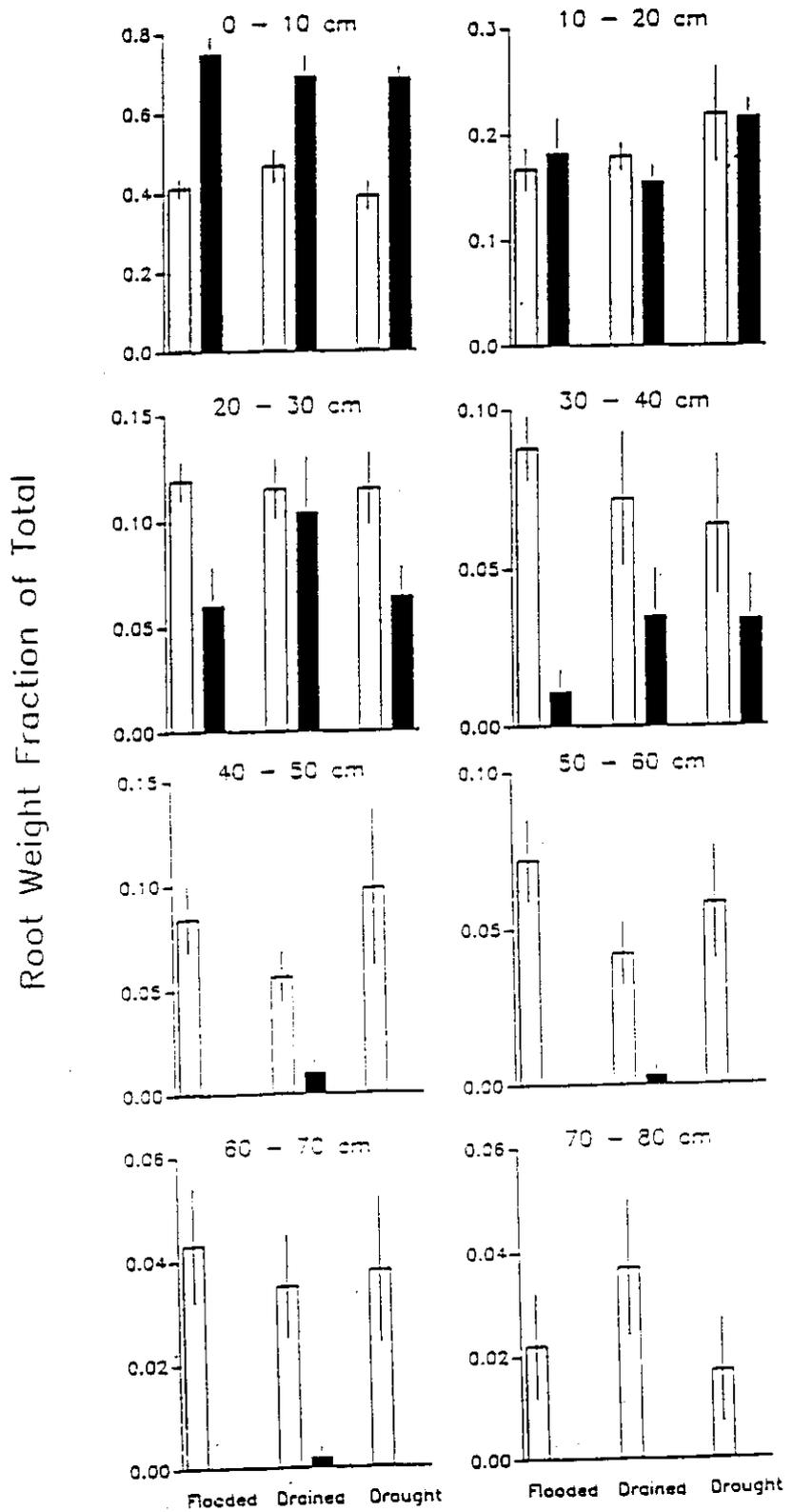


Fig.5 - Depth distribution of dry weight of roots of beans grown under air (open columns) or nitrogen gas (closed columns) purging through the root volume at three water supply levels. Means \pm S.E. of fraction of total root weight per plant.

How to Cope With a Low CO₂ Stress? A New Interpretation of an Old Observation

Yoav WAISEL, Department of Botany,
The George S. Wise Faculty of Life Sciences,
Tel Aviv University, Tel Aviv, ISRAEL.

Saline conditions induce severe stresses in plants. Such stresses are instantly reflected by closure of the stomata, by reduction of photosynthesis and by the inevitable inhibition of growth. Halophytes have developed a choice of mechanisms that enable them to cope with such deleterious stresses, and to evade the effects of excess ions and of water shortage (cf. WAISEL, 1991). Many of the halophytes, including all the species of the Tamaricaceae, have developed typical glands on their leaves. It is well accepted that those glands function as a disposal system for excesses of ions that have penetrated into the shoots of those plants. The appearance of salt crystals on top of the leaves of the salt secreting halophytes presents the visual expression for such features.

The existence of special glands on the leaves of *Tamarix aphylla* (L.) Karst. was first reported some 100 years ago. The original explanation, given to the functions of those glands, was that they are primarily involved in chalk precipitation. Accordingly, the glands were named "Kalkdrüsen" (VOLKENS, 1884, 1887; MARLOTH, 1887). Still, modern investigators have disregarded this "archaic" approach to chalk deposition. As similar glands were found in several other halophytes, they were renamed "salt glands" (cf. THOMSON 1975, FAHN 1979, LIPHSCHITZ and WAISEL, 1982; BRECKLE 1986). The idea that precipitation of calcium carbonate by such glands may have certain virtues of its own and that such a characteristic may contribute to the adaptation of halophytes by other means than recretion, was skeptically dismissed (cf. WAISEL 1972).

Plants of *T. aphylla* have to cope with two major environmental determinants: too much salt and too little CO₂.

The need for disposal of excess salts is most obvious for all plants of saline habitats. This was the basis for the rationale that the leaf glands were named "salt glands". As such, their main function was thought to be recretion of distressing ions that invade the plants (THOMSON et al., 1969; WAISEL, 1961, 1991; HAGEMEYER, 1990). However, as the efficiency of such glands was found to be rather low (cf. WAISEL, et al., 1986), the question was asked if the recretion of salt is really their main function?

Like many other perennial desert species, plants of *T. aphylla* have minute and stipule-like leaves. On the one hand such leaves guarantees the reduction of transpiration. On the other hand they provide the plants with a relatively small surface area for CO₂ uptake. The capability of *T. aphylla* to absorb CO₂ is further hampered because of its endogenous rhythm that keeps the stomata open for only some 10-11 h a day (cf. HAGEMEYER and WAISEL 1987). No doubt that such conditions hamper the photosynthetic capability of those plants and stress their carbon metabolism. Nevertheless, in spite of the apparent limitations of photosynthesis, growth of *Tamarix aphylla* plants is among the fastest known for desert trees (WAISEL 1972). How can two such contradictory features, low photosynthetic capability and fast growth, reconcile?

Four major factors enable plants to reach high rates of net carbon assimilation: (a) a large leaf area for CO₂ acquisition; (b) a high efficiency of the assimilating mechanism; (c) a low rate of respiration and (d) a high concentration of ambient CO₂. Which of those factors determines the rate of photosynthesis of *T. aphylla*?

Apparently, the leaves of *T. aphylla* have a small surface area, a trait that under the ambient atmospheric pCO₂ limits their rates of photosynthesis (WAISEL 1991a). Moreover, the photosynthetic mechanism of *T. aphylla* does not show an exceptionally high efficiency for CO₂ fixation. Comparative studies of Rubisco activity of various species of *Tamarix* (SOLOMON et al., 1993) have indicated that the rate of activity of this key enzyme is very much the same as in other non-halophytic plants. It is interesting to note that also the rates of respiration of *T. aphylla* plants remain in the "normal" range of respiration that was measured for most other plants. Thus, the fourth of the above alternatives, i.e., the enrichment of CO₂ around the green shoots, remains the only feasible explanation for the improved carbon assimilation by those desert stressed plants. Where from do those plants get their extra supply of CO₂? This is where the "salt glands" enter the story.

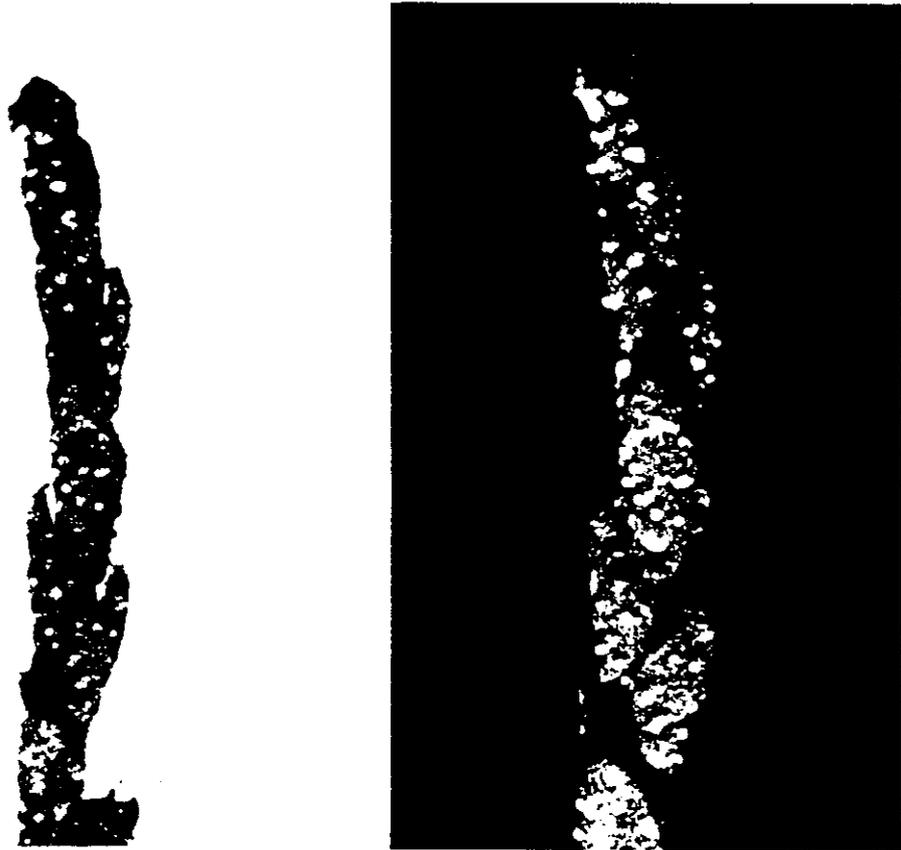


Figure 1. Chalk crystals covering a twig of *Tamarix aphylla*.

Tamarix aphylla is one of those halophytes whose leaves are covered by a thick crust of salt crystals (Fig 1). A significant part of this crust is comprised of CaCO₃. No doubt that the calcium of the crust is taken up from the soil and recreted by the glands. However, what is the source of the carbonate? Is it in the soil or is it somewhere else?

When twigs of *T. aphylla* were supplied, via the transpiration stream, with labelled carbon sources, i.e., either with ¹⁴C-bicarbonate or with ¹⁴C-sucrose, only insignificant quantities of the labelled carbon have reached the chalk crust. Even after 24 hours of constant ¹⁴C-supply via the shoot base, only less than 1.0% of the absorbed ¹⁴C reached the crust carbonate. However, when twigs of *T. aphylla*, were exposed to gaseous ¹⁴CO₂, large

quantities of the labelled carbon were trapped and incorporated in the crusts. This was particularly conspicuous under dark and humid conditions, that kept the twigs moist for several hours.

How is the CO_2 trapped?—The system seems to be rather simple. The glands of *Tamarix aphylla* secrete numerous droplets of brine that keep the twigs moist during the night. This solution is characterized by a high concentration of ions (HAGEMeyer and WAISEL, 1988) but also by its high alkalinity. The pH of this solution fluctuates between 8.0 and 10.5, being lower during the night and higher during the light hours. Apparently, this alkaline solution constitutes an efficient trap for atmospheric CO_2 . Thus, the chalks of *T. aphylla* crusts are the product of the gland-recreted-calcium and of trapped atmospheric CO_2 .

However, this is only one part of the story. The CO_2 which had been trapped in the secreted fluid is not retained there permanently. Upon the nightly drop in pH, some of this carbon, previously preserved as CaCO_3 , is transmuted into CO_2 and enriches the immediate surroundings of the green twigs of *Tamarix*. Is that carbon available to the plants? The answer is definitely yes. Following illumination, $^{14}\text{CO}_2$ that had been fixed in the crust during the previous night, can be recovered inside the shoots. The appearance of ^{14}C inside the photosynthesizing shoots confirms two of our basic hypotheses:

- (a) CO_2 is periodically released from the precipitated chalks.
- (b) CO_2 that is released from the chalks can be subsequently assimilated, in the light, by the green twigs.

The rates of assimilation of *T. aphylla* are positively correlated with the ambient pCO_2 . At the current CO_2 concentration of the air (330 ppm), net photosynthesis is suboptimal. Photosynthesis reaches maximal rates only at CO_2 concentrations of 500-600 ppm, depending on the ambient temperature (Fig 2). Can the glands induce the generation of enough CO_2 so as to raise its concentration around the twigs up to the optimal concentration? Can they improve photosynthesis by doing so? The answer to such questions is also yes! Upon the circadian drop in the pH of the gland-produced brine, the CO_2 concentration, at the boundary layer between the atmosphere and the surface of the scaly leaves, is assumed to rise to above 1000 ppm. Though such a concentration prevails around the twigs for only a few of the morning hours, its magnitude is large enough to enable the plants to photosynthesize at higher rates. Thus, plants of *T. aphylla* may benefit from this extra source of carbon, and probably exploit it for maximal assimilation under the stressing desert environment.

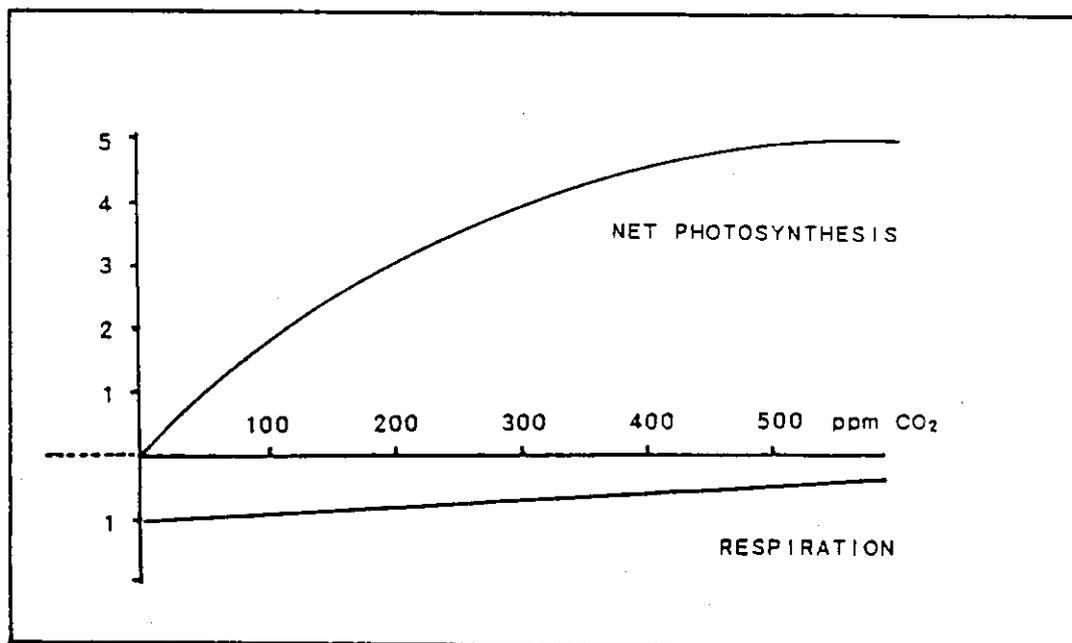


Figure 2. The effect of the ambient CO_2 concentration on the rates of net photosynthesis and of dark respiration of *Tamarix aphylla*. ($\mu\text{moles CO}_2 \text{ min}^{-1} (\text{g DW})^{-1}$)

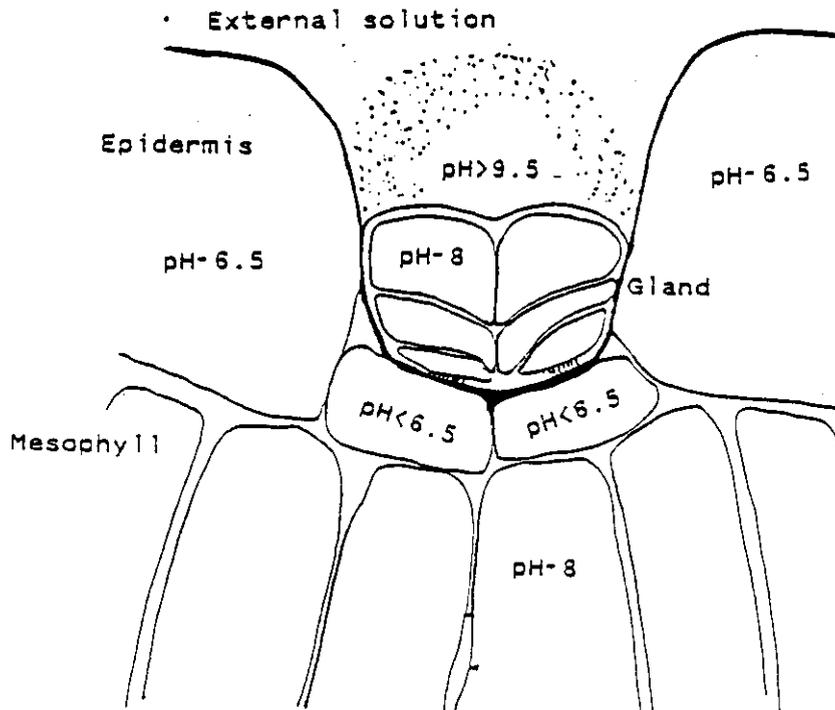


Figure 3. Differences in pH among various cells, in, and around a leaf gland of *Tamarix aphylla*.

The precise site of hydroxyl excretion by the leaves is not clear. Still, with the aid of a few pH indicators we were able to determine the pH gradient across the glands and the adjacent leaf cells. The pH of the upper epidermal cells, and that of the lower gland cells, was relatively low, i.e., about pH 6.5. The pH of the mesophyll cells was approximately 8, apparently reflecting the photosynthetic activity of those cells. The pH of the upper gland cells was also approximately 8, but the pH of the fluid that had accumulated on top of them was 9.5 or higher (Fig. 3).

Table 1. The effect of a pH change of one unit on the carbon content of the wetting solution around the twigs of *Tamarix aphylla*. Values for aqueous solutions at equilibrium with an atmosphere of 350 ppm CO₂. Data in $\mu\text{mol ml}^{-1}$. Calculated after BEER et al., 1977.

	pH 8.5	pH 9.5
Total C content	4.2	252
Carbon species:		
CO ₂	0.011	0.011
HCO ₃ ⁻	3.39	53.73
CO ₃ ²⁻	0.79	198.4

Large quantities of carbon are precipitated in the crust, chalks or can be released from them as CO₂ (Tab. 1). Even a modest drop of one pH unit (from pH 9.5 to pH 8.5), would release some 250 $\mu\text{mol CO}_2$ (cf. BEER et al., 1977) from each g DW of *T. aphylla* twigs. This should be enough to boost the pCO₂ around the twigs to such levels that would saturate their photosynthetic apparatus. Some of the trapped CO₂ is released into the embedding solution but some of it ends up in the ambient atmosphere. Direct absorption of

CO₂ from the engulfing solution would be the more efficient way because it would lessen the dispersal of the gas by blowing winds. In any case, both paths would guarantee the twigs with a CO₂ enriched environment. Thus, the chalk cover of *T. aphylla* leaves constitutes a store of carbon. Apparently, the glands contribute to the well-being of this species by their management of the carbon content of the chinks and by their act as a CO₂ concentrating system. Analogous systems were described in the past for Cyanobacteria or for algae (BADGER et al. 1978, OGAWA and KAPLAN 1987, REISKIND et al. 1989 etc.) as well as for C₄ and CAM plants. Of course, the circumstances of *Tamarix* are somewhat different, involving external chalk precipitates that play a conspicuous role in the optimization of photosynthesis. The quantitative beneficial aspects of such a system and their possible beneficial value in the field, need further investigation. However, understanding of the function of such a CO₂ enrichment system helps to explain the high productivity of *T. aphylla*, in spite of the small area of its photosynthesizing surface (WASEL 1991a).

Enrichment of the ambient CO₂ would not only increase the photosynthesis of *Tamarix aphylla* plants, but would also suppress their photorespiration (cf. SCHWARZ and GALE 1984) and even promote their salt tolerance (cf. ENOCH et al. 1973, ZERONI 1988). These two additional features of the "carbon concentrating system" may help *Tamarix* to cope with the salt stresses that it faces in desert environments.

Such a system for carbon enrichment is not restricted to *T. aphylla*. Preliminary observations of other plants have shown that this might be common also in other plants and can be expected to characterize many of the salt stressed halophytes.

Conclusions

The "salt glands" of *Tamarix*, i.e., those structures that are liable for the production of the alkaline secretum, promote a triplet of purposes:

- (a) They remove excess salts out of the live parts of the leaves.
- (b) They recrete hygroscopic solutes, thus, providing the plants with a moist cover. Altogether they reduce the time during which transpiration occurs.
- (c) They award the plants with a CO₂ enriched environment, which improves photosynthesis during the early morning hours, reduces respiration and may improve the salt resistance of those plants.

Acknowledgements

Sincere thanks are due to Prof. W.W. THOMSON of UC Riverside, California, and to Drs. S. BEER and A. ESHEL of Tel Aviv University for stimulating discussions.

References

- BADGER, M.R., KAPLAN, A. & BERRY, J.A. 1978. A mechanism for concentrating CO₂ in *Chlamydomonas reinhardtii* and *Anabaena variabilis* and its role in photosynthetic CO₂ fixation.- Carnegie Inst. Wash. YB 77: 251-261.
- BEER, S., ESHEL, A. & WASEL, Y. 1977. Carbon metabolism in seagrasses. I. The utilization of exogenous inorganic carbon species in photosynthesis.- J. Exp. Bot. 106: 1180-1189.
- BRECKLE, S.-W. 1986. Studies on halophytes from Iran and Afghanistan. II. Ecology of halophytes along salt gradients.- Proc. Roy. Soc. Edinburgh. 89B: 203-215.
- ENOCH, H.Z., ZIESLIN, N., BIRAN, Y., HALEVY, A.H., SCHWARZ, M., KESLER, B. & SHIMSHI, D. 1973. Principles of CO₂ nutrition research.- Acta Hort. 32: 97-118.
- FAHN, A. 1979. Secretory Tissues in Plants.- Academic Press, London. 302 pp.
- HAGEMEYER, J. 1990. Oekophysiologische Untersuchungen zur Salz- und Cadmiumresistenz von *Tamarix aphylla* (L.) Karst. (Tamaricaceae).- Diss. Bot. 155: 1-194
- HAGEMEYER, J. & WASEL, Y. 1987. An endogenous circadian rhythm of transpiration in *Tamarix aphylla*.- Physiol. Plant. 70: 133-138.

- HAGEMEYER, J. & WAISEL, Y. 1988. Excretion of ions (Cd^{2+} , Li^+ , Na^+ and Cl^-) by *Tamarix aphylla*.- *Physiol. Plant.* 73: 541-546.
- LIPHSCHITZ, N. & WAISEL, Y. 1982. Adaptation of plants to saline environments: salt excretion and glandular structure.- In: *Tasks for Vegetation Science*, vol. 2 (D.N. SEN AND K.S. RAJPUROHIT, eds), pp.197-214. Dr. W. Junk Publishers, The Hague.
- MARLOTH, R. 1887. Zur Bedeutung der salzabscheidenden Drüsen der Tamariscineen.- *Ber. Deut. Bot. Ges.* 5: 319-324.
- OGAWA, T. & KAPLAN A. 1987. A model for inorganic carbon accumulation in cyanobacteria.- In: *Progress in Photosynthesis Research* (J. BIGGENS, ed.), IV.6. pp. 296-297, Martinus Nijhoff Publ., Dordrecht.
- REISKIND, J., BEER, S. & BOWES, G. 1989. Photosynthesis and photorespiration in marine macroalgae.- *Aquat. Bot.* 34:131-152.
- SCHWARZ, M. & GALE, J. 1984. Growth response to salinity at high levels of carbon dioxide.- *J. Exp. Bot.* 35: 193-196.
- SOLOMON, A., BEER, S., WAISEL, Y., JONES, G.P. AND PALEG, L.G. 1993. Effects of NaCl on the carboxylating activity of Rubisco from *Tamarix jordanis*, in the presence and absence of proline-related compatible solutes.- *Physiol. Plant.* (In Press).
- THOMSON, W.W., BERRY, W.L. AND LIU, L.L. 1969. Localization and secretion of salt by the salt glands of *Tamarix aphylla*.- *Proc. Nat. Acad. Sci. (Wash.)* 63:310-317.
- THOMSON, W.W. 1975. The structure and function of salt glands.- In: *Plants in Saline Environments* (A. POLJAKOFF-MAYBER and J. GALE, eds), *Ecol. Stud.* 15: 118-146. Springer Verlag, Berlin.
- VOLKENS, G. 1884. Die Kalkdrüsen der Plumbagineen.- *Ber. Deut. Bot. Ges.* 2: 334-342.
- VOLKENS, G. 1887. Zu Marloth's Aufsatz "Ueber die Bedeutung der salzabscheidenden Drüsen der Tamariscineen".- *Ber. Deut. Bot. Ges.* 5: 434-436.
- WAISEL, Y. 1961. Ecological studies on *Tamarix aphylla* (L.) Karst. III. The salt economy.- *Plant & Soil* 4: 356-364.
- WAISEL, Y. 1972. *Biology of Halophytes*.- 395pp., Academic Press, New York.
- WAISEL, Y., ESHEL, A. and AGAMI, M. 1986. Salt balance of the leaves of the mangrove *Avicennia marina*.- *Physiol. Plant.* 67: 67-72.
- WAISEL, Y. 1991. Adaptation to salinity.- In: *Physiology of Trees* (A.S. RAGHAVENDRA, ed.), pp. 357-381. John Wiley & Sons Inc. New York.
- WAISEL, Y. 1991a. The glands of *Tamarix aphylla*: a system for salt secretion or for carbon concentration? - *Physiol. Plant.* 83:506-510.
- ZERONI, M. 1988. Plant tolerance to salinity in greenhouses: physiological and practical considerations.- *Acta Hort.* 229: 55-73.

Salinity-Stress and Salt-Recretion in Plants

Prof. Dr. S.-W. BRECKLE
Dept. of Ecology
Faculty of Biology
University of Bielefeld
D-4800 Bielefeld 1

- 1 Abstract
- 2 Introduction
- 3 Adaptations to Salt-Stress in Halophytes
 - 3.1 Halophyte-types
 - 3.1.1 Salt-glands and Bladders
 - 3.1.2 Anatomy
 - 3.2 Ecophysiological Aspects and Examples
 - 3.2.1 General Remarks
 - 3.2.2 Salt-glands in Limonium
 - 3.2.3 Bladders in Atriplex
- 4 General and ecological conclusions
- 5 References

1 Abstract

Saline soils are widespread on the globe. In climatically arid regions not only coastal areas but all erosion basins tend to become saline, as well as agricultural fields by irrigation (if without drainage).

Salinity stress is thus a worldwide phenomenon in organisms, espec. in plants. Plants have evolved various adaptations to cope with salinity stress. As an example the special group of salt-recreting plants is discussed here. Their anatomical features (salt-glands or bladder structures) are the basis for recreting salt from the mesophyll out of the leaf or to metabolically isolated vacuoles. The activity of the ecophysiological processes as well as the selectivity to ions is discussed. The adaptation to saline stands is documented also by the occurrence of the various halophyte types along the salt gradient in nature, as e.g. at salt lakes. The relative ecological advantages of salt-recreting halophytes are shown.

2 Introduction

Salinity-problems in arid countries are widespread. In many countries a high percentage of arable land exhibits increasingly low yields because of accumulation of salt from low quality river water for irrigation and by high evaporation rates of capillary water from the high phreatic water levels. Thus accumulation of salt during the last half century in many agricultural projects is an increasingly severe problem (RICHARDS et al. 1954, STROGOV 1964, JONES 1970, POLJAKOFF-MAYBER & GALE 1975, GRAETZ & HOWES 1979, SHAINBERG & SHALHEVET 1984).

To cope with stress needs special adaptations. These adaptations can be mainly structural or functional on various levels or both (LEVITT 1980).

Salinity stress needs a general adaptation to the lowered osmotic potential and special adaptations to the distinct ionic effects of Na and/or Cl (WAISEL 1972, ALBERT 1982).

Plants need water from the soil. This is available only when the osmotic gradient can be maintained (HADAS et al. 1973). Uptake of salty water leads inevitably to increasing salt concentrations in the plant-body. The plasmatic adaptation on the one side and the ecophysiologicaly efficient recreation on the other side is a precondition or a strategy of one group of plants growing on saline stands. But also other adaptations are known to be efficient to cope with salt-stress. This is especially the typical halo-succulence.

3 Adaptations to Salt-Stress in Halophytes

Higher plants which can grow on soils much higher in salt content than the average soils are termed halophytes. There is no other definition, which might be less weak. Various authors use many different parameters to distinguish halophytes from non-halophytes, but the delimitation is not sharp. The same is true for the various systems in classifying halophytes (WASEL 1972). The necessity of salt for growth was taken as a parameter for halophyte-types by KREEB (1964).

3.1 Halophyte-types

Since decades various classifications for halophytes were suggested. Certainly the control mechanisms in plants, which maintain a distinct level of sodium concentration in leaves and shoots is an important factor. According to those mechanisms dominant in specific plant-groups halophyte-types can be distinguished on a rather general scheme (Table 1)

Table 1 : Control mechanisms in plants for maintaining distinct levels of Sodium and Chloride in leaves and shoots and corresponding halophyte-type using predominantly that mechanism (acc. to WASEL 1972, BRECKLE 1976, BRECKLE 1990, WALTER & BRECKLE 1991)

mechanisms/strategy	Halophyte-Type	examples/species
selectivity of salt-absorption	(NON-HALOPHYTES)	more or less in all species
leaching of leaves	(NON-HALOPHYTES)	more or less in all species
disposal of older plant parts	GLYKO-, PSEUDO-HALOPHYTES	<i>Juncus, Zygophyllum</i>
recretion by roots		common? <i>Suaeda monoica, Salicornia</i>
increase in halo-succulence - in stems - in leaves	EU-HALOPHYTES	halo-succulents: - stem-succulents - leaf-succulents
recretion by salt-glands	CRINO-HALOPHYTES: EXO-CRINO-H.	<i>Cressa, Limonium, Glaux, Frankenia, Tamarix</i> etc.
recretion by bladder-hair	CRINO-HALOPHYTES: ENDO-CRINO-H.	<i>Atriplex, Halimione;</i> some <i>Mesembryanth.</i>

In the following we will discuss only those halophytes and their adaptations, which are secreting salt by glands or bladder.

3.1.1 Salt-glands and Bladders

In various separately evolved groups of angiosperms we can find structures for salt-recretion. Some of these structures in other genera of the same taxonomic group may have other functions as e.g. excretion of essential oils. Thus, in this respect a given specific structure, typical for a distinct systematic entity, may have changed its function by adaptation to specific ecological needs, e.g. to cope with salt-stress. In table 2 is given a list of various genera with species able to recrete salt is demonstrated.

Table 2 : Salt recretion in terrestrial halophytes by salt-glands or bladder structures
(partly from LIPHSCHITZ & WAISEL 1982): Plant-families and genera with
crinohalophytic species

in italics: mangrove-species;

* : only some species of the genus on saline soils

§ : rarely on saline soils

A. species with salt-glands

<i>Acanthaceae</i>	<i>Acanthus</i>
<i>Avicenniaceae</i>	<i>Avicennia</i>
<i>Combretaceae</i>	<i>Laguncularia</i>
Convolvulaceae	Cressa, Ipomoea*
Frankeniaceae	Frankenia
<i>Myrsinaceae</i>	<i>Aegiceras</i>
Plumbaginaceae	<i>Aegialitis</i> , <i>Armeria</i> *, <i>Limonium</i> (Statice), <i>Limoniastrum</i> , <i>Plumbago</i> * etc.
Poaceae	<i>Aeluropus</i> , <i>Distichlis</i> , <i>Spartina</i>
Chloridoideae §	<i>Bouteloua</i> , <i>Buchloe</i> , <i>Chloris</i> , <i>Cynodon</i> , <i>Coelachryum</i> , <i>Crypsis</i> , <i>Dactyloctenium</i> , <i>Dinebra</i> , <i>Eleusine</i> , <i>Enteropogon</i> , <i>Sporobolus</i> , <i>Tetrachne</i> , <i>Tetrapogon</i>
Panicoideae §	<i>Andropogon</i> , <i>Brachiaria</i> , <i>Cenchrus</i> , <i>Chrysopogon</i> , <i>Coix</i> , <i>Dichanthium</i> , <i>Digitaria</i> , <i>Echinochloa</i> , <i>Erianthus</i> , <i>Hyparrhenia</i> , <i>Panicum</i> , <i>Paspalum</i> , <i>Paspalidium</i> , <i>Saccharum</i> , <i>Setaria</i> , <i>Sorghum</i> , <i>Tricholaena</i>
Primulaceae	<i>Glaux</i>
<i>Rhizophoraceae</i>	<i>Ceriops</i> , <i>Bruguiera</i>
Scrophulariaceae	<i>Cordylanthus</i> , <i>Castilleja</i> *
<i>Sonneratiaceae</i>	<i>Sonneratia</i>
Tamaricaceae	<i>Reaumuria</i> , <i>Tamarix</i> *

B. species with bladder cells

Aizoaceae (s.l.)	<i>Mesembryanthemum</i> (s.l.) et al.*; §;
Chenopodiaceae	<i>Atriplex</i> (Obione)*, <i>Chenopodium</i> *, <i>Halimione</i> , <i>Salsola</i> , et al.?
Oxalidaceae	<i>Oxalis</i> (?)

3.1.2 Anatomy

Species with salt-glands in the various plant-families have evolved apparently several times independently in higher plants. The anatomical structure is often family-specific.

In Fig. 1 - 4 there are given four examples of such glands with only 2 cells or up to 20 cells. In all glands it is very typical that the gland cells are physiologically very active, with dense cytoplasm and high number of mitochondria. The gland apparatus is separated from the mesophyll by cutinized cell wall layers and the adjacent cells are dense in plasmodesmic joints.

In all salt glands during dry seasons a concentrated brine is recreted, which at the surface crystalizes. By dew in the morning, or by rain the salts are washed down.

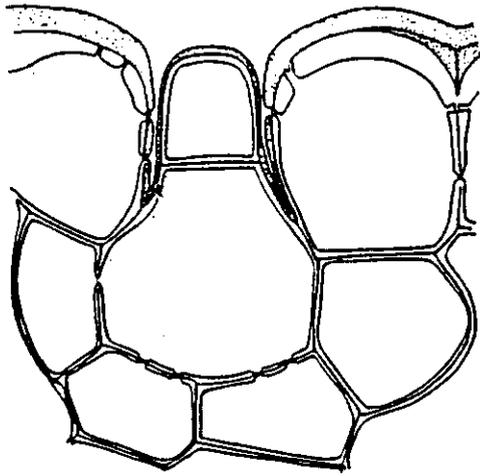


Fig. 1 : Two-cellular saltgland in *Spartina townsendii* (WASEL 1972)

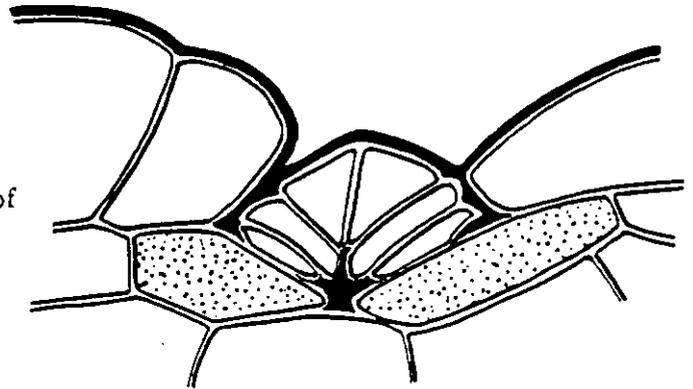


Fig. 2 : Eight-cellular saltgland of *Tamarix aphylla* (WASEL 1972)

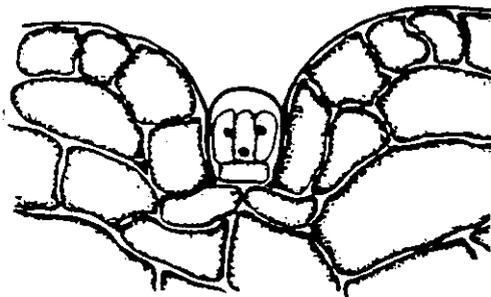


Fig. 3 : Eight-cellular saltgland in *Avicennia marina* (WASEL 1972)

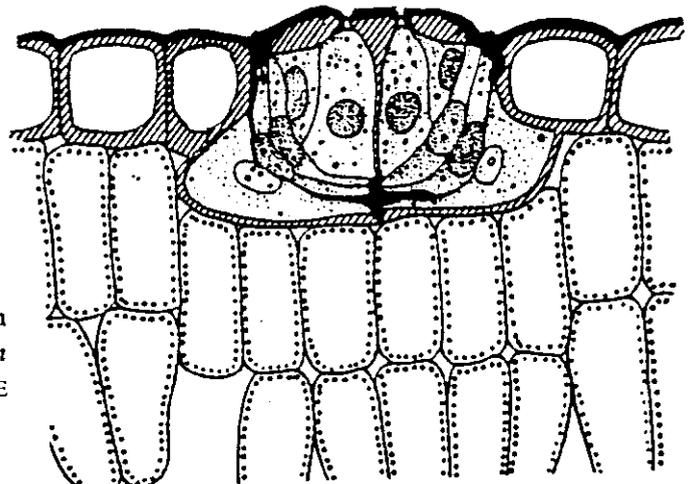


Fig. 4 : Twenty-cellular saltgland in species from the genus *Limonium* (RUHLAND 1915, WIEHE & BRECKLE 1990)

Species with bladders exhibit less variability in bladder structure. In all genera from the Chenopodiaceae the bladder apparatus consists of a large terminal bladder and 1 - 4 stalk cells. The stalk cell(s) function as metabolic active gland cells. They transport brine to the vacuole of the bladder, where it is concentrated and kept inside (Endo-Crino-Halophytes). It is possible that the bladder itself or the whole leaf is separated and thus the plant has got rid of the salt. In other cases the bladders collapse and form thick mats of salty crusts and cell remnants on the surface. These crusts were originally thought to be the main purpose for that structure in reflecting high sun light radiation. Perennial *Atriplex*-species form continuously new bladder cells, annual *Atriplex* species normally have only one generation of bladders on their leaves.

An extensive study on ecophysiology of *Atriplex* was done by OSMOND et al. (1980), also some of the functions of bladders were tackled.

In Fig. 5 the typical anatomical structure of an C4-leaf (*Atriplex mollis*) from the North-African deserts is shown. This is a perfect example of the bladder cover in these leaves.

In the genus *Chenopodium* also bladders are known. Their structure is similar. In Fig. 6 two different types are shown. In other genera of the Chenopodiaceae such bladder are scarcely occurring.

In the Aizoaceae/Mesembryanthemaceae there are some genera which exhibit large bladder cells, too. In this case the enlarged epidermal cells are not so perfectly isolated from the mesophyll as in the Chenopodiaceous bladders. In Fig. 7 the anatomy of epidermal bladder cells from this type is shown.

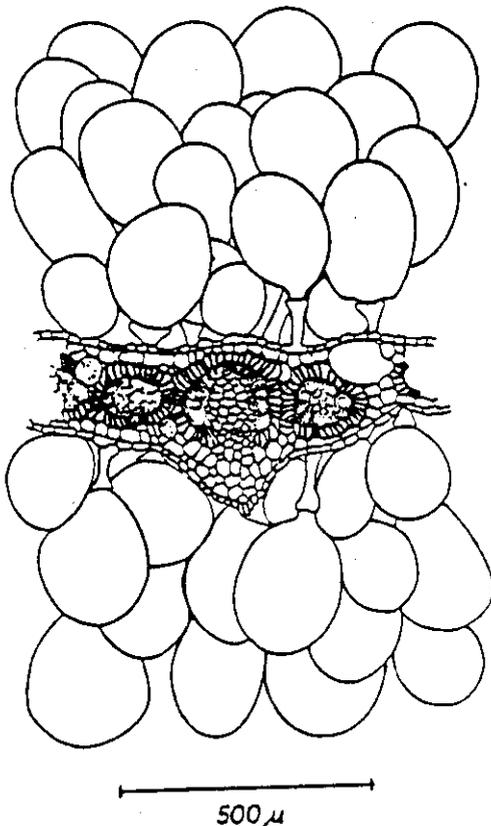


Fig. 5 : Leaf cross-section of *Atriplex mollis* with thick layers of bladder (acc. to BERGER-LANDEFELDT 1959)

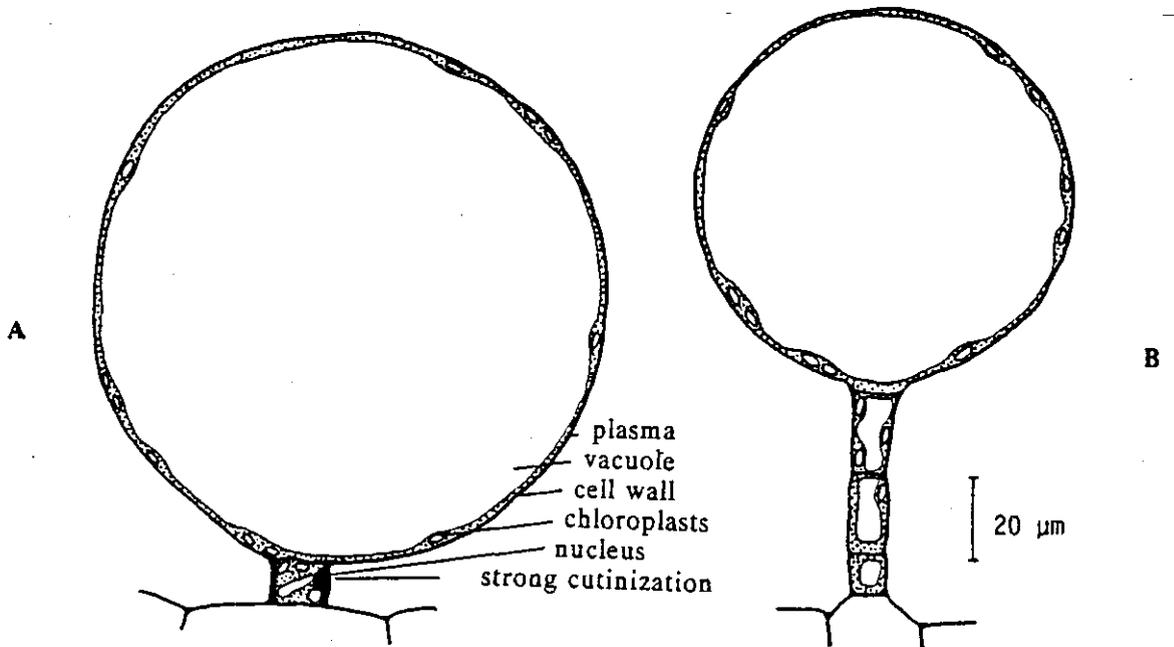


Fig. 6 : Types of bladder in *Chenopodium*. A: *Chenopodium album*, with glandlike structured stalk cell. B: *Chenopodium murale* with several slender, vacuolized stalk cells (REIMANN & BRECKLE 1987)

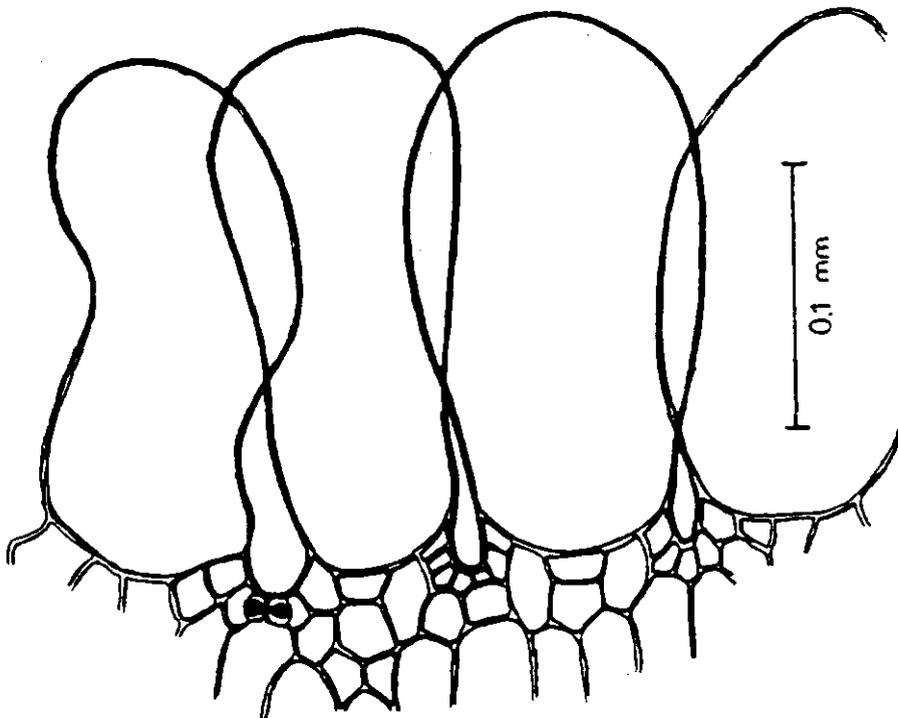


Fig. 7 : Epidermal bladder cells in *Psilocaulon salicornioides* (WALTER 1973)

3.2 Ecophysiological Aspects and Examples

3.2.1 General Remarks

The salt secretion only can be considered effective if the amount of salt within a given time is high enough to keep the salt concentration in photosynthetic active tissues low enough. Of course, this effectiveness is interdependent with many other ecological factors. In *Aeluropus litoralis* by POLLAK & WAISEL already in 1970 it was shown that the salt-concentration in leaves only to some extent can be kept low. Under higher salinities the leaf-concentration increases sharply and the turnover-time also. Thus, to some extent the salt-glands are effective enough but they are not able to cope with very high salt stress (Table 3)

Table 3 : Sodium Secretion and Sodium Content in Leaves from *Aeluropus litoralis* affected by NaCl-Concentration in culture-medium (recalc. from POLLAK & WAISEL 1970)

Na ⁺ conc. in culture medium [M]	Na ⁺ recreted [$\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$](d.m.)	Na ⁺ content in leaves [$\mu\text{M}\cdot\text{mg}^{-1}$](d.m.)	turnover-time [h] to recrete whole Na ⁺ -content
0	0.03	0.17	137
0.005	0.08	0.18	54
0.05	0.19	0.25	32
0.1	0.48	0.43	22
0.2	0.85	0.51	14
0.3	0.99	0.88	21

3.2.2 Salt-glands in *Limonium*

The genus *Limonium* is very widespread in various salty places, along coasts as well as in salt-deserts.

The salt-gland in *Limonium* is a very complex structure, which was studied in many details from various authors (VOLKENS 1884, LÜTTGE 1971, HILL 1967, ZIEGLER & LÜTTGE 1967). WIEHE & BRECKLE (1990) have demonstrated that the whole apparatus of this salt gland consists of 20 cells and that this structure is rather different in effectiveness in different species. In Fig. 8 the selectivity to Na and K in shoots and salt glands in three *Limonium* species is shown. It is remarkable that the degree of selectivity differs widely. In general the salt glands keep the cytoplasmic mesophyll low in sodium, but much less effective in *Limonium sinuatum*.

A second interesting effect beyond ion selectivity is the fact that the activity of the salt glands apparently is retarded. They only start beyond a specific threshold value of salt concentration in leaves. In Fig. 9 it is clearly indicated that with increasing Cl⁻-concentration in leaves only beyond 250 μM Cl⁻ per g H₂O the concentration in the recret fluid is sharply increasing.

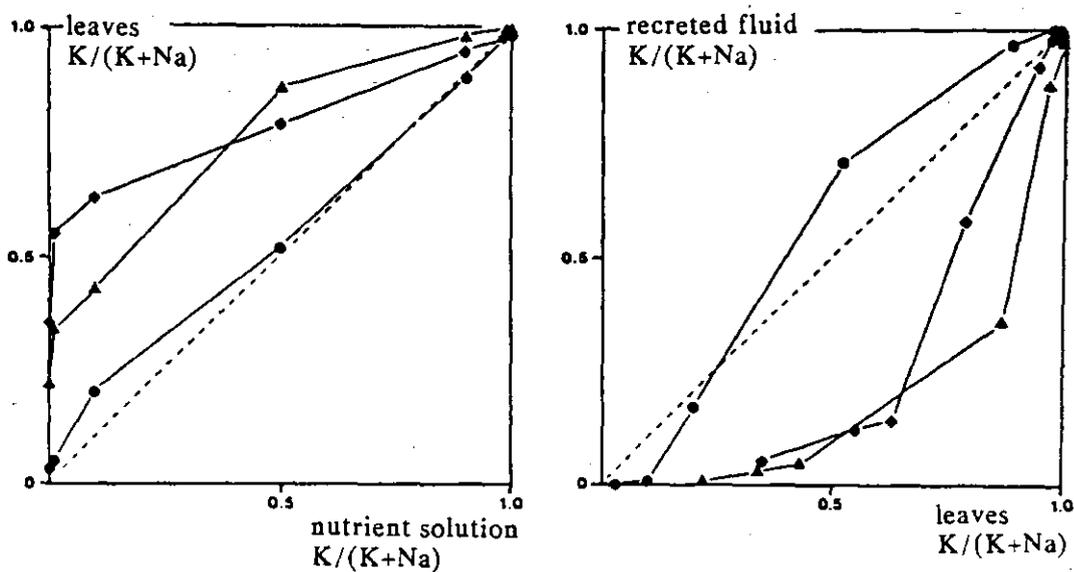


Fig. 8 : Sodium/Potassium selectivity in shoots and saltglands of three *Limonium* species (WIEHE 1986); triangles: *Limonium ramosissimum*; squares: *Limonium gmelinii*; circles: *Limonium sinuatum*; diagonal indicates 1 : 1 - selectivity. Left side: ion ratios from nutrient solution to leaves; right side: ion ratios from leaf to recreted fluid.

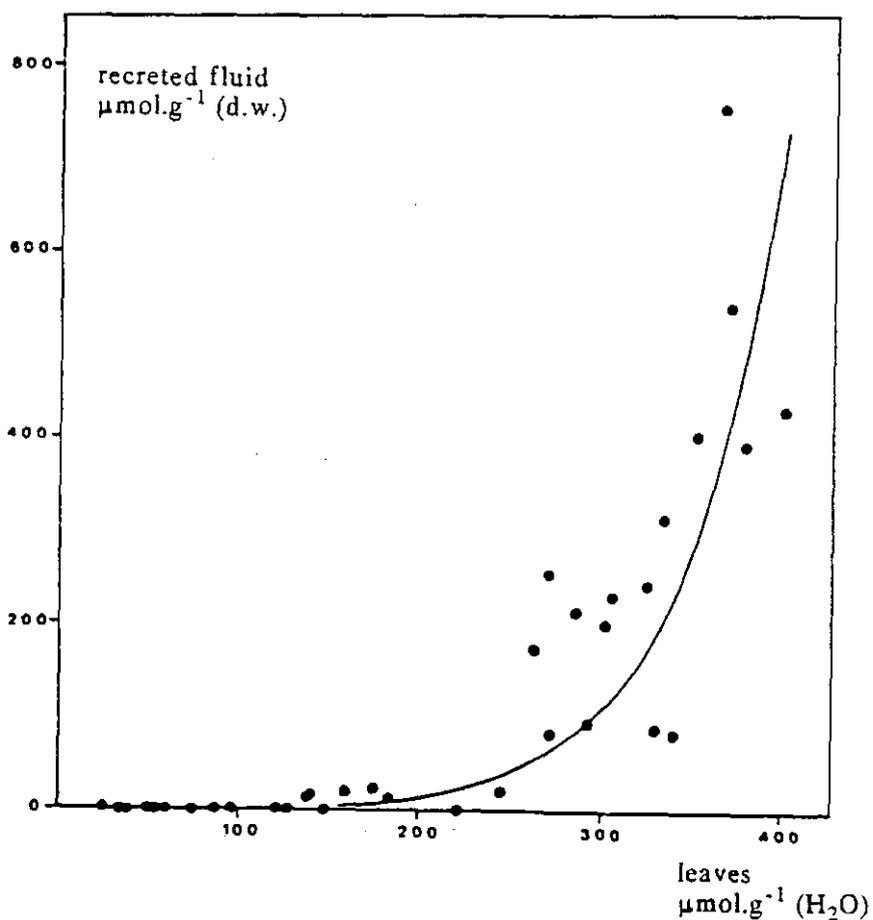


Fig. 9 : Retarded increase of chloride-concentration in recreted sap from *Limonium sinuatum* with increase of leaf-chloride-concentration in leaves (WIEHE 1986)

3.2.3 Bladders in *Atriplex*

Whereas plants with salt-glands apparently cope with salt-stress by an increased activity under higher salinities, those plants characterized by bladders have another metabolic feature. They recrete salt from the beginning, from low salt-stress on, sometimes reaching saturation already under low salt-stress (100 mM NaCl), others far beyond 250 mM. (Fig. 10).

In all *Atriplex*-species the enrichment-factor of salt in the bladder corresponding to the nutrient solution drops with increasing salinity. In Fig. 11 this is shown for *Atriplex falcata*, an dwarf-shrub of the semi-deserts in Utah, which was cultivated in sand-culture experiments with increasing salinities (BRECKLE, 1976). The same was true for *A. confertifolia*, and a similar behaviour could be shown for the annual *Atriplex hortensis* (SCHIRMER & BRECKLE 1982).

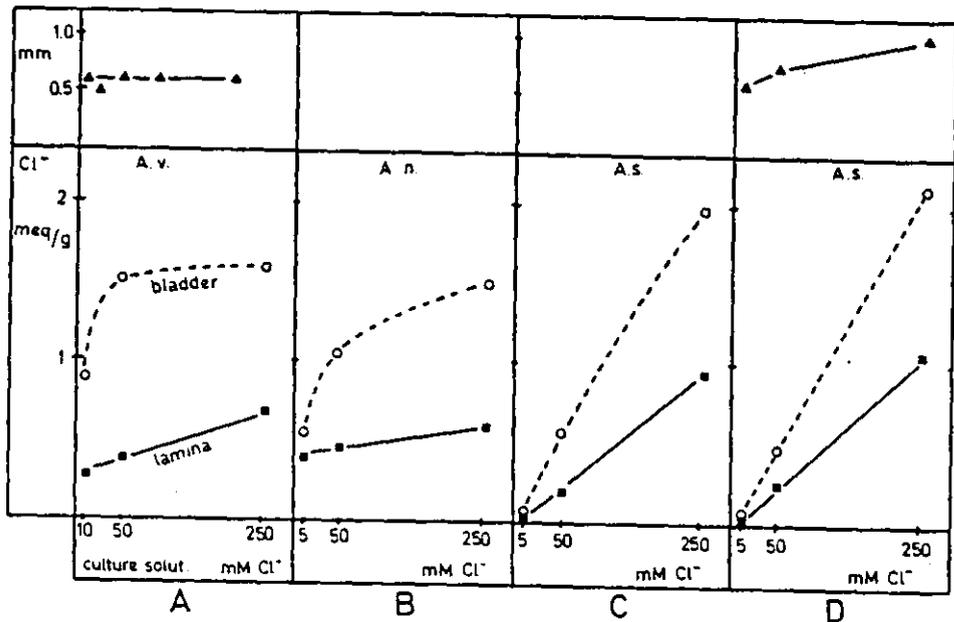


Fig. 10 : Concentrations of Cl⁻ in lamina and bladders of *Atriplex vesicaria* (A.v.), *A. nummularia* (A.n.) and *A. spongiosa* (A.s.) grown in nutrient solution containing NaCl. In A and D thickness of leaf lamina is indicated (OSMOND et al. 1980)

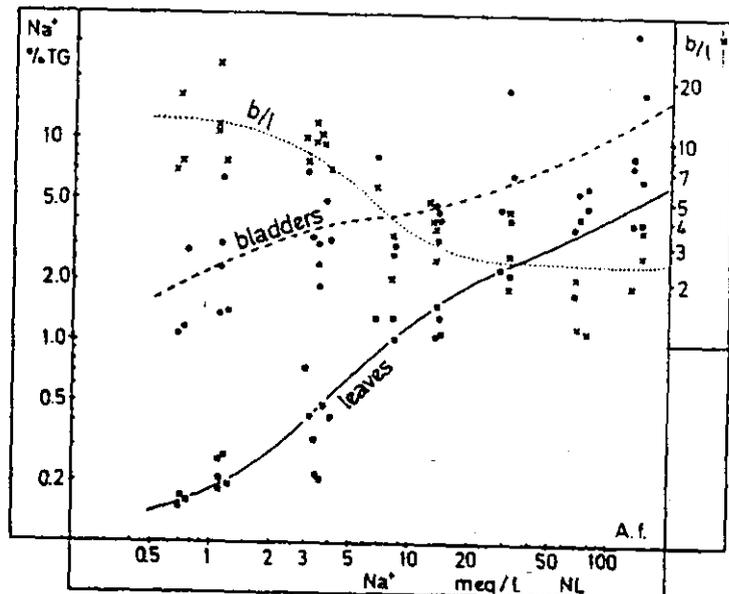


Fig. 11 : Sodium percentage in leaves (l) and bladders (b) from *Atriplex nutallii* (= *A. falcata*), cultivated in quartz sand with increasing sodium concentration in nutrient solution (BRECKLE 1976). Indicated is also the molar ratio of sodium: b/l

It can be shown that the accumulation ratio by bladder bearing plant species is relatively higher in *Atriplex* than in *Chenopodium*. In Tab. 4 the accumulation of Cl⁻ -ions in bladder hair of some Chenopodiacean genera is shown.

Table 4 : Accumulation of Chloride-Ions in Bladder Hair of *Atriplex*-,
Halimione- and *Chenopodium*-species (B: Bladder; L: Mesophyll)

NS: mM NaCl in nutrient-solution;

AR1: accumulation-ratio = $B/L+B$; AR2: accumulation-ratio = B/L

species	NS	AR1	AR2	Author
<i>Atriplex nummularia</i>	50	0.73	2.8	OSMOND et al. 1969
<i>A. spongiosa</i>	50	0,70	2.3	dto.
<i>Halimione portulacoides</i>	100	0.71	2.5	BAUMEIST. & KL. 1974
<i>Atriplex confertifolia</i>	100	0.69	2.1	BRECKLE 1976
<i>Atriplex confertifolia</i>	50	0.73	2.8	dto.
<i>Atriplex falcata</i>	100	0.59	1.4	dto.
<i>Atriplex inflata</i>	250	0.76	3.1	OSMOND 1979
<i>Atriplex nummularia</i>	50	0.71	2.5	dto.
<i>Atriplex spongiosa</i>	50	0.68	2.1	dto.
<i>Atriplex vesicaria</i>	50	0.76	3.1	dto.
<i>Atriplex spongiosa</i>	50	0.67	2.0	OSMOND et al. 1980
<i>Atriplex vesicaria</i>	50	0.71	2.5	dto.
<i>Atriplex hortensis</i>	100	0.70	2.4	SCHIRM./BRECK. 1982
<i>Chenopodium album</i>	100	0.58	1.3	REIMANN/BRECK. 1985
<i>Chenopodium giganteum</i>	100	0.60	1.5	dto.
<i>Chenopodium murale</i>	100	0.59	1.4	dto.
<i>Atriplex littoralis</i>	50	0.75	3.0	FREITAS/BRECK. 1992
<i>Atriplex calotheca</i>	50	0.84	5.3	dto.

Under moderate salinity-stress the huge amount of bladders is able to accumulate a rather high proportion of salts in the vacuoles of the bladders. Thus, these salts are removed, mainly from meristematic tissues in the plant. This seems to be the main function of bladders in those species. This is strengthened by the observation, that bladders during ontogenesis are very early formed. In very young leaves close to the vegetation tip the volume of bladders may be more than double of that of the rest of the leaf. In adult leaves the volume may be equal to that of the mesophyll.

4 General and ecological conclusions

A typical halo-series along a salt-gradient was shown by KEARNEY et al. already in 1915 from the Great Salt Lake area (Fig. 12).

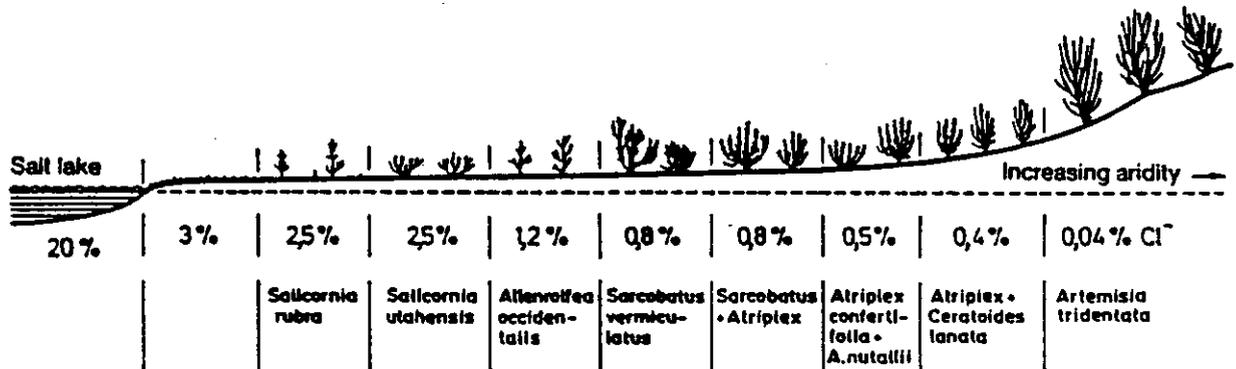


Fig. 12 : Diagrammatic profile of the haloseries on the shore of the Great Salt Lake, Utah (USA) showing chloride percentage of dry soil weight in the individual vegetational belts (KEARNEY et al. 1914, BRECKLE 1976)

Under natural conditions with high competition it was shown by BRECKLE (1986, 1989, 1990), that the halo-series in those floristic areas, where enough halophytic species and types are present (this is dependent on vegetation history), a distinct sequence of life-forms and halophyte-types develops. This can be taken as proof for the competitive force of the halophyte-types. Almost in all investigated salt-deserts (Namib, Australia, Atacama, Afghanistan, Iran, Uzbekistan, Negev, Sahara, Utah) or along salty lakes (Spain, Romania, Turkey, Israel, South Africa) the sequence of halophyte-types was more or less similar. At the most saline end (which is also more moist) stem-succulent Eu-Halophytes dominate and are within the halo-series intermixed with leaf-succulent ones, which often have a rather wide range. On moderate salinities (often more dry) mainly the various types of recretating halophytes occur and have to cope with a wide amplitude of salt-stress and partly as well as with drought-stress. To the less saline side in the sequence other pseudo-halophytes and non-halophytes intergrade and lead to the zonal vegetation mosaic.

Summarizing conclusions:

- salt-glands have evolved several times independantly in higher plants and are found in several different plant families -- forced by salt-stress
- bladder structures with glandlike stalk cells are typical for some genera in Chenopodiaceae
- activity and selectivity for ions differs from genus (species) to genus (species)
- Along salt gradients (natural stands, salt lakes) recretating halophytes have their ecological optimum (with few exceptions) mostly on moderate salinities (paralleled by moderate aridity?)
- Thus recretion of salts is only one of several adaptations to cope with salt-stress, but a very interesting one: with a very close correlation of structure and function!

5 References

- ALBERT, R. (1982) Halophyten; in KINZEL, H.: Pflanzenökologie und Mineralstoffwechsel.- Ulmer p.33-215
- BAUMEISTER, W. & G. KLOOS 1974 Über die Salzsekretion bei *Halimione portulacoides* (L.) Aellen. - Flora 163: 310-326
- BERGER-LANDEFELDT, U. 1959 Beiträge zur Ökologie der Pflanzen nordafrikanischer Salzpflanzen.- Vegetatio 9: 1-48
- BRECKLE, S.-W. 1976 Zur Ökologie und zu den Mineralstoffverhältnissen absalzender und nichtabsalzender Xerohalophyten (unter besonderer Berücksichtigung von Untersuchungen an *Atriplex confertifolia* und *Ceratoides lanata* in Utah/USA).- Diss.Bot. 35: 169pp.
- BRECKLE, S.-W. 1986 Studies on halophytes from Iran and Afghanistan. II. Ecology of halophytes along salt gradients.- Proceed. Royal Soc. Edinburgh 89B: 203-215
- BRECKLE, S.-W. 1989 Role of salinity and alkalinity in the pollution of developed and developing countries.- in "Plants and pollutants in developed and developing countries, by ÖZTÜRK, M.A., (ed.) p. 389-409
- BRECKLE, S.-W. 1990 Salinity tolerance of different halophyte types.- in BASSAM, N.EL (eds.): Genetic aspects of plant mineral nutrition, Kluwer/NL. p. 167-175
- BRESLER, E., B.L.MCNEAL & D.L.CARTER 1982 Saline and Sodic soils.- Adv. Ser. Agric. Science 10, Springer, 236p.
- CHAPMAN, V.J. 1974 (Repr.) Salt Marshes and Salt Deserts of the World.- Cramer 392p.
- CHAPMAN, V.J. 1976 (Repr.) Mangrove Vegetation.- Cramer 447p.
- DIJKEMA, K.S.(ed.) 1984 Salt Marshes in Europe.- EG, Strasbourg 178p.
- ELLENBERG, H. 1986 Vegetation Mitteleuropas mit den Alpen (4. Aufl.).- Ulmer 989p.
- FREITAS, H. & S.-W.BRECKLE 1992 Importance of bladder hair for salt tolerance of field-grown *Atriplex* species from a Portuguese salt marsh.- Flora 187: (in press)
- GRAETZ, R.D. & K.M.W.HOWES 1979 Studies of the Australian Arid Zone IV. Chenopod Shrublands.- CSIRO 196p.
- HADAS, A., D.SWARTZENDRUBER, P.E.RIJTEMA, M.FUCHS & B.YARON 1973 Physical Aspects of Soil Water and Salts in Ecosystems.- Ecol. Studies 4: 460p.
- HILL, A.E. 1967 Ion and water transport in *Limonium*. I.-IV. Biochem. Biophys. Acta 135: 454-460, 461-465; 136: 66-72, 73-79
- Jones, R. 1970 The Biology of *Atriplex*, CSIRO, Canberra 128p.
- KREEB, K.H. 1964 Ökologische Grundlagen der Bewässerungskulturen in den Subtropen.- Fischer/Stuttgart 150p.

LEVITT, J. 1980 Responses of plants to environmental stresses, vol. I, II, 497, 606pp.; Acad. Press NY, London

LIPHSCHITZ, N. & Y. WAISEL 1982 Adaptation of plants to saline environments: salt excretion and glandular structure.- in Tasks for Vegetation Science, vol. 2 (ed. by D.N.SEN & K.S.-RAJPUROHIT), Junk/Nl p. 197-214

LÜTTGE, U. 1966 Funktion und Struktur pflanzlicher Drüsen.- Naturwiss. 53: 96-103

OSMOND, C.B. (1979) Ion uptake, transport and excretion. in PERRY, R.A. & D.W.GOODALL (eds.): Arid Land ecosystems; structure, functioning and management. IBP 16, vol. I, Cambridge Univ. Press, p. 607-625

OSMOND, C.B., U.LÜTTGE, K.R.WEST, C.K.PALLAGHY & B.SHACHER-HILL 1969 Ion absorption in *Atriplex* leaf tissue. II. Secretion of ions to epidermal bladders.- Austr. J.Biol. Science 22: 797-814

OSMOND, C.B., O.BJÖRKMAN & D.ANDERSON 1980 Physiological Processes in Plant Ecology: Towards a synthesis with *Atriplex*.- Ecol. Stud. 36, Springer 468p.

POLJAKOFF-MAYBER, A. & J.GALE 1975 Plants in Saline Environments.- Ecol. Stud. 15, Springer 213p.

POLLAK, G. & Y.WAISEL 1970 Salt secretion in *Aeluropus litoralis* (Willd.) Parl. - Ann.Bot. 34: 879-888,

REIMANN, C. & S.-W. BRECKLE 1987 Salt secretion in some *Chenopodium*-species.- Flora 180: 289-296

REIMOLD, R.J. & W.H.QUEEN 1974 Ecology of Halophytes.- Acad.Pr. NY/London 605p.

RICHARDS, L.A. (ed.) 1954 Diagnosis and Improvement of Saline and Alkali Soils.- USDP Handbook No. 60, Wash.DC, 160p.

SCHIRMER, U. & S.-W. BRECKLE 1982 The role of bladders for salt removal in some Chenopodiaceae (mainly *Atriplex*-species) in Tasks for Vegetation Science, vol. 2 (ed. by D.N.SEN & K.S.RAJPUROHIT), Junk/NL p. 215-231

SEN, D.N. & K.S.RAJPUROHIT 1982 Contributions to the Ecology of Halophytes. Tasks for Vegetation Science, Junk/NL, vol. 2, 272pp.

SHAINBERG, I. & J.SHALHEVET 1984 Soil Salinity under Irrigation.- Ecol. Stud. 51, Springer 349p.

STROGONOV, B.P. 1964 Physiological Basis of Salt Tolerance of Plants.- (engl. transl. by IPST) 280p.

UNGAR, I. 1991 Ecophysiology of vascular halophytes.- CRC Press/London, 250 pp.

VOLKENS, G. 1884 Die Kalkdrüsen der Plumbagineen.- Ber. Dtsch. Bot. Ges. 2: 334-342

WAISEL, Y. 1972 Biology of Halophytes.- Acad.Pr.NY/London 395p.

WALTER, H. 1973 Die Vegetation der Erde in ökophysiologischer Betrachtung.- (3. Aufl.) Fischer/Stuttgart-Jena 743pp.

Walter, H. & S.-W. Breckle 1991 Ökologie der Erde, Bd. 1 (2. Aufl.).- Fischer/Stuttgart 238p.

WIEHE, W. 1986 Untersuchungen zur Salzsekretion bei ausgewählten *Limonium*-Arten.- Dipl.Thesis Univ. Bielefeld, 136 pp.

WIEHE, W. & S.-W. BRECKLE 1990 Die Ontogenese der Salzdrüsen von *Limonium* (Plumbaginaceae).- Botanica Acta 103: 107-110

ZIEGLER, H. & U. LÜTTGE 1966, 1967 Die Salzdrüsen von *Limonium vulgare*. I., II.- Planta 70: 193-206; 74: 1-17

The Black Bedouin Goat

Amiram SHKOLNIK.

Department of Zoology, Tel-Aviv University,
Ramat-Aviv 69978, Israel

In areas of extreme aridity that are characterized by abrupt transition from abundance of both lush forage and plentiful water supply to prolonged period where both adequate food and water are at a premium, the small body sized sheep and goats profoundly outnumber any breed of cattle. In the dry deserts of the Middle East, only small body sized black goats (11-25 kg) accompany man into the harsh wilderness. These dwarf ruminants were first described, as a distinct breed of goats, by EPSTEIN (1946) in the Hajaz and Midian, both areas of extreme aridity in the Arabian peninsula. SHKOLNIK et al (1972) have described the same dwarf breed dominating the herds of the Bedouin tribesmen in the Sinai and the southern parts of the Negev desert of Israel.

The abundance of the herds of the black goats in the barren, sun scorched terrain arises many questions: Is a small body size advantageous to a ruminant in such a stressful environment? Were these goats selected, by the indigenous inhabitant of the desert, because their small body size lend them some relative productive merits in the arid environment? Is there some optimal size which will maximize the productive merits, i.e. the efficiency at which energy in food is being converted to energy in meat or milk (BRODY 1945), of a ruminant selected to be productive under stressful arid condition?

In most arid regions of the world, including the East Mediterranean Basin, abundance of food is available only during a limited period of time, that follows a short and erratic rainy season. The first rain that adds moisture to the dry soil triggers, in these areas, extensive growth of both annual and perennial plants and induces intensive photosynthetic activity. Within a few days, lush green pasture dramatically change the former barren terrain. In the Middle East shepherds refer to such exuberant period as "Esheb" or "Rabia" meaning: grass, pasture and regard it as god's blessing. Very rarely however will such a blessing last more than a couple of months. The heat spells that mark the beginning of the dry season, enhance the exhaustion of soil moisture and wilting of the plants rapidly ensues. Meager dry hay and emaciated dwarf shrubs will comprise the bulk of the forage available to livestock throughout the rest of the year. A capacity to maximize production during the short period of lush pasture on the one hand and to survive during the lean period on the other, are the characteristics a herdsman in arid areas will select most eagerly. The small body sized breed of goat appear to have been selected according to such criteria. This assumption was often challenged on the basis of allometric considerations.

As the energy required for the production is often regarded to be "superimposed" on the maintenance energy of the animal, one tends to argue that a large body sized ruminant should be expected to be a more efficient producer than its small body sized kindred. The logic behind such arguments is usually based on KLEIBER's widely known "Mouse to Elephant" equation. This equation implies that the energy a mammal requires for maintenance, instead of being proportional to body mass, is allometrically related to it as a three quarter power of the animal's body mass: $MR = K \cdot M^{0.75}$

In such allometric perspective, increase in size within the ruminants can be reviewed as an energy sparing mechanism (table 1.). It implies that a 400kg cow is likely to require for maintenance only 10 times more energy than a 20 kg goat. In other words: the cost of maintenance of one kg of tissue in the 20 kg ruminant is likely to be twice as costly as to maintain the same mass of tissue in the 400 kg cow.

Table 1 : Metabolic rate and mass specific metabolic rate of a goat and a cow, calculated from KLEIBER's equation

	body mass (kg)	kcal.day ⁻¹	kcal.kg ⁻¹ .day ⁻¹
goat	20	662	33
cattle	400	6261	16
ratio	1/20	1/10	2/1

On the basis of such generalizations one may be tempted to conclude that small ruminants wastes more food than the large one. Goats, according to such reasoning, are likely to be far inferior to cows in their capacity to cope with the shortage of food that characterizes arid habitats. KLEIBER himself, in his monumental book "The Fire of Life" (1961) has refuted this fallible reasoning (fig 1.). KLEIBER compared the ratio at which one ton of hay is converted into body mass by 300 rabbits, to the ratio it is converted into body mass by one steer; the collective body mass of the the 300 rabbits being equivalent to the mass of the one steer (590kg). As demonstrated in fig. 1, the collective metabolic rate of the rabbits was four times that of the steer. Within one month, they have exhausted the ton of hay that was offered to them and collectively gained 109 kg. For the steer, the ton of hay lasted for four months, but its gain in body mass during this time was, nevertheless, the same as in the rabbits, namely 109 kg.

	1 ton of hay	1 ton of hay
Animals	1 steer	300 rabbits
Total body weight	590 kg	590 kg
Food consumption per day	7.6 kg	30 kg
Duration of 1 ton of food	120 days	30 days
Heat loss per day	20,000 kcal	80,000 kcal
Gain in weight per day	0.9 kg	3.6 kg
Gain from 1 ton of food	109 kg	109 kg

Fig.1: The example quoted by Max KLEIBER to demonstrate that productive merits (the efficiency at which food is being converted to an agricultural product) are about the same in animals of different size. Small animals, according to this example, can, however, convert the same amount of food into the same amount of protein much more rapidly than large animals can do. (Modified from KLEIBER 1961)

While, according to KLEIBER's enlightening proposition (also known as "Kleiber's law"), body size does not effect the efficiency of food utilization, it still has a profound effect on the length of time required for accomplishing the process. In the example Kleiber quoted, the time required by the small body sized rabbits to produce the 109 kg of meat was only one quarter the time required, for the same accomplishment, by the steer. According to these principles we may conclude that body size in ruminant may not be a factor determining the efficiency at which food is utilized for production. Nevertheless it still profoundly affects the length of time required for converting a given amount of food into agricultural products. In other words, food consumption and its conversion to ruminant products, are both accelerated in the small body sized animal. On these grounds we should examine, now, the advantage of tending small, rather than large animals in the arid zones of the world.

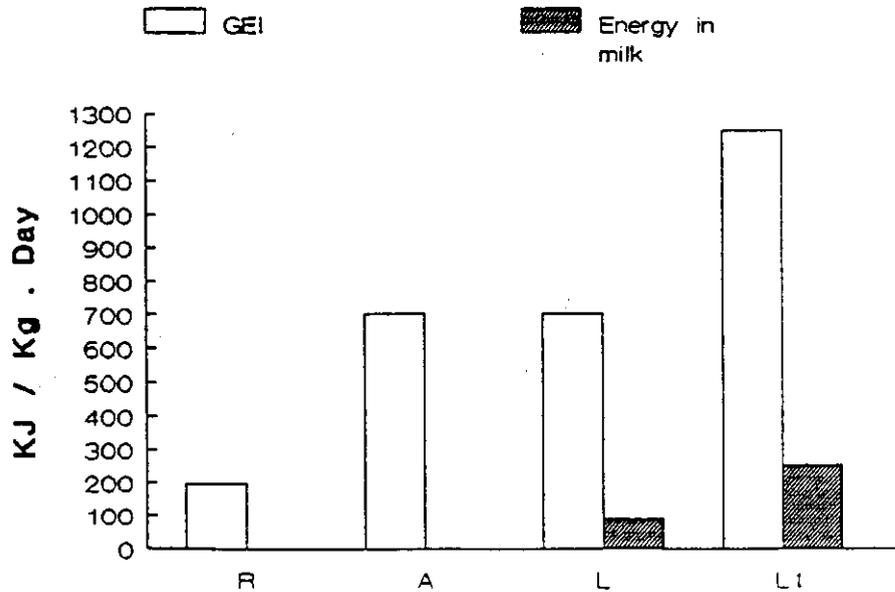


Fig.2 : Gross energy intake of Bedouin goats maintained on different planes of nutrition. R - Non-lactating on an energy intake restricted to 30% the ad lib consumption (BROSH et al.1986)
 A - Non lactating on ad lib food consumption (BROSH et al 1986)
 L - Lactating on food consumption restricted to the ad lib consumption of a non lactating goats (MALTZ et al 1982).
 L1 - Lactating on ad lib consumption (MALTZ et al 1982).

Kidding and lambing in arid countries are usually timed to the the period when pasture is plentiful. The energy intake of a goat, that during the dry season will amount to only 4160 kJ.day⁻¹, will increase during peak lactation to 25000 - 30000 kJ.day⁻¹ (fig.2.). Concurrently, the daily milk yield of the lactating goat may often exceed 10% of the animal body mass (MALTZ et al 1982).

According to BRODY's (1945), mammals capable of converting 25% of their digestible energy intake into energy in milk are considered as possessing superior dairy merits. In the Bedouin goat, a conversion coefficient ranging from 34% at peak lactation (2nd month post partum), to 25% at the end of the 5th month post partum, was reported by MALTZ et al (1982).

It may be concluded that during the short period adequate for production in the desert, the small body size of the black Bedouin goat can indeed be regaded as a time sparing mechanism. It enables these ruminant to fully exploit the desert resources for the benefit of their owner. A question remained to be answered: how these goats survive the prolonged harsh period that follows the one of favorable conditions?

When environmental condition deteriorate at the onset of the dry season, production gradually diminishes. Energy requirement drops to maintenance levels (fig.2.). Even on this plane of nutrition, lactating goats produce a volume of milk sufficient to meet the demand of a young kid. However, when food availability deteriorates further, the meeting even of these requirement becomes a severe challenge. Indeed in desert adapted ruminants a "near maintenance energy requirement" far more frugal than those of their relatives that are traditionally raised in temperate countries is repeatedly reported. Livestock adapted to the arid condition, have demonstrated in the laboratory, a capacity to maintain a constant body mass even when their food intake drops to 50-40% their ad lib food consumption. This they can do by virtue of two major strategies, complementary to each other: economizing their energy expenditure, and efficiently digest low quality forages.

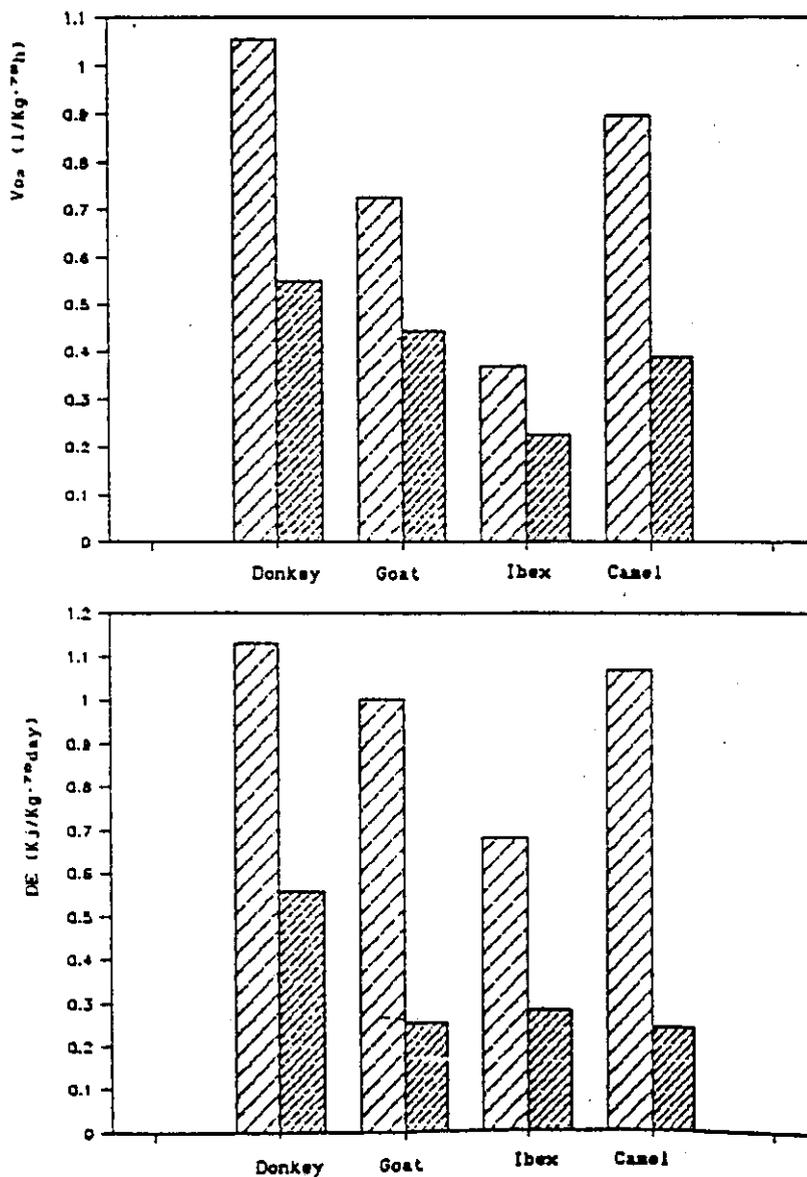


Fig.3: Digestible energy intake and oxygen consumption of donkeys goats ibexes and camels, fed either alfalfa hay (a high quality roughage; left bar) or wheat straw (a low quality one; right bar)

A so called, "Basal Metabolic Rate" lower than the value expected from the animals body mass (according to KLEIBER's equation mentioned above), is an adaptation that distinct a wide range of desert mammals and most livestock, indigenous to the arid environment, from their relative that reside in temperate and mesic habitats (SHKOLNIK 1988). The maintenance energy expenditure of desert adapted sheep ,goats, camels and donkeys is regularly 20-40% lower than that of their non desert kindred. Furthermore: under nutritional stress, like the one to which they are subjected to during the prolonged dry season , the metabolic rate of these animals drops further untill it balances the 50-60% reduction in energy intake (fig. 3).

The ways deserts dwelling mammals manage to lower their energy expenditure far below "Basal" levels, is still obscure. It is however an instrumental element in their capacity to survive the prolonged lean season and withstand even continous years of draought that, in the arid zones only too often occur.

Now, let us turn to digestive efficiency? Can it be that a small body size is related also to efficient digestion of low quality forage? Allometric consideration postulate the opposite. DEMMENT and VAN-SOEST (1983, 1985) in a number of publications have argued that the time the digesta are retained in the gut,subjected to bacterial degradation (the so called mean retention time, MRT), is likely to be longer, and consequently digestive efficiency to be higher, the larger the animal is. This they base on the asumption that gut capacity is isometric to body size (PARA 1978) while energy requirements are related allometrically, as power function (0.75), to it. As a result the ratio of gut capacity to food requirement will be higher in the big body sized animal than in the small one. This will allow the digesta to stay longer in the gut of the large body sized animal than in that of the small body sized one.

A comparison of digestive efficiency in Bedouin and in Swiss Saanen goats contradict the above mentioned generalization (SILANIKOVE 1985). The MRT and consequently digestive efficiency are both significantly higher in the Bedouin goat than in the twice as large Swiss one (table 2).

Table 2 : Water turnover, Food (dry Rhodes grass) intake and digestibility in Bedouin Goat and in Swiss Saanen goat (from SILANIKOVE 1980)

	Bedouin	Saanen
Body mass (kg)	19.5	49.1
DM intake ($\text{kg} \cdot \text{kg}^{-0.75} \cdot \text{day}^{-1}$)	72.7	72.5
Digestibility (%)	68.5	61.5
WTO ($\text{ml} \cdot \text{kg}^{-0.75} \cdot \text{day}^{-1}$)	59.0	75.4
Rumen: Fluid flow ($\text{ml} \cdot \text{kg}^{-0.75} \cdot \text{h}^{-1}$)	37.6	51.5
Rumen: MRT (h)	41.6	31.6
MRT in total GIT (h)	56.8	45.4

The contention proposed by DEMMENT & VAN-SOEST, apparently overlooks disparate characteristics that distinguish certain species and breeds. It is well established that goats as well as sheep possess, relatively, much more spacious rumen than that of cattle and other ruminants. From extensive comparative studies in the wild as well as in domestic ruminants, it is also known that the volume of the rumen in ruminants that are predominantly grazers

exceeds those found in browsers and concentrate selectors (KEY et al 1980). ENGELHARDT (1985) demonstrated a profound direct effect of diet on reticulo-rumen of sheep. Two weeks on a high fiber diet induced, according to this report, a 14 to 22% body weight increase in the reticulo-rumen volume. It appears that adaptations to a stressful environment overrides the theoretical principles.

The rumen in ruminant that thrive in the arid zones, serves not only as a spacious fermentation vat. In Bedouin goats (CHOSHNIAC and SHKOLNIK 1978) and in Australian Sheep (HECKER et al 1964) it was described as also playing a role of a voluminous water reservoir and is viewed as an important adaptation to life in the desert. In water depleted arid vistas, such a reservoir enables sheep and goats withstand prolonged period of water deprivation and helps the animals widen their grazing range around the sparse water points. Such a capacity to withstand prolonged period of water deprivation was found coupled to a capacity to rapidly rehydrate and to replenish huge water deficits within one continuous drinking bout (SHKOLNIK and CHOSHNIAC 1985).

Similar to their frugal energy requirements, ruminant native to the arid environment possess also an economic water metabolism. Coupled to the ability to store water, the frugal water economy of the black Bedouin goats enables them to graze at great distance from the watering sites and evenly exploit the meager pasture. A high rate of stocking can consequently be achieved (SHKOLNIK and CHOSHNIAC 1985). A low rate of water turnover and a capacity to drink infrequently affect food utilization in many ways: a basic linear relationship between water requirements and digestible energy intake was demonstrated by SILANIKOVE (1989) in goats, irrespective of their breed. MOUALEM et al (1990) have shown in goats a negative relationship between water consumption and roughage digestibility that exist not only irrespective of the breed but also of the body size of the animal (fig. 4).

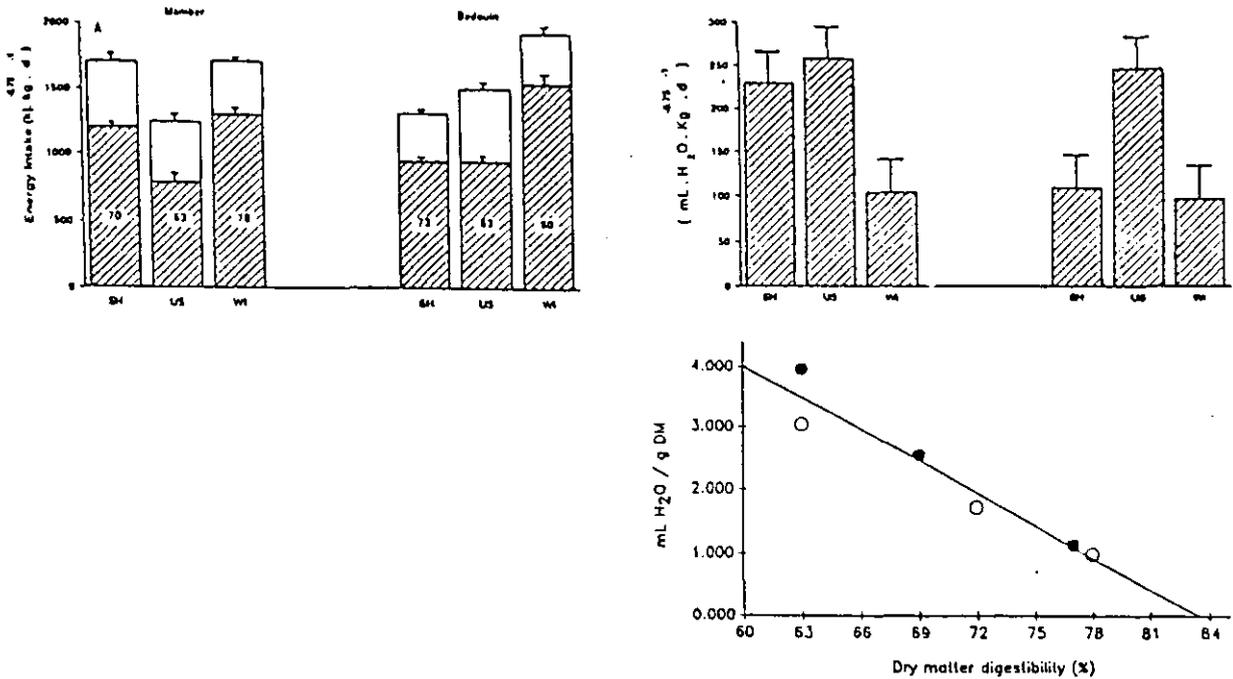


Fig.4: Gross energy intake, dry matter digestibility (panel A), and water intake (panel B), in Mammer goats from a Mediterranean temperate region and of Bedouin goats from the extreme desert, exposed to different heat loads. SH - summer in shade; US - unshaded during summer; WI - winter conditions, temperature below 15°C. Panel C: The ratio of water consumption to dry matter intake (Y) plotted against dry matter digestibility obtained under the different heat loads. The regression demonstrates that irrespective of the goats Breed or size a frugal water economy favors utilization of roughage by goats.

The effects of infrequent drinking on the energy balance of Bedouin goats, maintained on either high (alfalfa hay) or low quality (wheat straw) roughages, was extensively studied by BROSH et al (1986). These authors found that infrequent drinking hamper food intake only when the animal is maintained on high quality roughage (table 3). Its effect on the consumption of forage high in fiber, such as the one available during the lean season, is negligible. Concomitantly, infrequent drinking was shown to improve fiber digestibility.

Table 3 : Factors affecting digestive efficiency of black Bedouin goats when fed either high quality roughage (Alfalfa hay: 25% fiber, 20% protein) or low quality ones (Wheat straw: 40% fiber, 3.5% protein) and watered either once daily or only once every 4 days

watering:	once daily	once daily	once every 4 days	once every 4 days
feed quality:	high	low	high	low
water consumption (ml.kg ^{-0.75} .day ⁻¹)	248	101	138	93
Flow through R.O.O. (ml.kg ^{-0.75} .day ⁻¹)	1243	832	604	494
M.R.T. (h)	47	70	63	90
particle size; % smaller than 1 mm	68	--	82	--
% smaller than 0.4 mm	37	--	52	--

It was suggested that infrequent drinking favours digestibility of rough aged by inducing a marked reduction in the rate of the gut's fluid flow and consequently an extension of the MRT and an increase of the share of the small sized particles in the digesta. Under such an extension of the MRT even lignin digestibility was demonstrated (SILANIKOVE and BROSH 1989). Indeed BROSH et al (1986) have shown that Bedouin goats exposed to outdoors conditions, are capable of maintaining a constant body mass only when they are watered infrequently. MITTAL and GOSH (1986) in the arid country of Rhagastan maintained Magra and Marwari sheep for a period of 39 months during which the animals were watered twice a week. They have investigated the effect of infrequent drinking on reproductive indices of their animals and found no ill effect. No ill effect of infrequent drinking on the reproductive indices was demonstrated in these sheep.

It may be concluded that when ever sheep and goats encounter adverse nutritional conditions, infrequent drinking is by no means disadvantageous. This conclusion is of great importance to the management of sheep and goats in the arid environment where water projects are being developed in order to enhance the economy. Increasing the number of watering sites facilitates frequent drinking. An increase in energy expenditure and in water turnover rates follows. Digestibility is suppressed and an increasing demand for food ensues. The animals, now restricted to the close vicinity of the water, rapidly denude the area close to the watering point and vast areas of pasture remain unexploited. Mal nutrition becomes inevitable.

SCHENKER and GOUMANDAKOYE (1987) report from Niger and other Sahalian countries that a project involving the development of many new borholes resulted in overgrazing, trampling and excessive loping of browse trees. Lack of pasture became the main problem. Incipient soil erosion indicated the beginning of the desertification process.

A similar report, this time from the Lake Chad Basin, was published by WANZIE (1990) "concentration of animals in such areas, especially in the dry season has only resulted in over-utilization of limited areas of the ecosystem and the death of animals". Basic physiological consideration teach us that the development of water project in arid country must be accompanied by the development of the food resources. Otherwise it may be deleterious to pastoralism as well as to the entire ecosystem. Most likely that the goats will be blamed for it.

References

- BRODY, A. 1945. Bioenergetics and growth. Hefner Pub. N.Y.
- BROSH, A., SHKOLNIK, A., CHOSHNIK, I. 1986. Metabolic effects of infrequent drinking and low quality feed on Bedouin goats.- *Ecology* 67:1086-90
- CHOSHNIK, I. and SHKOLNIK, A. 1978. The rumen as a protective osmotic mechanism during rapid rehydration in the black Bedouin goat. in: *Osmotic and Volume regulation*. Alfred Benson Symposium XI; Munksgaard Copenhagen.
- DEMMENT, M.W. and VANSOEST, P.J. 1983. Body size, digestive capacity and feeding strategies of herbivores. Winrock International. Morrilton Arkansas.
- DEMMENT, M.W. and VANSOEST, P.J. 1985. A nutritional explanation for body size pattern of ruminant herbivores.- *Am. Natural.*: 125: 641-72.
- ENGELHARDT, W.V. DELLOW, D.W., HOELLER, H. 1985. The potential of ruminants for the utilization of fibrous low quality diets.- *Proc. Nut. Soc.* 44:37-43.
- EPSTEIN, H. 1946 The Hejaz dwarf goat.- *J.Hered.* 37:345.
- HECKER, J.F., BUDTZ-OLSEN, D.E., OSTWALD, M. 1964. The rumen as a water storage in sheep.- *Aust. J. Agric. Res.* 15:961.
- KEY, R.N.B., ENGELHARDT, W. A. and WHITE, R.G. 1980. Digestive Physiology and Metabolism in Ruminants.- pp. 743-61. (Eds) ROUCKEBUSCH, Y. and THIVEND, P.- Lancaster MTP Press Ltd.
- KLEIBER, M. 1961. The fire of life. John Wiley and Sons Inc. Publishers.
- MALTZ, E., SILANIKOVE, N., SHKOLNIK, A. 1982. Energy cost and water requirements of black Bedouin goats at different levels of production.
- MOUALEM, R., CHOSHNIK, I., SHKOLNIK, A. 1990. Environmental heat load, bioenergetics and water economy of two breeds of goats: The Mamber goat and the black Bedouin goat.- *Rev. Anim. Prod.* 25: 91-95. Wld.
- PARRA, R. 1978. Comparison of foregut and hindgut fermentation in herbivores. in: G.G. Montgomery (ed) *The Ecology of Arboreal Folivores*. pp 205-230 Smithsonian Ins. Press
- SHKOLNIK, A. 1988. Physiological adaptations to the environment: the Israeli experience.- in: (Eds) YOM-TOV, Y., TCHERNOV, E. *The Zoogeography of Israel*.- Dr.W.Junk Pub. Dordrecht.
- SHKOLNIK, A., BORUT, A., CHOSHNIK, I. 1972. Water economy of the Bedouin goat.- *Symp. Zool. Soc. Lond.* 31:229-242.
- SHKOLNIK, A., CHOSHNIK, I. 1985. Physiological responses and productivity in goats. in *Stress Physiology in Livestock* (Ed) YOUSEF, M.K. Vol.I pp 39-57 CRC Press.
- SILANIKOVE, N., 1983. Feed Utilization and Energy and Nitrogen Balance in the Desert Black Bedouin Goat.- Ph.D. Thesis submitted to Tel-Aviv University (Hebrew).
- SILANIKOVE, N. 1989. Interrelationships between water, food and digestive energy intake in desert and temperate goats.- *Appetite* 12:163-170.
- SILANIKOVE, N., BROSH, A. 1989. Lignocellulose degradation and subsequent metabolism of lignin fermentation products in the black Bedouin goat fed wheat straw as a single component diet.- *Brit.J.Nut.* 63:509-520.
- WANZIE, C.S. 1990. Water resources management and wildlife conservation in the Lake Chad

**Physiological Adaptations to Underground Life:
Reptilian Embryos, Tunnel Nesting Birds, and Fossorial
Mammals as Examples.**

Amos AR

Department of Zoology, Faculty of Life Sciences
Tel Aviv University

Present Address: Department of Physiology and School of Medicine & Biomedical Sciences
correspondence : 114 Sherman Hall, Buffalo, New York 14214 (USA) (until September 1993)
Phone: + (716) 829 2752; Fax: + (716) 829 2344; E-Mail: aar@ubmedc.buffalo.edu

Introduction

A single stimulus, or a group of stimuli, that change, disturb or interfere with the normal physiological, mental and/or emotional steady state of an organism, may be defined as a biological adaptagent, and, in cases of clearly unfavorable stimulus - biological stress. The majority of these stresses originate in the environment surrounding the organism. Environmental stress may cause impairment, injury or some weakening within the living organism - namely, a biological strain - to which an organism has to react in order to restore the degree of steady state essential for its survival. An organism may react only if the strain is somehow sensed by a detector within it. In cases where this information is transmitted to an effector either directly, or via a regulatory mechanism, a response results (ADOLPH, 1964).

Responses to environmental stresses may be learned, somatic or inherent. They may act at individual or at population levels. They may change the micro- or the macro environment, the population structure, behavior, morphology, anatomy, physiology, cellular properties and the biochemistry of organisms. They may be specific and nonspecific. But, most importantly, if successful, they relieve the stress.

The vast array of organismal responses to environmental stresses may be arranged in three main groups: conforming, avoiding, or coping with stress (PROSSER, 1973).

Conformity usually marks incapability to respond because of the absence of a detector, transmitter of information, effector, or the necessary feedback mechanism. It is typical, among other forms, of animals which inhabit stable environments, embryos, and neonates. These forms lack, or have not developed responding mechanisms; or may be indifferent to a given stress.

Avoidance involves a certain behavior which usually allows the animal to actively "walk away" and prevent confrontation with a given stress.

Coping implies that animals are capable of physiological responses which are acquired as a result of either an adjustment, acclimation, acclimatization or adaptation (BOUVEROT, 1985).

Scope

I have chosen to illustrate how are the three types of responses manifested in three land vertebrates exposed to the same stress: The stress exerted by the special living conditions below the surface of the ground. Both the choice of the examples, and the literature cited represent my own bias and do not, in any way, pretend to fully cover the subject.

Living underground, while solving some existential problems, raises others that have to be solved. Most animals are active or move when they forage for food, reproduce, or defend themselves. An appropriate shelter reduces activity (and energy) requirements as it protects from both enemies and other environmental stresses. Since the degree of protection needed is higher in eggs, newborns and young, it is not surprising to find out that caves and other underground shelters are widely used for reproductive purposes even among relatively large animals. This strategy may have led to the development of, sometimes exclusive, underground lifestyle in a wide variety of species (NEVO, 1979; BOGGS et al., 1984; AR, 1987).

The animals chosen -- reptile embryos, tunnel nesting birds, and fossorial mammals -- represent three different vertebrate classes, differing in morphology, anatomy, maturity and basically in almost every respect including the strategy of coping with their environment. However, I will limit my discussion mainly to their physiology.

The Medium

For those animals, which live in or visit the underground habitat, soil is not only a substrate but also a special living medium.

Soils differ in their physical and chemical properties. They may differ in their minerals, organic matter and inorganic components; in particle size distribution, density, compaction, cohesiveness, porosity and air filled porosity; in water capacity, content, potential and vapor pressure; in temperature, heat conductance and capacity. As a result, they differ in the absolute and relative amounts of microorganisms, fauna and flora, which may also change as a function of the soil profile and following short term, seasonal or long term climatic changes (HILLEL, 1978).

A comparative analysis shows that "light" (coarse grain and poor cohesiveness) and "dry" (low water capacity and good drainage) soils have more air filled pores and are therefore more permeable to respiratory gases than "heavy" and "wet" soils. The latter provide more stable shelters and moist conditions but exact a high cost of digging, poor aeration, and high heat conductance.

The interplay between gas permeability, water content and heat conductance is of special importance to soil dwellers. There is very little gas convection in soil in general. Some convection may occur following rain fall when gas in soil is temporarily replaced with water, and some is due to gas expansion and contraction following temperature and pressure changes. Temperature changes may be due to the daily cycle but also to the metabolic heat production of the living inhabitants (WHITE et al., 1978). Pressure changes may be related to barometric pressure, to the lift effect of wind (VOGEL et al., 1973), and to the movement activity of the dwellers (OLSZEWSKI and SKOCZEN, 1956). However, gas movements within the soil (including water vapor movements in the gas phase) are normally accomplished by diffusion. This diffusion is negligible in soil particles and is very slow in the water phase when compared with air (REID et al., 1977).

Air filled porosity may reach up to 2/3 of the total volume in loose dry soil. But, since not all air spaces are interconnected within the soil, and the diffusional path in a given vectorial direction is not strait, gas diffusivity in soil may amount up to about 1/3 that of free air. However, most soils are wet, and compact to some degree and may hinder gas diffusion by several orders of magnitude (CURIE, 1984).

The presence of water in the soil not only determines the gas diffusivity and therefore oxygen availability, but is also linked to marked changes (2-3 orders of magnitude) in heat capacity and heat conductance (WIERENGA et al., 1969; HILLEL, 1978). Water cohesion and adhesion to and between soil particles, the composition of soil minerals dissolved in the water, and to some extent, gravitational force, are responsible to the apparent water potential of the soil. Together with soil temperature they determine the water vapor pressure in the soil, and therefore the direction of water movements.

The medium of mixed Earth, Air and Water must indeed pose a special challenging stress to the Fire of Life.

The Stress

Is living underground stressful? In many aspects it is not: The soil environment provides shelter from predators and its high thermal capacity mediates surface temperature fluctuations. There do exist problems such as underground intraspecific communication, the huge investment in digging and maintaining the tunnels, and the confinement to a restricted home range (and thus, restricted resources). However, there seem to be three main physiological factors which may be defined as stresses: thermoregulation, water budget, and gas exchange.

The increase in heat conductance and capacity (= reduced insulation), with the increase in soil compaction and water content, make it difficult to thermoregulate in soil and requires increased metabolic heat production. This reduced insulation can be offset to a degree due to the fact that there is almost no forced convection which strips off protecting boundary layers in underground tunnels (SOHOLT, 1974). The task of evaporating, is the only means of cooling in warm conditions, is difficult in the wet atmosphere of the underground. All these factors dictate a narrow range of temperatures that animals can tolerate. Avoidance by migrating along a thermal (usually vertical) gradient may be the only solution (JARVIS and SALE, 1971).

Although soils contain normally water, and although evaporative water loss is limited, (as described in the previous paragraph), fossorial animals do not usually find drinking water, and must resort to nutritional sources. Eggs laid in the soil exchange water with their surroundings and may face an unavoidable danger of desiccation.

Gas exchange limitations due to restricted diffusion may be a problem, especially in animals of high metabolic rate, and during activity such as digging, during which they cannot escape to more aerated areas. The accumulation of CO₂ may augment the problem caused by the reduced O₂. Even an open tunnel ceases to be efficient in diffusive gas transfer at a distance of about 3 body lengths from the opening (WILSON and KILGORE, 1978). Fossorial animals of high metabolic rates have no choice but to physiologically cope with the gas conditions.

The Responses

Reptile Embryos -- Conformity

Many reptiles lay their eggs, either singly or in clutches, within the soil. Reptile eggs have an external integument that falls into two main categories (with intermediates): a "hard" calcified shell covering eggshell membranes, or a "soft" or parchment-like shell composed mainly of the equivalent to eggshell membranes of birds (PACKARD and PACKARD, 1980). "Hard" eggshells occur in most geckos, crocodylians, and many turtle species, while lizards, snakes, and some turtles lay "soft" shelled eggs. "Hard" shelled eggs have relatively

low gas and water vapor diffusive conductances, while those of the "soft" shelled eggs are relatively high (ACKERMAN et al., 1985a). It is not surprising to find that hard shelled eggs tend to be limited in their water loss but are also limited in their oxygen uptake while soft shelled eggs may exchange large amounts of water and seem to be less restricted in their gas exchange (ACKERMAN et al., 1985b).

It stands for reason that eggs, which conduct heat and are permeable to gases and water, tend to equilibrate with their immediate surrounding. They either lose or gain water at a rate which is proportional to the direction of the water potential difference between the soil and the egg content, and the gradient intensity (e.g.: LESHEM et al., 1986; PACKARD, 1991). The proportionality constant, which determines the absolute water fluxes is defined as the diffusive shell conductance. The literature is not very conclusive about whether the phase by which water is transported across the shell is liquid or vapor. Recent evidence indicates that (unlike seeds), in most field situations, water is exchanged in the form of vapor (ACKERMAN, 1991; pers. obs.). But whatever the mechanism is, the presence or the absence of the embryo in the egg makes only a little difference to egg water gain or loss. This indicates that the embryo is not capable of exerting control on the process. In fact, it is a common observation that very often during experiments, eggs may either explode from excess water or completely dehydrate.

The only deviation from this complete conformity resides, not in the physiology of the embryos but in the parent behavior during egg laying: It seems that certain laying sites and seasons are preferred by females over the others in relation to rainfall, soil water table, and soil seasonal temperatures at the depth of laying. However such field observations have not been verified in controlled experiments.

Tunnel Nesting Birds -- Behavioural Avoidance

Although incubation or dwelling in holes and cavities is not uncommon among birds in groups such as woodpeckers, parrots, hornbills, and bee-eaters, and in spite of the fact that this behavior has attracted the attention of researchers, sound information concerning the composition of the atmosphere in nesting chambers and the ways oxygen is delivered there, is by and large unknown. It has been found that the partial pressures of O_2 and CO_2 may be lower and higher respectively in the cavities as compared to the free atmosphere nearby (WICKLER and MARSH, 1981; HOWE and KILGORE, 1987; HOWE et al., 1987). However, relevant calculations reveal that diffusion through the walls and the opening may not be sufficient to explain the gas composition of the chamber. WHITE et al., (1978) was able to show, using smoke, that winds blowing over the opening may cause an outward convection. HOWE and KILGORE (1987) have demonstrated temperature difference driven free convection in nest cavities. Both these mechanisms are seemingly unreliable when it comes to long term securing of sufficient aeration in the nest cavity. The Syrian woodpecker was found to move up and down its cavity at a rate of about 5-6 times per hr throughout the night. It has been speculated that this behavior helps the gas exchange by ventilating the cavity (BARNEA, 1982).

AR and PIONTKIEWITZ, (1992) tested the hypothesis, that adult birds actively ventilate their nest cavities, for the European Bee-eater. These birds dig a long (up to 2.5m) horizontal tunnel into a soil bank some 0.5m below the surface, at the end of which they form a chamber for nesting. Incubation starts from the laying of the first of the typical 6 eggs clutch. Eggs are laid every 2 days, and therefore hatching and fledging occur sequentially also every second day. The first egg hatches after 20 days, the first hatchling fledges after additional 30 days and the total breeding period is 60 days.

From laboratory measurements of the daily change in oxygen uptakes of eggs, hatchlings and adults, and from the reconstructed daily nest budget of O_2 uptake; from field measurements of nest volumes, and the daily change in nest O_2 and CO_2 partial pressures; and from

the rate of increase in O₂ partial pressure in empty nests which were flushed with nitrogen, a calculation was made of the amount of nest ventilation mandatory to maintain the relatively high O₂ and a low CO₂ pressures found in the nest atmosphere.

The model of AR and PIONTKIEWITZ (1992), predicts a peak nest ventilation of 1 L.min⁻¹ when the first hatchling is about to fledge and the total oxygen uptake in the nest is maximal. At that time, the frequency of feeding visits of the parents to the nest peaks to 40 per hr. Thus, each visit convects up to 1.5 L of air by movement along the tunnel leading to the nesting chamber. This amount of air is about 7% of the total nest volume. It is interesting to note that in normal lung ventilation of man at rest, about the same proportion of gas is convected at every breath.

Birds are known to be sensitive and responsive to changes in levels of CO₂. Do these birds ventilate their nests in response to the changing atmosphere within? All that can be said at this stage is that as the young grow and their metabolic demands increase, both their oxygen and food uptakes increase in the same proportion. Thus, the caring behavior of the parents brings about an increase in the linked functions of feeding and nest ventilation.

Fossorial Mammals -- Coping

About 150 species of mammals of different groups such as rodents and insectivores are known to reside in the underground for more than temporary shelter. They had converged independently to fossorial life (NEVO, 1979). Food sources such as roots, tubers, bulbs, insects and worms are available but have to be excavated. Since digging seems to be a costly task, and the cost/benefit ratio should be kept at the advantage of the burrower, foraging in tunnels must be very efficient. Digging costs are minimized by choosing a tunnel depth of a potentially high food availability, and by restricting the cross section of the burrow to minimum, namely, to body diameter (VLECK, 1979; VLECK, 1981). In many cases the tunnels do not open to the surface or the openings are plugged. After rains, during activity and especially while digging at the end of a narrow tunnel, the animals may consume oxygen at a rate which severely reduces its availability (AR, 1987). Their survival under such conditions must be linked to special physiological adaptations.

Many fossorial mammals have lower than predicted (75-85%) resting metabolic rates at their thermoneutral zone (MCNAB, 1979). This means that animals may save energy if they find favorable temperatures for resting. While this may be the case, they also have an increased thermal conductivity which is of advantage in the still, insulating air of the burrows (many species possess short fur and some have naked skin). This may help thermoregulation in relatively high temperatures and during activity. However, in cold conditions, heating costs may be increased due to thermoregulation. Some burrowers reduce body temperature in cold conditions, while others enter into a state of torpor. Both strategies serve as means of saving energy (JOHANSEN et al., 1976; JENSEN, 1983).

Mammals living in open air are sensitive to the elevated levels of CO₂ and increase their ventilation in response. However, in the chronically hypoxic and hypercapnic atmosphere of the burrows (ARIELI, 1979), lung hyperventilation is of little help. Hence, the CO₂ and the O₂ responses of fossorial mammals are both blunted and have a high threshold. Moreover, their lung ventilation, measured under "normal" conditions (e.g.: in room air) is lower than predicted for mammals in general, mainly due to lowered frequency (ARIELI and AR, 1979; BOGGS et al., 1984) and may indicate that what we call normal is actually hyperoxic and hypocapnic for fossorial mammals.

The lack of pronounced ventilatory response indicates that adaptations to oxygen delivery at low ambient pressure must be found elsewhere along the oxygen path to the mitochondria. It is therefore surprising to find that in normoxia, nervous control keeps heart rate (STORIER et al., 1981) and calculated cardiac output much lower than in comparable mammals. On the other hand, this low heart rate can increase dramatically in hypoxia

(AR et al., 1977; ARIELI and AR, 1981a) showing an elevated scope for oxygen transport in the circulation of burrowers.

Only in some burrowers can a similar trend be shown in blood properties, where hemoglobin and erythrocyte concentrations and hematocrit value are increased (JOHANSEN et al., 1976; QUILLIAM et al., 1971). The more significant change was in the blood affinity to oxygen: The typical half O₂ saturation value (P₅₀) is on the average only 70% that of the predicted (AR, 1987). High oxygen affinity of blood helps loading oxygen in the lungs but may interfere with its unloading in the tissues where it is utilized. In order to maintain efficient unloading under such conditions a low tissue oxygen pressure is required. This was found to be the case in the mole rat *Spalax ehrenbergi* where low tissue O₂ pressure was found, accompanied by a high muscle capillarization index and high myoglobin concentrations (AR et al., 1977; ARIELI and AR, 1981b).

Concluding Remarks

Subterranean animals share the same basic traits as those of their relatives living in the free atmosphere, but they further exploit these traits for life underground. We cannot point towards any novel organ or function in these animals. (The evolution of behavior might have been different). All we can observe are changes which either extended or degenerated existing behaviors, structures and functions so as to fit them into the context of underground life. The mere fact that animals exist and reproduce successfully in the underground may be taken as a proof that in the course of the evolution the "Underground Stress" has been eased.

Acknowledgements

The administrative support and the encouragement of the Department of Physiology, School of Medicine, State University of New York at Buffalo, is acknowledged.

References

- ACKERMAN, R.A. (1991). Physical factors affecting the water exchange of buried reptile eggs.- In: Egg incubation: its effects on embryonic development in birds and reptiles; edited by P.C. DEEMING and M.W.J. FERGUSON, Cambridge University Press, pp. 193-211.
- ACKERMAN, R.A., R. DMI'EL and A. AR (1985a). Energy and water vapor exchange by parchment-shelled reptile eggs.- *Physiol.Zool.* 58: 129-137.
- ACKERMAN, R.A., R.C. SEAGRAVE, R. DMI'EL and A. AR (1985b). Water and heat exchange between parchment-shelled reptile eggs and their surroundings.- *Copeia* 3:703-711.
- ADOLPH, E.F. (1964). Perspectives of adaptation: some general properties. in: Handbook of adaptation to environment; edited by D.B. DILL, E.F. ADOLPH AND C.G. WILBER.- *Amer. Physiol.Soc.*, Washington, DC, pp. 27-35.
- AR, A. (1987). Physiological adaptations to underground life in mammals. A case of mammalian neoteny? - In: Comparative physiology of environmental adaptations; edited by P. DEJOURS. Karger, Basel, pp. 208-221.

- AR, A. AND Y. PIONTKIEWITZ (1992). Nest ventilation explains gas composition in the nest-chamber of the European Bee-eater.- *Respir.Physiol.* 87:407-418.
- AR, A., R. ARIELI AND A. SHKOLNIK (1977). Blood-gas properties and functions in the fossorial mole rat under normal and hypoxic- hypercapnic atmospheric conditions.- *Respir.Physiol.* 30: 210-218.
- ARIELI, R. & A. AR (1979). The ventilation of the fossorial mammal (*SPALAX EHRENBORGII*) in hypoxic and hypercapnic conditions.- *J.Appl.Physiol.* 47: 1010-1017.
- ARIELI, R. & A. AR (1981a). Heart rate responses of the mole rat (*Spalax ehrenbergi*) i hypercapnic, hypoxic and cold conditions.- *Physiol.Zool.* 54(1): 11-21.
- ARIELI, R. & A. AR (1981b). Blood capillary density in heart and skeletal muscles of the fossorial mole rat.- *Physiol.Zool.* 54(1): 22-27.
- BARNEA, A. (1982). On the biology of the Syrian woodpecker in Israel.- M.Sc. thesis; Tel Aviv University.
- BOGGS, D.F., D.L. KILGORE, G.F. BIRCHARD (1984). Respiratory physiology of burrowing mammals and birds.- *Comp.Biochem.Physiol.* 77A:1-7.
- BOUVEROT, P. (1985). Adaptation to altitude-hypoxia in vertebrates.- Springer, Berl., 176p.
- CURRIE, J.A. (1984). Gas diffusion through soil crumbs: the effects of compaction and wetting. *J.Soil Sci.* 35:1-10.
- HILLEL, D. (1978). Soil and water. New York: Academic Press
- HOWE, S., D.L. KILGORE, C. COLBY (1987). Respiratory gas concentrations and temperature within the nest cavities of the northern flicker (*Colaptes auratus*). *Can.J.Zool.* 65:1541-1547.
- HOWE, S. & D.L.JR KILGORE (1987). Convective and diffusive gas exchange in nest cavities of the northern flicker (*Colaptes auratus*). *Physiol.Zool.* 60:707-712.
- JARVIS, J.U.M. & J.B. SALE (1971). Burrowing and burrow patterns of East African mole rats *Tachyoryctes*, *Heliophobis* and *Heterocephalus*.- *J.Zool.Lond.* 163:451-479.
- JENSEN, I.M. (1983). Metabolic rates of the hairy-tailed mole, *Parascalops breweri* (BACHMAN, 1842).- *J.Mamm.* 64:453-462.
- JOHANSEN, K., G. LYKKEBOE, R.E. WEBER, G.M.O. MALOY (1976). Blood respiratory properties in the naked mole rat *Heterocephalus glober*, a mammal of low body temperature. *Respir.Physiol.* 28:303-314.
- LESHEM, A., A. AR, R.A. ACKERMAN (1991). Growth, water and energy metabolism of the soft-shelled turtle (*Trionyx triunguis*) embryo: effects of temperature.- *Physiol.Zool.* 64:568-594.
- M McNAB, B.K. (1979). The influence of body size on the energetics and distribution of fossorial and burrowing mammals.- *Ecology*, 60: 1010-1021.

NEVO, E. (1979). Adaptive convergence and divergence of subterranean mammals.- A.Rev. Ecol.Syst. 10: 269-308.

OLSZEWSKI, J.L. & S. SKOCZEN (1965). The airing of burrows of the mole; *Talpa europaea* LINNAEUS.- Acta Theriol. 10:181-193.

PACKARD, G.C. (1991). Physiological and ecological importance of water to embryos of oviparous reptiles. In: Egg incubation: its effects on embryonic development in birds and reptiles; edited by P.C. DEEMING & M.W.J. FERGUSON. Cambridge, Cambridge University Press, pp. 213-228.

PACKARD, G.C. & M.J. PACKARD (1980). Evolution of the cleidoic egg among reptilian antecedents of birds.- Amer.Zool. 20:351-362.

PROSSER, C.L. (1973). Comparative animal physiology. 3rd ed. Saunders, Philadelphia, 966p.

QUILLIAM, T.A., J.A. KLARKE, A.J. SALESBURY (1971). The ecological significance of certain new hematological findings in the mole and the hedgehog.- Comp.Biochem. Physiol. 40A:98-102.

REID, R.C., J.M. PRAUSNITZ, T.K. SHERWOOD (1977). The properties of gases and liquids.- 3rd edn. McGraw-Hill, New York, 688p.

SOHOLT, L.F. (1974). Environmental conditions in an artificial burrow occupied by merriam's kangaroo rat *Dipodomys merriami*.- J.Mamm. 55:859-864.

STORIER, D., Z. WOLLBERG, A. AR (1981). Low and nonrhythmic heart rate of the mole rat (*Spalax ehrenbergi*) control by the autonomic nervous system.- J. Comp. Physiol. 142: 533-538.

VLECK, D. (1979). The energy cost of burrowing by the pocket gopher *Thomomys bottae*.- Physiol.Zool. 52:122-136.

VLECK, D. (1981). Burrow structure and foraging costs in the fossorial rodent *Thomomys bottae*.- Oecologia 49:391-396.

VOGEL, S., C.P. ELLINGTON, D.L. KILGORE (1973). Wind ventilation of the burrow of the prairie dog *Cynomys ludovicianus*.- J.Comp.Physiol. 85:1-14.

WHITE, F.N., G.A. BARTHOLOMEW, J.L. KINNEY (1978). Physiological and ecological correlates of tunnel nesting in the European bee-eater, *Merops apiaster*.- Physiol.Zool. 51:140-154.

WICKLER, S.J. & R.L. MARSH (1981). Effects of nestling age and burrow depth on CO₂ and O₂ concentrations in the burrows of bank swallows (*Riparia riparia*).- Physiol.Zool. 54:132-136.

WERENGA, P.J., D.R. NIELSEN, R.M. HAGAN (1969). Thermal properties of a soil based upon field and laboratory measurements.- Soil Soc. Am. Proc. 33:354-360.

WILSON, K.J. & D.L.JR KILGORE (1978). The effect of location and design on the diffusion of respiratory gases in mammal burrows.- J.Theor.Biol. 71:73-101.

**Stress-induced Alterations in both Peripheral and Central Nervous System:
Experimental Stress and the Mesoprefrontal Dopamine Projection.**

Gertraud TEUCHERT-NOODT

Introductory comments to DAWIRS' report on studies of "Stress and Synaptic Rearrangement" (same volume,): It was the mechanism of natural selection that fit men with highest stress tolerance in animal kingdom. But nowadays it proves civilized men's undoing. What's the matter? Tracing men's ancestry, in Stone Age homo sapiens adapted to first class performance of hunters and gatherers by optimizing both central and peripheral nervous system function and cooperation. Power to act in the dangers of jungle, traffic or job equally comes from transmitter release at peripheral and brain level: The secretion of sympathetic amines stimulates breathing activity and blood circulation and at the same time brain aminergic activity increases alertness of mind, concentration and motivation. Peripheral and central ergotropic and trophotropic interaction will function in any situation and even with consequences at night. Both peripheral organs and brain will fail to function if stress tolerance is exceeded and this will result from long-term effects as a sum of several stressors. Synthesized neurotransmitters then come into the state of exhaustion. Principally, stress disease is nothing else than a supply problem of neurotransmitters.

There are individual dispositions to stress disease with either sympathicotonic or vagotonic overstrain. In the first case blood pressure might enhance the risk of cardiac infarction besides other disorders in men. In the vagotonic person on the other hand a lowering of circulation also causes feelings of dizziness, perhaps with attacks of migraine at weekend. Anyway temporary impotence of reproduction may result from long-term stress. Now focussing to brain: Some patients may suffer from brain impotence but with quite different failures. Stress induced brain disfunction may produce depression perhaps with blocking of mental activity or on the other hand aggressive behaviour. The morphological correlate for these mental disorders in any case is the limbic system to its greatest extent including the rostral forebrain which is the prefrontal cortex (PFC).

The physiology of mental disorder is not well understood. We are working on this problem in our lab and we are experimenting with gerbils. The PFC also exists in rodents. As in men, a prominent dopamine projection triggers mental functions predominantly. Principally, three main projections originate from specific sets of dopamine neurons in the mesencephalic brainstem tegmentum and trigger endocrine, motor and mood functions by separate pathways. The mesoprefrontal dopamine projection being mostly involved in the limbic reafferent system appears to play

a role in learning and memory. Laboratory animals which get low electric currents by an electrode implanted into this particular pathway answer with highest rates of self-stimulation by treadle pressing . Experiments show that just dopaminergic fiber system and not the adrenergic one supports self-stimulation behaviour and is the substrad of brain reward mechanism. Like self-stimulation by electric current any stressors and moreover even dopamine effecting drugs manipulate the reward-system of the brain and appear to alleviate psychotic behaviour.

References

1. DAWIRS R.R. (1993). Stress and Synaptic Rearrangement: A Mutual Principle of Adaptation to Normal and Aberrant Stimuli. Same Volume.
2. SELYE H. (1974). Stress Without Distress. J.B. Lippincott Comp., Philadelphia & New York.
3. BRADFORD H.F. (1986). Chemical Neurobiology - An Introduction to Neurochemistry, 507, W.H. Freeman and Company, New York.
4. ROUTTENBERG A. (1978). The Reward System of the Brain. In: The Workings of the Brain Development, Memory and Perception (R. R. Linas ed.) 75 - 87, Scientific American.

Stress and Synaptic Rearrangement: A Mutual Principle of Adaptation to Normal and Aberrant Stimuli

Ralph R. Dawirs

In mammals, the whole prefrontal cortex is activated by the ascending mesocortical dopamine (DA) system (Fig.1). This is realized by modulation of the spontaneous firing rates and the overall excitability of prefrontal pyramidal cells¹. In order to understand the biological function of this it is important to know that when animals are exposed to stressful conditions the mesoprefrontal DA system becomes selectively activated².

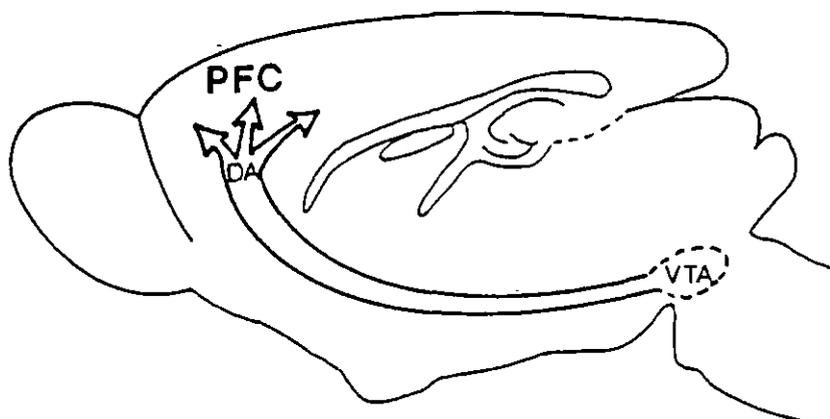


Fig.1. Schematic view of a parasagittal section of the gerbil brain and the mesoprefrontal dopamine (DA) projection: prefrontal cortex (PFC); ventral tegmental area (VTA).

Evidence is accumulating that in the embryonic brain certain phenomena of structural regression contribute to progressive development³. On the basis of this, synaptic degeneration and rearrangement both may be morphological tools in changing the structural substrate of behavioral development. From a variety of experimental data it has been supposed that non-damaging normal patterns of functional activity induce structural modification, so that even in the mature nervous system synaptic plasticity

seems to be an ongoing natural process⁴. In this concept, there is a continuous loss and reacquisition of synaptic contacts which result in structural remodelling of functional circuits in response to normal physiological interaction between organism and environment.

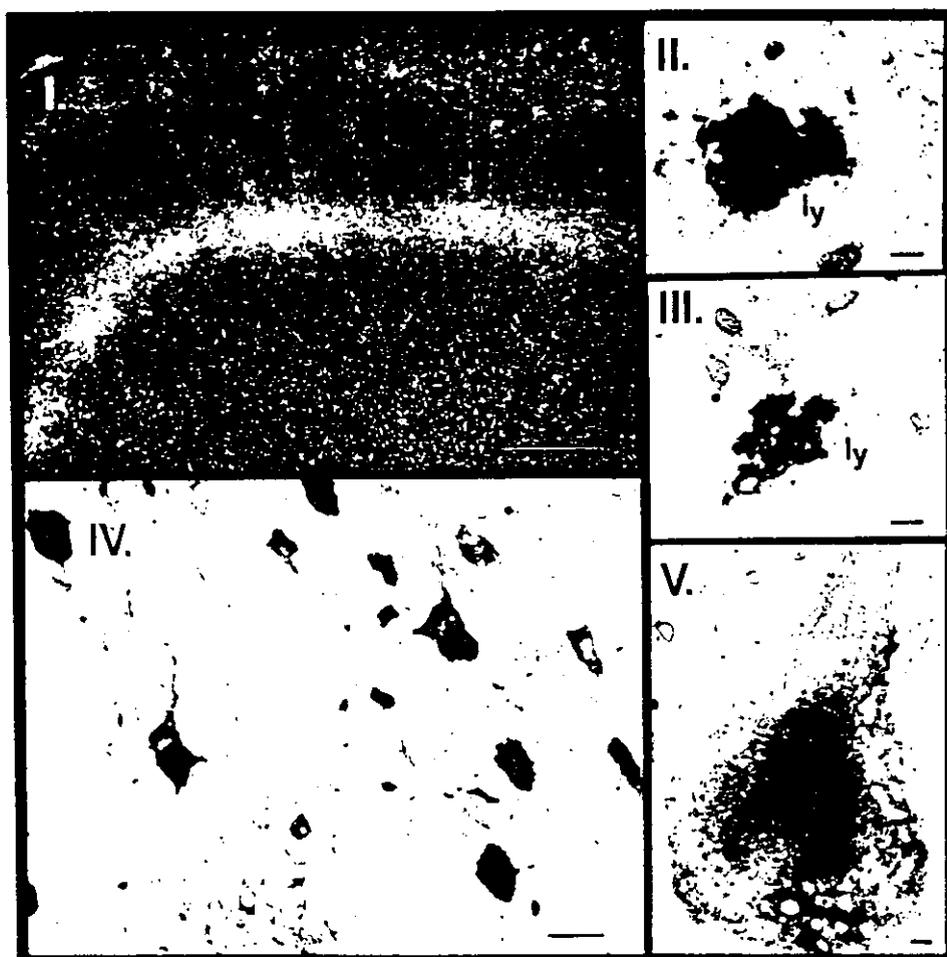


Fig.2. I: Intense silver staining of degrading dopamine fibre terminals in the prefrontal cortex 3 days after a single dose of methamphetamine; II,III: electron micrographs of degrading synaptic profiles; lysosome (ly); brightfield (IV) and electron micrographs (V) of dark neurons, probably responding to degradation of dopamine fibres; bars: 40 μm (I), 200 nm (II,III), 20 μm (IV), 1 μm (V).

Against this background, we are especially interested in the development of the prefrontal cortex (PFC) including the mesoprefrontal DA system. In this connection we wondered what might happen after a selective destruction of DA terminals in the PFC. For that purpose we applied a single and unrepeated dose of methamphetamine to young adult gerbils.

What happened was (1): massive acute degeneration of axon terminals mainly in layer III of the medial PFC. This is indicated by means of a sensitive silver staining technique selectively labelling degrading profiles (Fig.2). Electron micrographs of these degrading synaptic profiles were characterized by large reactive lysosomes. From a long list of indications there is now reasonable evidence to assume that axon terminals affected by this methamphetamine induced toxicity were selectively DAergic (Fig.3). Acute effects of large doses of methamphetamine on the mammalian

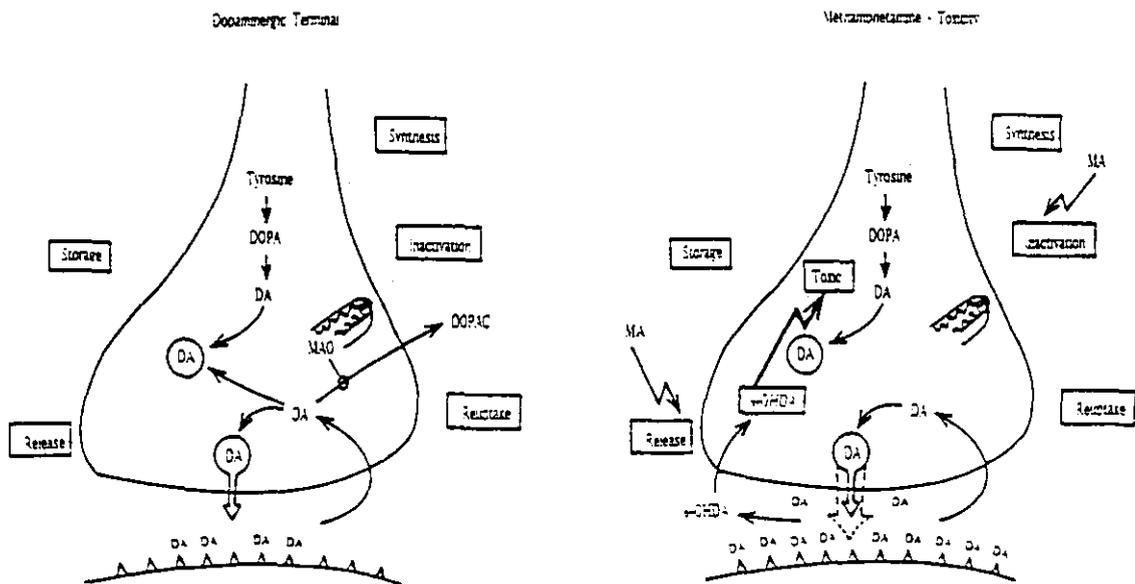


Fig.3. Normal functioning of a dopaminergic axon terminal (left) and acute effects of a large dose of methamphetamine (right); dopamine (DA), 3,4-dihydroxy phenylalanine (DOPA), dihydroxy phenylacetic acid (DOPAC), 6-hydroxy dopamine (6-OHDA), methamphetamine (MA), monoamine oxidase (MAO).

brain are described as massive release of DA from presynaptic pools and blockade of the degrading enzyme monoamine oxidase (MAO). Although DA might re-enter the terminals by high affinity re-uptake under these conditions, it will rather be restored but immediately released again, due to persisting large effective concentrations of the drug in the tissue. It has been shown that when MAO is absent and DA concentration in the synaptic cleft is high, DA might be metabolized non-enzymatically to 6-OHDA. This endogenously produced 6-OHDA is recognized as a so-called "false transmitter" and enters the presynaptic terminal again where it exerts its toxic degrading effects.

Besides presynaptic responses, there was (2) indication of methamphetamine induced effects on postsynaptic sites, with prefrontal pyramidal cells showing all signs of degradation (indicated by so-called "dark neurons") (Fig.2). It cannot be ruled out so far that this postsynaptic responses were actually caused by methamphetamine induced deafferentiation. Nevertheless, these effects were reversible and symptoms completely vanished after at least 10 days following application of the drug.

What further happened (3) was: at the same time the density of dendritic spines dramatically increased on prefrontal pyramidal cells, which are located in the very same area of the PFC (Fig.4). Today we have enough reason to argue that methamphetamine induced deafferentiation of DA axon terminals cause this postsynaptic increases in spine density.

The question is whether or not a single pharmacological impact by methamphetamine, which results both in presynaptic DAergic deafferentiation and proliferation of postsynaptic contact sites, may serve as a trigger of functional changes by means of compensatory neuroplasticity. Therefore, we now look at the maturation of DA innervation in the PFC of gerbils. For that purpose, DAergic nerve fibres were visualized by means of a specific antibody against DA, and were counted throughout the postnatal development in juveniles and young adults (Fig.5).

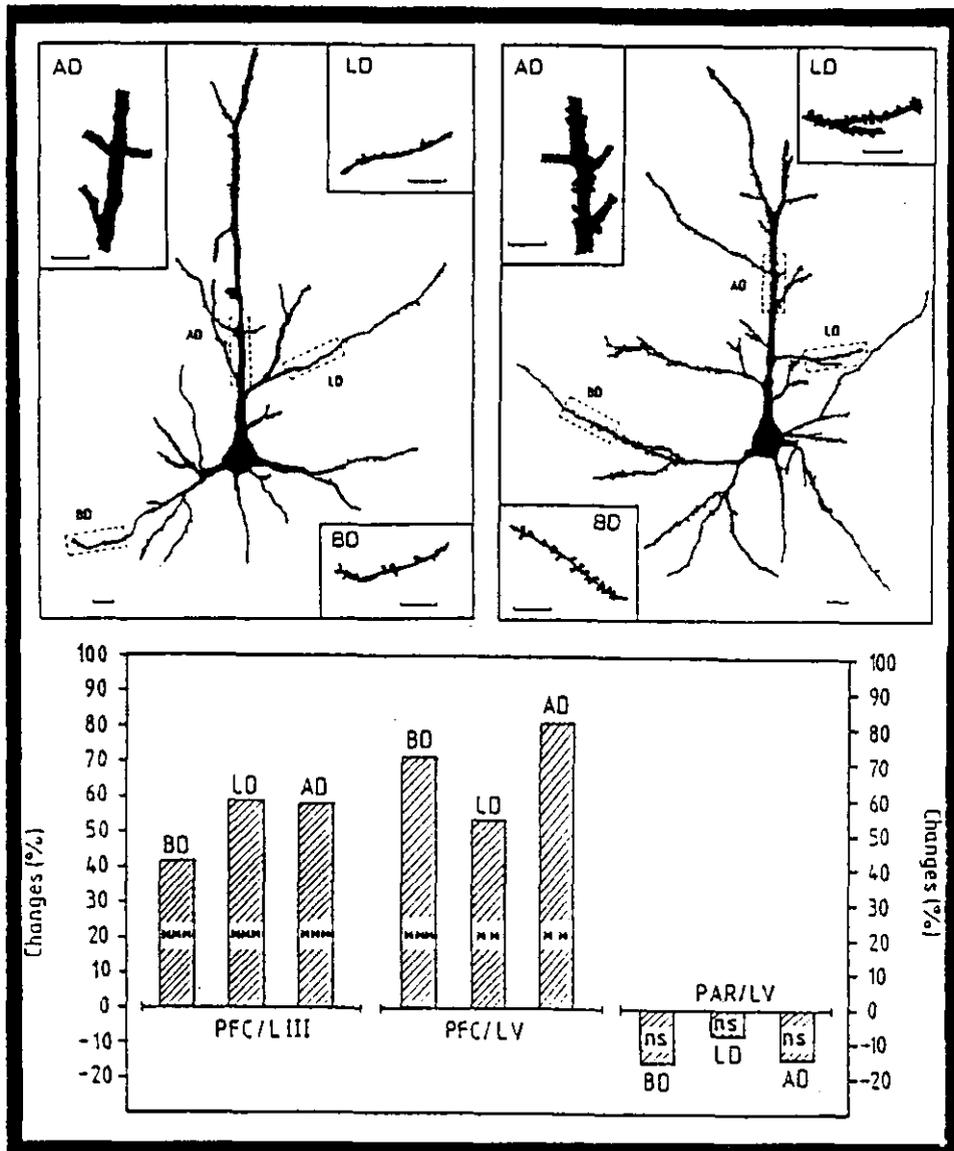


Fig.4. Top: typical golgi stained pyramidal cells (layer III) in saline (left) and methamphetamine treated gerbils (right); apical (AD), lateral (LD) and basal dendrites (BD); bars: 10 μ m; below: densities of dendritic spines significantly increased in both layers III and V of the prefrontal cortex (PFC); no significant changes were found in the parietal cortex (PAR).



Fig.5. Immunostained DAergic cell somata in the VTA (top) and DAergic nerve fibres in the medial PFC (below) of young adult gerbils.

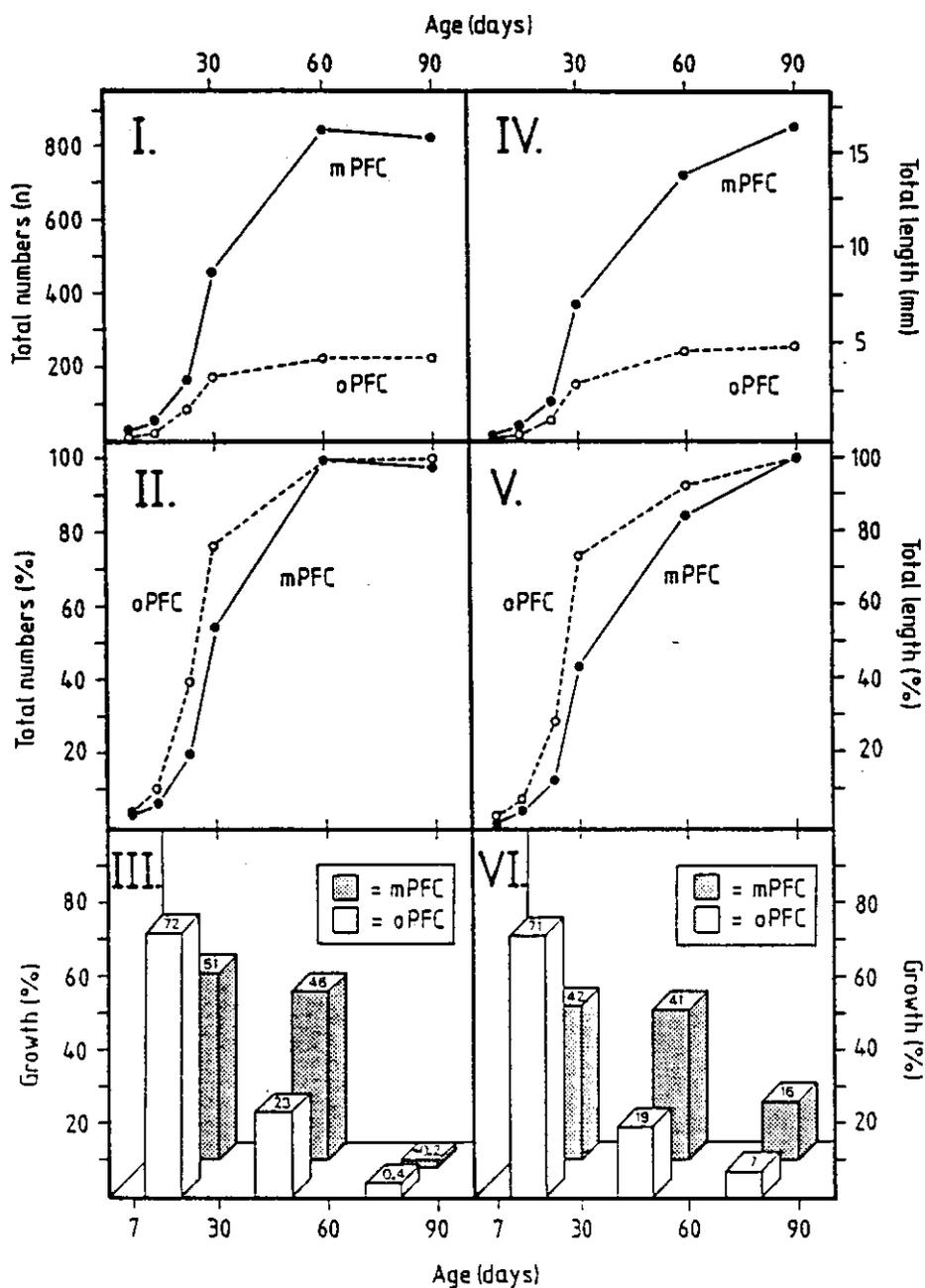


Fig.6. Absolute and percentage growth pattern of total numbers (I,II) and total length (IV,V) of DA fibres in the orbital (oPFC) and medial prefrontal cortex (mPFC); proportionate gain in total numbers (III) and total length (VI) during subsequent periods of development (P7-30, P30-60, P60-90) in both prefrontal subareas; mean values per standard 40 μ m-slice.

We found that total numbers of DA fibres continually increase postnatally and attained about adult level on postnatal day 60 (Fig.6). This obviously delayed maturation of prefrontal DA innervation appears as a structural correlate of the well established retarded functional maturation of this brain area.

Mindful of the fact that methamphetamine is selectively toxic to highly active functional DA synapses, we asked what might be the influence of an early massive stimulation of DA transmission in the PFC on subsequent development of prefrontal DA function. For that purpose, juvenile gerbils received a single methamphetamine challenge at the age of day 14 and DA fibres were counted on postnatal day 90. What we actually found was that this single stimulus severely influenced subsequent maturation of the mesoprefrontal DA systems, ending up in a dramatically reduced adult innervation pattern when compared with untreated controls (Fig.7). This long-term effect of an early vulnerable stimulus was even more pronounced in the orbital PFC. This may be well attributed to the fact that in this subarea DA innervation achieves maturity earlier than in the medial PFC.

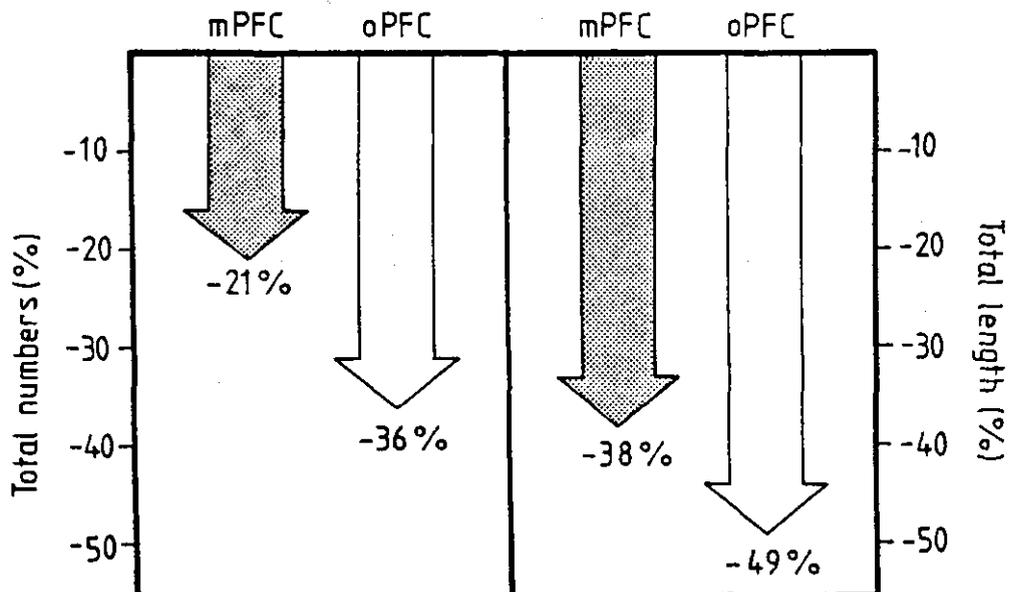


Fig.7. After a single dose of methamphetamine in juveniles (P14) during subsequent development fewer DA fibres innervate the prefrontal subareas (oPFC, mPFC) leading to significantly reduced DA innervation on P90 when compared with untreated controls.

In conclusion, present results show that the mesoprefrontal DA fibres are particularly vulnerable against high DA concentrations in the synaptic cleft, which as we know may also occur in response to chronic high stress levels. The PFC is involved in a variety of emotional, cognitive and social behaviors in which the integrity of the DA innervation appears to be most important for normal functioning^{5,6}. Therefore, we believe that under certain circumstances stress might become a naturally occurring kind of aberrant stimulus with significant neurogenetic and thus behavioral consequences.

References

1. Thierry A.M., Tassin J.P., Blanc G. and Glowinski J. (1976). Selective activation of the mesocortical DA systems by stress. *Nature* 263, 242-243.
2. Thierry A.M., Godbout R., Mantz J. and Glowinski J. (1990). Influence of the ascending monoaminergic systems on the activity of the rat prefrontal cortex. In: *Progress in Brain Research* (eds. Uylings H.B.M., Van Eden C.G., De Bruin J.P.C., Corner M.A. and Feenstra M.G.P.), Vol.85, pp. 357-365. Elsevier, Amsterdam.
3. Teuchert-Noodt G., Breuker K.-H., Dawirs R.R. (1991). Neural lysosome accumulation in degrading synapses of sensory-motor and limbic subsystems in the duck *Anas platyrhynchos*: Indication of rearrangements during avian brain development? *Dev Neurosci* 13, 151-163
4. Dawirs R.R., Teuchert-Noodt G., Kacza J. (1992). Naturally occurring degrading events in axon terminals of the dentate gyrus and stratum lucidum in the spiny mouse (*Acomys cahirinus*) during maturation, adulthood and aging. *Dev Neurosci*, in press

5. Bertolucci-D'Angio M., Serrano A., Driscoll P. and Scatton B. (1990). Involvement of mesocorticolimbic dopaminergic systems in emotional states. In *Progress in Brain Research* (eds. Uylings H.B.M., Van Eden C.G., De Bruin J.P.C., Corner M.A. and Feenstra M.G.P.), Vol. 85, pp. 405-417. Elsevier, Amsterdam.

6. Fibiger H.C. and Phillips A.G. (1986). Reward, motivation, cognition: psychobiology of mesotelencephalic dopamine systems. In: *Handbook of Physiology, Section 1: The Nervous System, Intrinsic Regulatory Systems of the Brain* (ed. Bloom F.E.), Vol. IV, pp. 647-675. American Physiological Society, Bethesda MD.

**Stress adaptation to moderate altitude conditions. I.
Sympathetic activity and metabolic response.**

Elke Zimmermann#, Christiane Stock*

**# Fak. Psychologie und Sportwissenschaft, AB Sportmedizin,
Universität Bielefeld**

*** Fak. Biologie, Abt. Neuroanatomie, Universität Bielefeld**

Introduction

From the view of sports medicine the research of stress reveals big methodical problems, but at the same time offers a great chance. Attention is mainly focussed on stress-reactions of selected organ functions (e.g. heart- and immune system). The system responsible for stress-related alterations of organ functions, the autonomic nervous system, is not well understood until today, because reading its information still remains rather difficult. The general understanding of information given by the autonomic nervous system is looked at in the sense of thrill, stimulation or calming, when given by its sympathetic part, or as support of recreation, when given by the parasympathetic part.

In relation to stress our special interest focusses on the sympathetic part of the autonomic nervous system. There are two ways to transfer information from the central nervous system to the sympathetic end organs or tissues. First there are sympathetic nervous fibers, which release noradrenaline (NA) as neurotransmitter. Second there is the possibility to secrete adrenaline (A) by activating the adrenal medulla. The distribution of the transmitter adrenaline by using the blood stream as a transport system, leads to a general activation of the organism. Measuring NA- and A- concentration in blood and urine serves as an indicator of what way of information transfer is predominantly used in reaction to a stressful event.

There are several methods of catecholamine determination in use, but most of them are not sensitive enough to measure changes of concentration within a physiological range. Therefore we developed a method that combines a gas-chromatographical separation with a mass-spectrometrical detection (9).

While noradrenaline is primarily increased during physical stress such as ergometry (6), adrenaline serves as an indicator of psychological stress such as time stress (7) mental stress (4) or anxiety (3). As the total excretion of catecholamines depends from several factors such as diurnal variation or training state (8), an estimation of sympathetic activity can best be done by looking at the ratio of NA/A. As a result of our studies with high performance athletes we developed a scale of NA/A-ratio from low to high degrees. This enables us to distinguish between state of nervousness indicated by a Na/A-ratio below 2 (that means: NA:A < 2:1), grade of best activation indicated by values from 3 to 6, and mental relaxation accompanied by high ratios NA/A > 7 (10).

In general stress adaptation is looked at as a modulation of metabolic functions and/or morphological structures in order to maintain homeostasis (2). Reaching homeostasis allows an economization of energy expenditure and therefore attracts the attention of sports sciences and medicine. Physical exercise stimulates adaptation processes and an additional exposure to altitude is accepted as a supporting stimulus for bettering aerobic capacity by endurance training (5,1). But one has to pay attention on some risks related with altitude training. An underestimation of the reduced oxygen pressure might lead to a training under predominantly anaerobic conditions, with the consequence of a lowered aerobic performance. In this context the present study focusses on the effects of an exposure to moderate altitude in combination with physical training on some hematological parameters, aerobic work capacity and sympathetic nervous system activity.

Subjects and Methods

A group of 14 students of sport sciences has been studied during a 2-weeks ski-mountaineering-course in the Swiss Alps.

Subjects:

	age (years)	weight (kg)	height (cm)
7 male	31+/-8	70+/-5	179+/-2
7 female	25+/-3	57+/-3	170+/-4

Stress conditions:

duration: 14 (10) days --- altitude: 1850 - 3000 m

physical activity: 4-7 h daily (ski mountaineering, alpine skiing, long distance skiing)

Physiological assessment:

Samples of morning urine were collected from 8 subjects before and after the altitude stay and at 4 days during the altitude exposure to determine nocturnal catecholamine excretion. Determination of catecholamine concentrations was done by gaschromatography/ masspectometry.

6 subjects performed an incremental graded exercise test on a treadmill ergometer as a pre- and post- examination. After each exercise intervall of 3 min duration a capillary blood sample was drawn from the earlobe to measure lactate concentrations enzymatically. The aerobic/anaerobic threshold was defined as running velocity at 4 mmol/l lactate concentration.

Hemoglobin and hematocrit were determined photometrically from venous blood samples drawn before the altitude stay and at day 2 after return to sea level.

Results and conclusions

Exposure to altitude results in an 8.5% increase in hematocrit and a 6.5% increase in hemoglobin. These increases are both significant (Fig.1), demonstrating, that even moderate altitude is an acceptable stimulus to cause the a desired adaptation of oxygen transport capacity. Nevertheless this can be accompanied by a non-desirable elevation of hematocrit. The 4 mmol/l lactate level is considered as a value that expresses an equilibrium between production and elimination of lactate in the working muscle mass of an exercising person. That means, that as long as an athlete shows lactate concentrations below 4 for a given running velocity, aerobic restauration of ATP predominates. With a shift of the lactate curves to the right, treadmill ergometry shows, that altitude training leads to an increase of the aerobic capacity in low-trained subjects (Fig.2 a-d). In one person no shift of the lactate curve occurred (Fig.2 e). The lactate curve of the best trained athlete slightly shifted to the left, indicating a decrease in aerobic capacity (fig.2 f). Training intensity and/or duration at altitude obviously was too low for this high performance female marathon runner to gain an improvement in endurance.

The basal catecholamine excretion at altitude is characterized by low nocturnal adrenaline excretion and slightly elevated noradrenaline excretion compared to the morning values at sea-level (Fig.3 a+b). Whereas elevated noradrenaline excretion is probably caused by extended physical exercise, the low adrenaline excretion may indicate a psychological relaxation.

The NA/A-ratio of morning urine samples further supports this explanation: the high ratios (> 10) are typical for good, regenerating sleep. The significant elevation of NA/A-ratio at the 5th night represents the nocturnal situation after a heavy, long-lasting ascent, answered with an elevation of noradrenaline even after the end of physical activity.

We can conclude, that for the present group a 2-weeks ski mountaineering course at moderate altitude did not mean stress in the sense of weakening regeneration or acclimatization, but has stimulated adaptive processes in order to recreate homeostasis.

Literature

- (1) BURTSCHER M, NACHBAUER W, BAUMGARTL P, PHILADELPHY M, VEITL V (1992): Beeinflussung spiroergometrischer Parameter durch Höhen- beziehungsweise Taltraining. In: Jahrbuch 1992 (Hg.) JENNY E, Österr. Gesellsch. f. Alpin- und Höhenmedizin
- (2) CANNON WB (1935): Stresses and strains of homeostasis. *Am J Med Sci* 189:1-7
- (3) EDMONDSON D, ROSCOE B VICHERS MD (1972): Biochemical evidence of anxiety in dental patients. *Br Med J* 4:7-9
- (4) LEBLANC J, COTE J, JOBIN M, LABRIE A (1979): Plasma catecholamines and cardiovascular responses to cold and mental activity. *J Appl Physiol* 47:1207-1211
- (5) MELLEROWICZ H, MELLER W, WOWERIES J, ZERDICK J, KETUSINH O, KRAL B (1970): Vergleichende Untersuchungen über die Wirkungen von Höhenttraining auf die Dauerleistung auf Meereshöhe. *Sportarzt und Sportmed* 9:207
- (6) PLUTO R, CRUZE SA, THOME C, WEICKER H (1986): Inter-individuelle Unterschiede venöser Plasmakatecholaminspiegel. *Dtsch Z Sportmed* 86:22-30
- (7) TIMIO M, GENTILI S, PEDE S (1979): Free adrenaline and noradrenaline excretion related to occupational stress. *Brit Heart J* 42:471-474
- (8) TOWNSEND AM, SMITH AF (1972): Factors influencing the urinary excretion of free catecholamines in man. *Clin Sci* 44:253-265
- (9) ZIMMERMANN E, SCHÄNZER W, DONIKE M (1983): Streßfaktoren vor und nach Wettkampf- bzw. Trainingsbelastung. In: *Sport-Leistung und Gesundheit*. (Hg.) HECK, Deutscher Ärzte Verlag Köln
- (10) ZIMMERMANN E, DONIKE M, SCHÄNZER W (1985): Katecholaminspiegel, psychische Aktivierung und Wettkampfstabilität. In: *Training und Sport zur Prävention und Rehabilitation in der technisierten Umwelt*. (Hg.) FRANZ, Springer Berlin Heidelberg

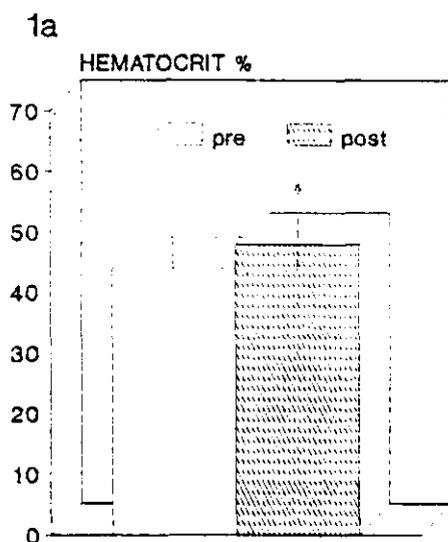


Fig.1a. Effect on hematocrit. Bars represent means \pm SD from pre- (n=14) and post- (n=14) examinations. * $p < 0.02$.

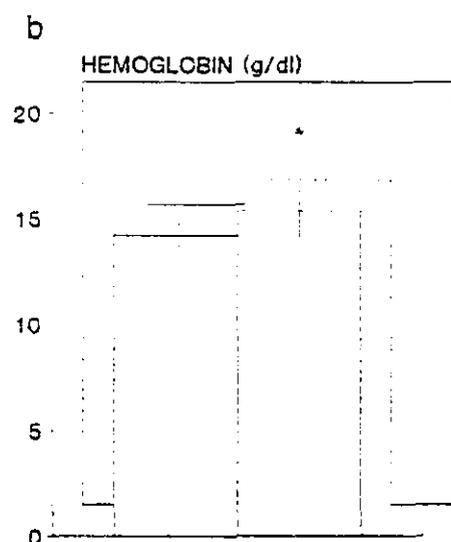


Fig.1b. Effect on hemoglobin concentration. Bars represent means \pm SD from pre- (n=14) and post- (n=14) examinations. * $p < 0.04$.

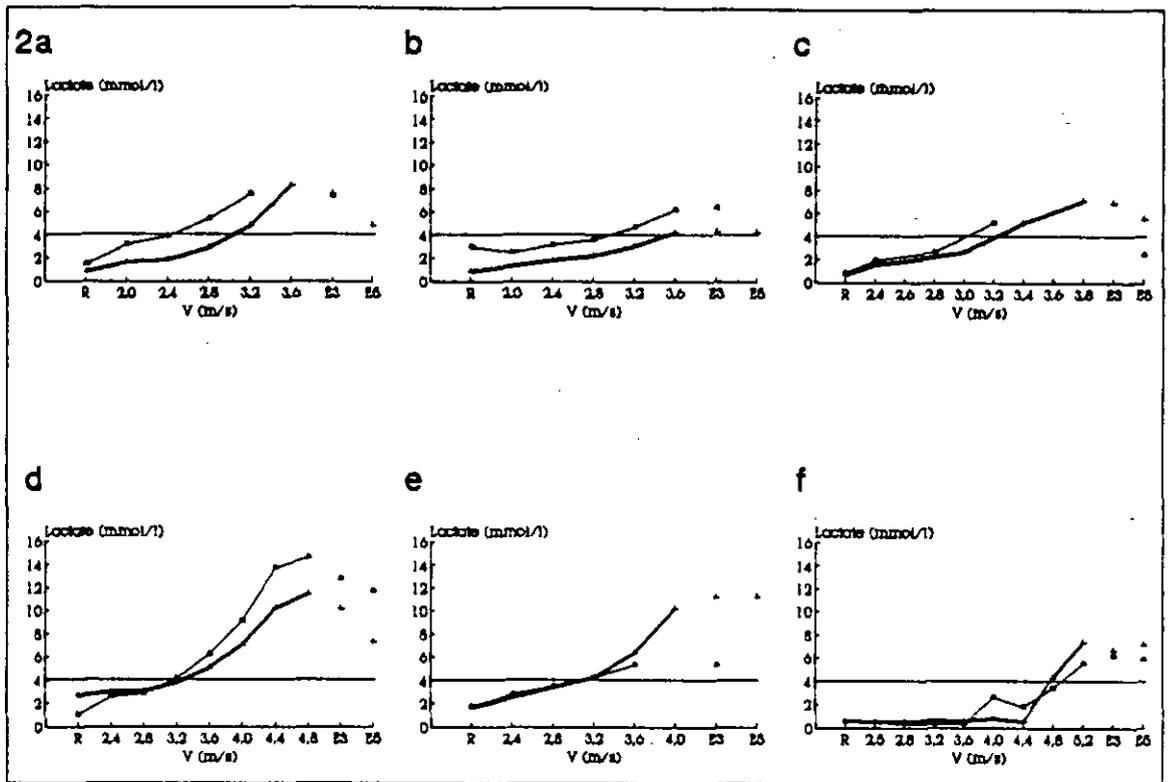
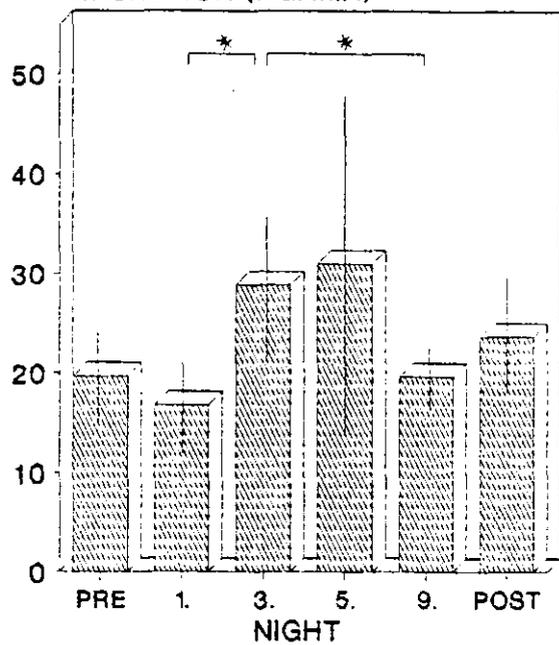


Fig.2a-f. Lactate curves from 6 subjects from pre- (thin lines) and post- (thick lines) graded exercise tests. Lactate concentrations are shown at rest (R), at various running velocities, and after a recovery of 3min (E3) and 5min (E5). Intercepts with the horizontal line indicate running velocities at 4mmol/l lactate concentrations (aerobic-anaerobic threshold).

3a

NORADRENALINE

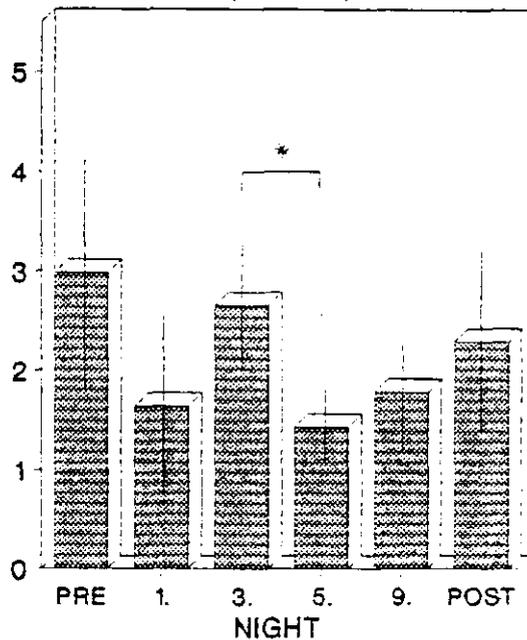
EXCRETION (NG/MIN)



b

ADRENALINE

EXCRETION (NG/MIN)



c

RATIO

NA/A

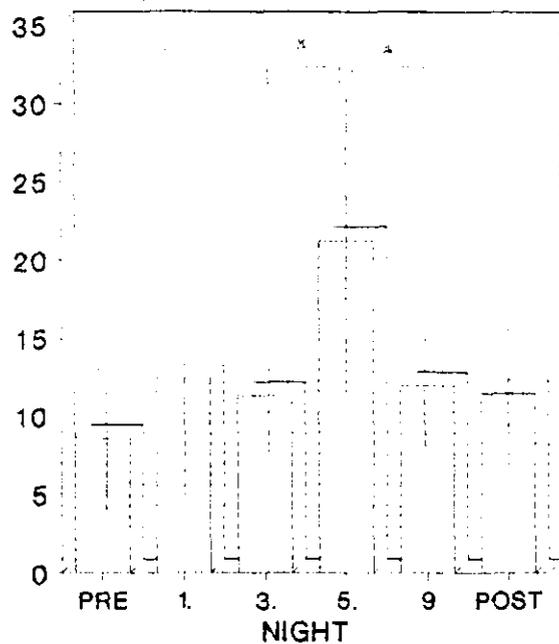


Fig.3a-c. Noradrenaline excretions, adrenaline excretions and NA/A-ratios from nights before (pre), after (post) and during the 14-days altitude stay (1,3,5,9.night).

* $p < 0.05$.

Stress adaptation to moderate altitude conditions. II. Effect on blood lymphocytes and β -adrenergic receptors.

Christiane STOCK*, Elke ZIMMERMANN#

*** Fak. Biologie, Abt. Neuroanatomie, Universität Bielefeld;
Fak. Psychologie und Sportwissenschaft, AB Sportmedizin,
Universität Bielefeld**

Introduction

A wide range of cardiorespiratory alterations are known to occur on exposure to high altitude. Humoral and endocrine factors have been implicated as being major mediating and contributory mechanisms. A number of workers have studied the pattern of sympathoadrenal activity on exposure to altitude, postulating an elevation of sympathetic activity (3,2,9). We investigated the effect of moderate altitude on baseline sympathetic activity and found an elevation of nocturnal noradrenaline excretion in urine (16). As high doses of catecholamines are known to down-regulate the sensitivity of cells to adrenergic stimulation (4,11), we asked further, to what extent this longer-lasting alteration in sympathetic activity effects the density of β -adrenergic receptors on the tissue. In order to get some information about receptor regulation due to altitude adaptation, we quantified the density of β -receptors on circulating mononuclear leukocytes (MNL) before and after an altitude stay. As the MNL-fraction consists of a set of subpopulations and the composition is known to be altered by different stressors, such as physical exercise (7,10), it is also necessary to quantify alterations of lymphocyte subsets in order to characterize receptor alterations.

Methods

A group of 14 normal, healthy volunteers were studied before and after a 14-days stay in the Swiss Alps at an altitude of 1800-3000m. Details of the protocol are reported in ZIMMERMANN & STOCK 1992 (16). Blood samples were drawn a few days before the altitude stay and at day 2 (n=10) and day 5 (n=4) after return to sea level. Mononuclear leukocytes from EDTA-anticoagulated blood were separated by density centrifugation. The saturation binding assay was performed with ^3H -Dihydroalprenol (DHA) concentrations ranging from 0.5 to 6.0 nM. 5×10^6 cells/ml were incubated in duplicates at 27°C for 30 min in the absence (total binding) or presence (nonspecific binding) of $10\mu\text{M}$ (-) propranolol. Bound radioactivity was separated by rapid vacuum filtration through glasfaser filters. Samples were counted in a scintillation counter.

Analysis of lymphocyte subsets was done by flow-cytometry.

Following monoclonal antibodies coupled with fluorescein or phycoerythrin were used: Hle-1, Leu M3, Leu2, Leu3, Leu4, Leu12, Leu11/19, HLA-DR (Becton Dickinson, Heidelberg).

Results

Saturation curves of binding data from pre- and post-examinations show a reduced binding of the radioligand after the altitude stay (fig.1a). In the corresponding scatchard plot the ratio of bound to free ligand versus the concentration of bound ligand gives a straight line indicating a homogenous population of binding sites (fig.1b). The intercept with the abscissa is the estimated value of maximal binding. The intercepts of pre- and post examinations clearly differ from each other indicating a reduction of total binding sites of about 25 %. The parallelity of the regression lines shows, that no alteration in the affinity of DHA to the receptor has occurred.

The calculation of receptor numbers per cell from pre- and post-examination values shows, that there is a significant decrease in receptor number at day 2 after return ($p < 0.01$) compared to control values, which is also found in blood samples drawn at day 5 after return (fig.2). Due to small sample numbers day 5 values have not been statistically evaluated. The reduction in receptor number was very similar in all test persons (fig.3).

The results of a classical blood count show, that there is a slight but not evident increase of total leukocytes after the altitude stay, which is also seen in granulocytes and mononuclear leukocytes (fig.4). A further separation of mononuclear leukocytes by flow-cytometric analysis shows, that altitude has no effect on the number of total T-cells and B-cells. There is a slight, but not significant decrease in T4-helper and a slight increase in T8-suppressor-cells. Activated T-cells, which express the interleukin-2-receptor on their surface, remain unchanged, as well as natural killer cells and monocytes (fig.5).

Slight alterations are seen in the T4/T8-ratio. The mean of the post examination values has shifted to the lower limit of the clinically used reference ranges (fig.6). Fig.7 presents the individual changes in T4/T8-ratio. Whereas three subjects show a clear reduction, three others show only a slight reduction and one subject shows an increase. There is also a remarkable reduction in the deviation of values after the altitude stay.

Discussion

The density of adrenergic receptors correlates with the sensitivity of a tissue to adrenergic stimulation (8). Although blood lymphocytes are not innervated by the sympathetic nervous system, they have β -receptors, that are stimulated by circulating catecholamines (15). This raises the question of the functional significance of these receptors on white blood cells. Most investigators clearly show, that adrenergic stimulation suppresses immune functions, like cytolytic activity (5,14) cell proliferation (1) or antibody production (12). This means, that a high density of β -receptors is correlated with a high potency of immune suppression. In this context a reduction of receptor density after physical activity under altitude conditions may represent a stress induced adaptation to an elevation in sympathetic activity. This adaptation may prevent an immune suppressive effect of increased levels of circulating catecholamines. In fact no subject suffered from infectious diseases at the time of the post examination. The adaptive response, expressed by a reduction in receptor number, remain stable over a period of time, at least 5 days, and may reflect longer lasting processes of adaptation.

In cell counts of lymphocyte subsets no significant alterations were found. All pre- and post-examination values are within the clinically used reference ranges and overall represent a stable state of immune function. The only sign of destabilization gives the T4/T8-ratio, showing a shift to the lower limit of the reference range. A decrease of T4/T8 ratio below 1 is found as an effect of strong physical load, like a marathon race (10). As T4-cells play a central role in the infection defence, a low ratio indicates a stress induced immune destabilization. This raises the question, whether this decrease in T4/T8-cell ratio is responsible for the decrease in total receptor number per cell. As T4-cells have only few β -receptors and T8-cells have at least the two-fold number (6,13) a decrease in T4/T8-cell ratio should result in an increase in the total β -receptor number. This means that the actual observed decrease in receptor number is not a consequence of a redistribution of lymphocyte subsets, but reflects a real change in receptor density per cell.

Literature

- (1) CARLSON SL, BROOKS WH, ROSZMAN TL (1989): Neurotransmitter-lymphocyte interactions: dual receptor modulation of lymphocyte proliferation and cAMP production. *J Neuroimmunol* 24:155-162
- (2) CLAUSTRE J, PEYRIN L (1982): Free and conjugated catecholamines and metabolites in cat urine after hypoxia. *J Appl Physiol* 52(2):304-308
- (3) CUNNINGHAM WL, BECKER EJ, KREUTZER F (1965): Catecholamines in plasma and urine at high altitude. *J Appl Physiol* 20(4):607-610
- (4) GREENACRE JK, CONOLLY ME (1978): Desensitization of the beta-adrenoceptors of lymphocytes from normal subjects and patients with phaeochromocytoma: studies in vivo. *Br J Clin Pharmacol* 5:191-197
- (5) HELLSTRAND K, HERMODSSON S (1989): An immunopharmacological analysis of adrenaline-induced suppression of human natural killer cell cytotoxicity. *Int Arch Allergy Appl Immunol* 89:334-341
- (6) MAISEL AS, HARRIS T, REARDEN CA, MICHEL MC (1990): β -adrenergic receptors in lymphocyte subsets after exercise. *Circulation* 82:2003-2010
- (7) KINDERMANN W, URHAUSEN A, RICKEN KH (1989): Einfluß von mehrtägigem intensiven Training und nachfolgender Regeneration auf Lymphozytensubpopulationen, *Deutsch Z Sportmed* 40:30-33
- (8) LEFKOWITZ RJ, LINDENLAUB E (1985): Adrenergic receptors: molecular properties and therapeutic implications. Schattauer Stuttgart
- (9) MANZL G, GEWALT B, BUB A, FORSSMANN WG, WEIKER H (1988): Der Einfluß von Höhe auf die sympathoadrenerge Regulation und das Renin-Aldosteron-System. *Dtsch Z Sportmed* 39:19-24
- (10) ORDER U, RIEDEL H, LIESEN H, WIEDENMAYER W, HELLWIG T, GEIST S, (1989): Leukozyten- und Lymphozytensubpopulationen. *Dtsch Z Sportmed* 40:22-29
- (11) SBIRRAZZUOLI V, LAPULUS P (1989): Human lymphocyte and myocardial beta-adrenoreceptors: up and down regulation. *Biomed Pharmacother* 43:369-374
- (12) SHERMAN NA, SMITH RS, MIDDLETON E (1973): Effect of adrenergic compounds, aminophylline and hydrocortisone on in vivo immunoglobulin synthesis by normal human peripheral lymphocytes. *J Allergy Clin Immunol* 52:13-22
- (13) WERLE E, JOST J, KOGLIN J, WEISS M, WEIKER H (1989): Modulation der zellulären Immunabwehr auf Rezeptorebene während akuter körperlicher Belastung. *Dtsch Z Sportmed* 40:14-22
- (14) WHALEN MM, BANKHURST AD (1990): Effects of β -adrenergic receptor activation, cholera toxin and forskolin on human natural killer cell function. *Biochem J* 272:327-331
- (15) WILLIAMS LT, SNYDERMAN R, LEFKOWITZ RJ (1976): Identifikation of β -adrenergic receptors in human lymphocytes by (-)- 3 H-alprenolol binding. *J Clin Invest* 57:149-155
- (16) ZIMMERMANN E, STOCK C (1992): Stress adaptation to moderate altitude conditions. I. Hormonal and metabolic responses; see this volume.

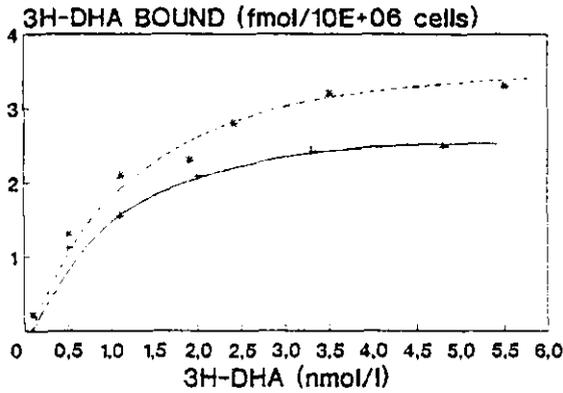


Fig.1a. Lymphocyte 3H-DHA binding from pre- (dotted line) and post- (continued line) examinations. Each data point represents the mean of 14 values.

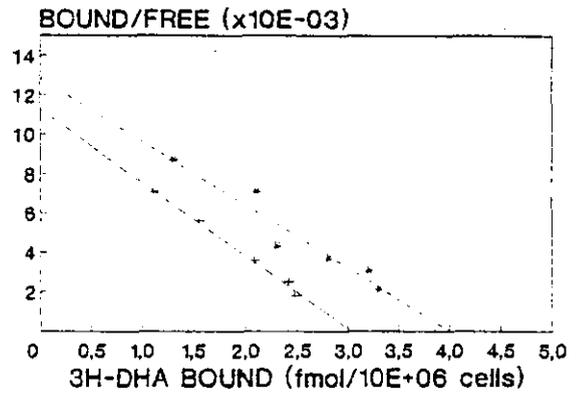


Fig.1b. Scatchard plots of the binding data from pre- (dotted line) and post- (continued line) examinations. Each data point represents the mean of 14 values.

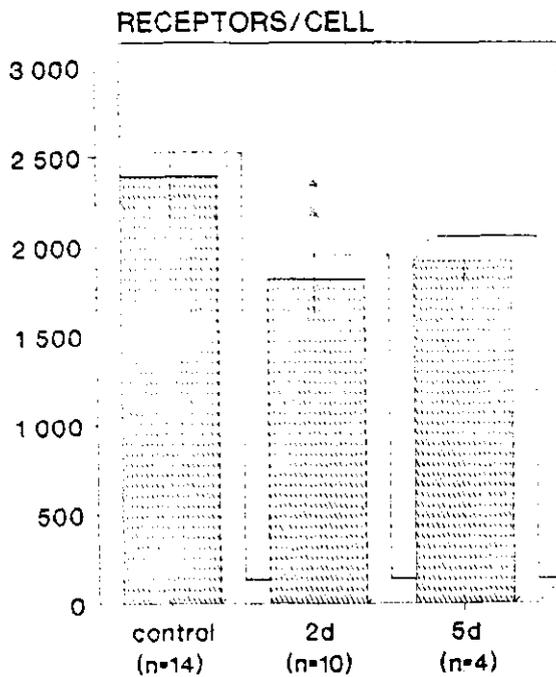


Fig.2. Density of β -receptors on human MNLs before the altitude stay (control, n=14) and at day 2 (2d, n=10) and day 5 (5d, n=4) after return. Bars represent the means \pm SD. ** p<0.01.

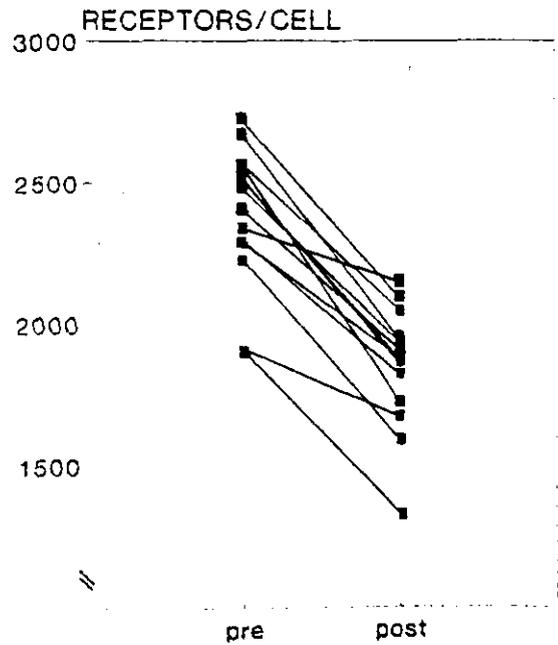


Fig.3. Density of β -receptors on mononuclear leukocytes of pre- and post-examinations from 14 subjects.

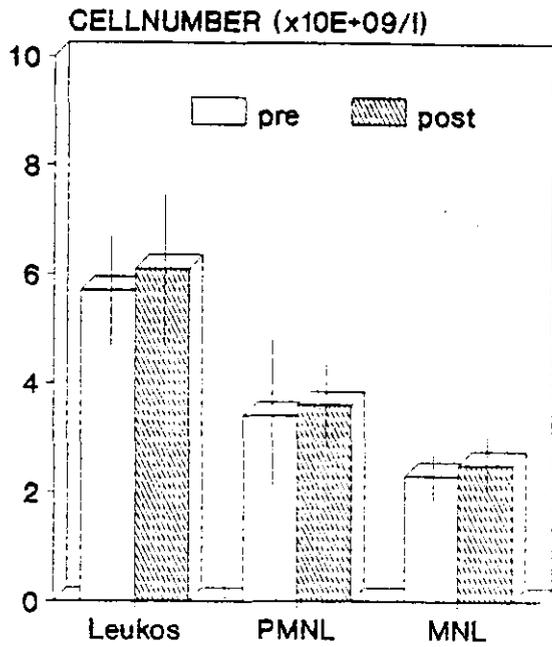


Fig.4. Effect the 14-days stay at altitude on total leukocytes (leukos), polymorphnuclear leukocytes (PMNL) and mononuclear leukocytes (MNL). Values are means \pm SD.

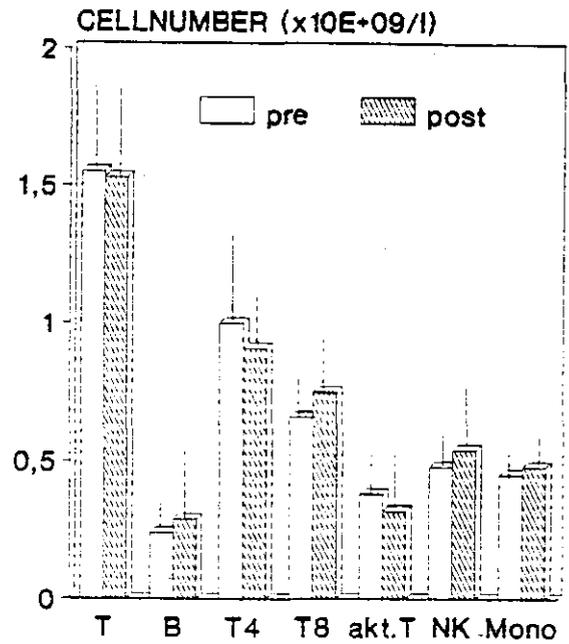


Fig.5. Effect of the 14-days stay at altitude on T-cell (T), B-cell (B), T4-cell (T4), T8-cell (T8), activated T-cell (akt.T), natural killer cell (NK) and monocyte (Mono) counts. Values are means \pm SD.

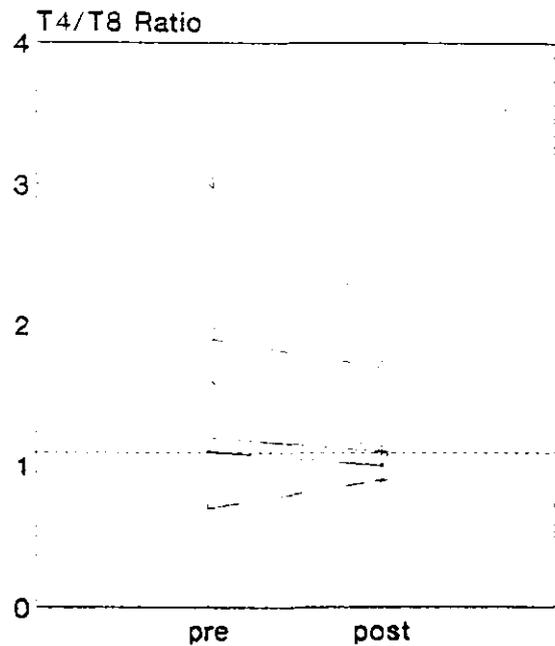
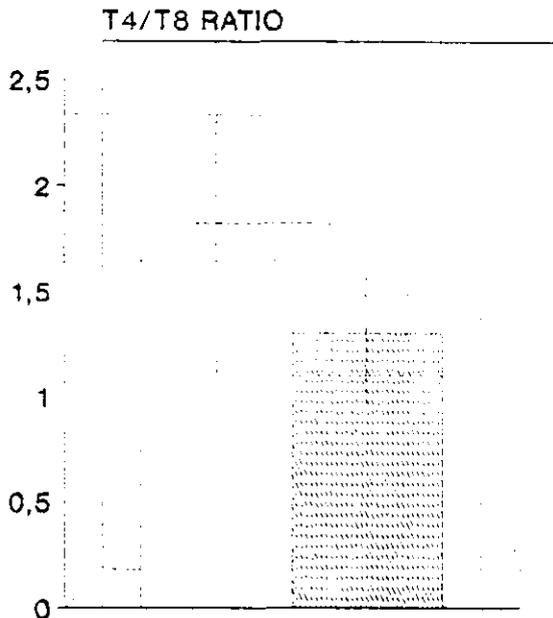


Fig.6. + Fig.7. Effect of the 14-days stay at moderate altitude on the T4/T8-ratio. Values in Fig.6 are means \pm SD. Dotted lines indicate the reference ranges.

**Antarctic Isopoda (Crustacea: Peracarida):
Stress in a polar environment?**

by J. W. Wägele, University of Bielefeld
Abteilung Morphologie und Systematik der Tiere

Introduction

Isopods are an important element of the crustacean fauna of the Southern Ocean. While the large decapods that are so common in shallow habitats of warmer seas are absent in Antarctica - with the exception of a few species of shrimps (Arntz & Gorny 1991) - brooding peracarids, especially the Amphipoda and Isopoda, occur with a large number of species nearly everywhere, from the sub-fast-ice communities to the deep-sea benthos. Today about 230 species of isopods are known from the continental shelf of Antarctica. This diversity suggests that cold might per se be no stressing factor for poikilotherm animals. Nevertheless the polar climate seems to be a disadvantage for many taxa. Most fish families do not occur in the Southern Ocean, several taxa of Malacostraca, Mollusca, Echinodermata etc. are absent. While most terrestrial animals obviously can not survive on the Antarctic continent because of the absence of food, the marine mammals and birds need adaptations to reduce and compensate the loss of heat and the marine invertebrates must adapt to the extreme seasonality and the slowness of biological processes at low temperatures. Studies on the biology and systematics of the Isopoda help to understand the peculiarities of this ecosystem.

Environmental conditions

The physical properties of the Southern Ocean are rather uniform throughout the year, the temperature fluctuating between about +1 and -2°C, the salinity between 34 and 35 ppm (e.g. Hellmer & Busch 1985). A pronounced vertical stratification does not exist, large changes in temperature that serve as barriers in other oceans are absent. Very important is the seasonality of light intensity and of the extension of the sea ice. Phytoplankton blooms are rich, but restricted to a few weeks per year (e.g. von Bodungen et al. 1988). Secondary production depends entirely on these blooms, the input of terrigenous organic material is of no importance, virtually absent in most coastal areas (review e.g. in Hempel 1985).

Peculiarities of the Antarctic benthos

Species diversity is unexpectedly high in taxa as Porifera, Polychaeta, Amphipoda, Isopoda, Bryozoa and Acidiacea, distinctly higher than in the Arctic Ocean (Knox & Lowry 1977). This is one reason why research on benthic ecology is so difficult and why we are very interested in the

compilation of taxonomic monographs, for which only few specialists remain as potential authors. The reasons for the high diversity discussed in literature are the age of the ecosystem, the predictable seasonality and relative stability of climatic conditions, which allow an evolutionary adaptation (e.g. Lipps & Hickman 1982). Speciation might also be a consequence of low mobility and slow reproduction. An indication for the existence of this mechanism is polymorphism in widely distributed species (e.g. Wägele 1986) (Fig. 1).

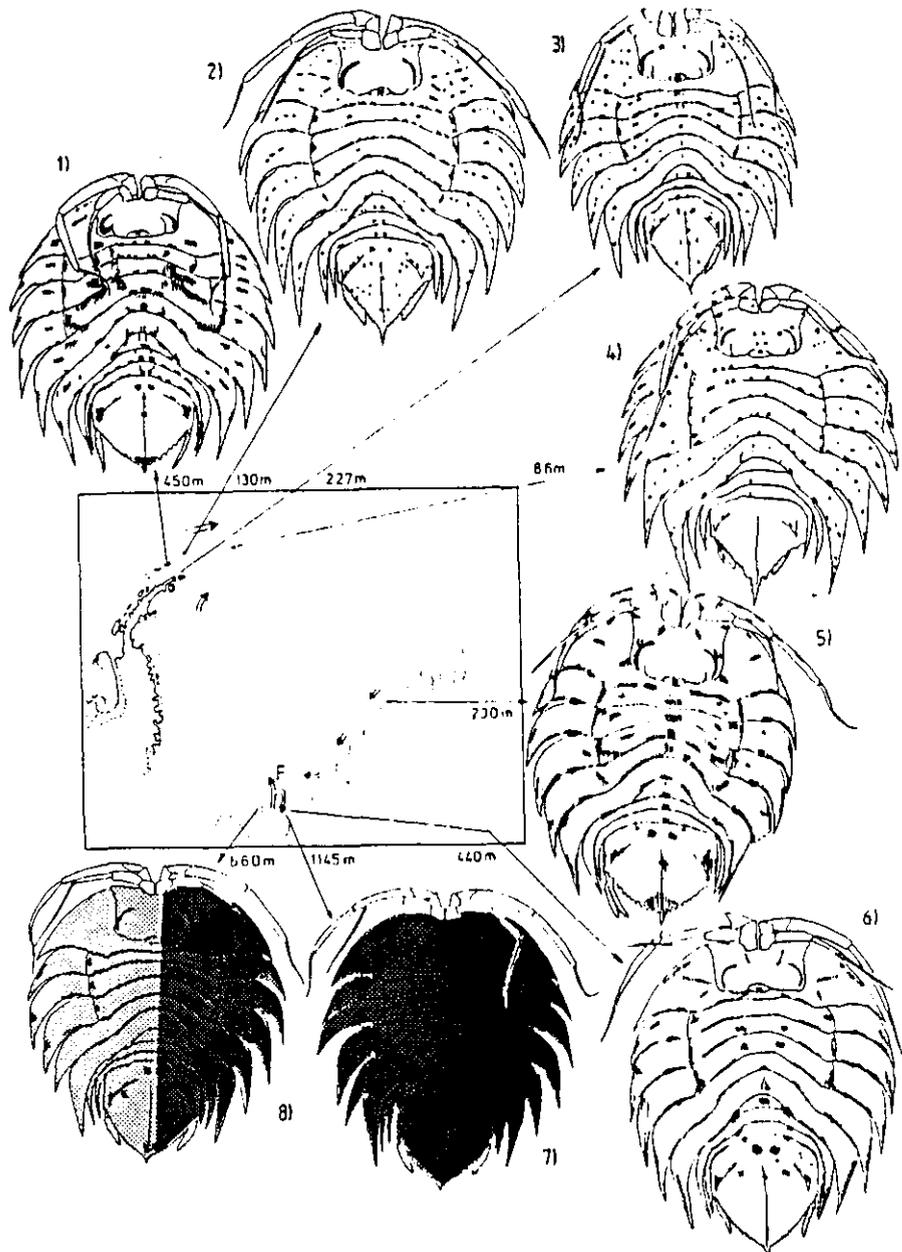
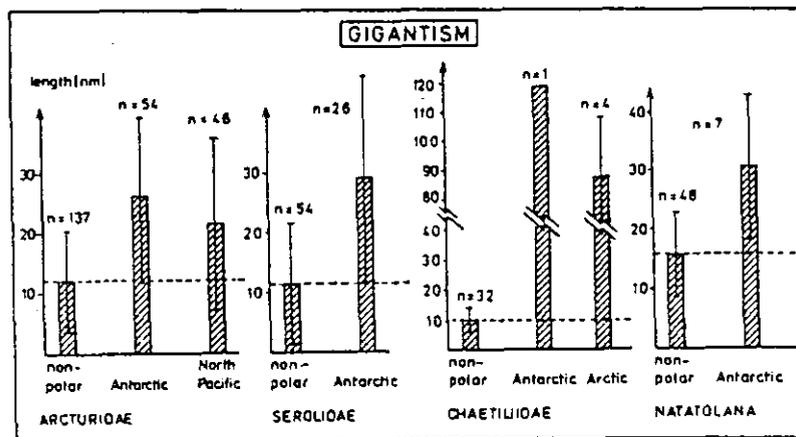


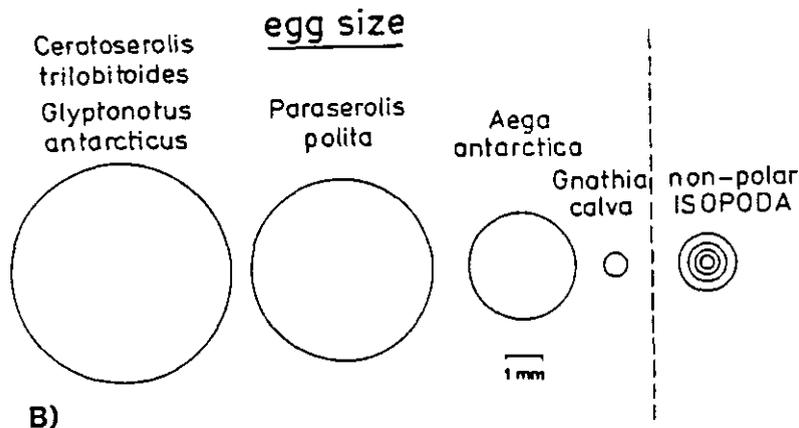
Fig. 1: Polymorphism of *Ceratoserolis trilobitoides*. Within local populations morphology varies little (from Wägele 1986). F: Filchner depression; arrows: currents

Most invertebrates are endemic for Antarctica (summary in Brandt 1991), probably also a consequence of the age and of the singular climate of the ecosystem.

The trophic relationships and productivity in the benthic communities are still poorly known. Standing stock is high (e.g. Jazdzewski et al. 1987), but hitherto observed cases of slow growth suggest that this biomass has accumulated in many years and has a low turnover. Interestingly suspension feeders (sponges, bryozoans, hydrozoans, dendrochirotid holothurians) are dominant in terms of biomass on the continental shelf of many areas (e.g. Voß 1988); and are preyed-on by specialized Amphipoda, Asteroidea, Nudibranchia (e.g. Coleman 1989, 1991, Dayton et al 1974, H. Wägele 1989, 1990). Another frequent mode of nutrition is necrophagy (e.g. Pressler 1986): a large number of species can be collected with baited traps. In deeper water an important source of energy must be detritus originating from sedimentation. This depends of the seasonal productivity of phytoplankton and has been studied e.g. in the Bransfield Street (von Bodungen et al. 1987).



A)



B)

Fig. 2: A: Average maximum length of isopod taxa from Antarctica in comparison with other regions. Note that species from colder oceans (Antarctica, North Pacific, Arctic) are larger than other species.

B: Egg size of Antarctic isopods in comparison with other species.

Biology of Antarctic Isopoda

When collecting specimens from trawl samples or by SCUBA diving one of the first impressions is that many species of invertebrates attain an unusual large size. Among isopods the largest species of the Paranthuridae, Chaetiliidae, Arcturidae, Serolidae und Gnathiidae live in Antarctica. To show that gigantism is a rule and not a subjective impression, the average maximal length of non-polar species can be compared with that of polar species (Fig. 2). The correlation between large size and low temperature even has an effect within populations of a single species: southern populations of *Ceratoserolis trilobitoides* have larger (but fewer) eggs than populations from Subantarctic localities (Wägele 1987). This is accordance with Thorson's rule (Thorson 1936, 1950).

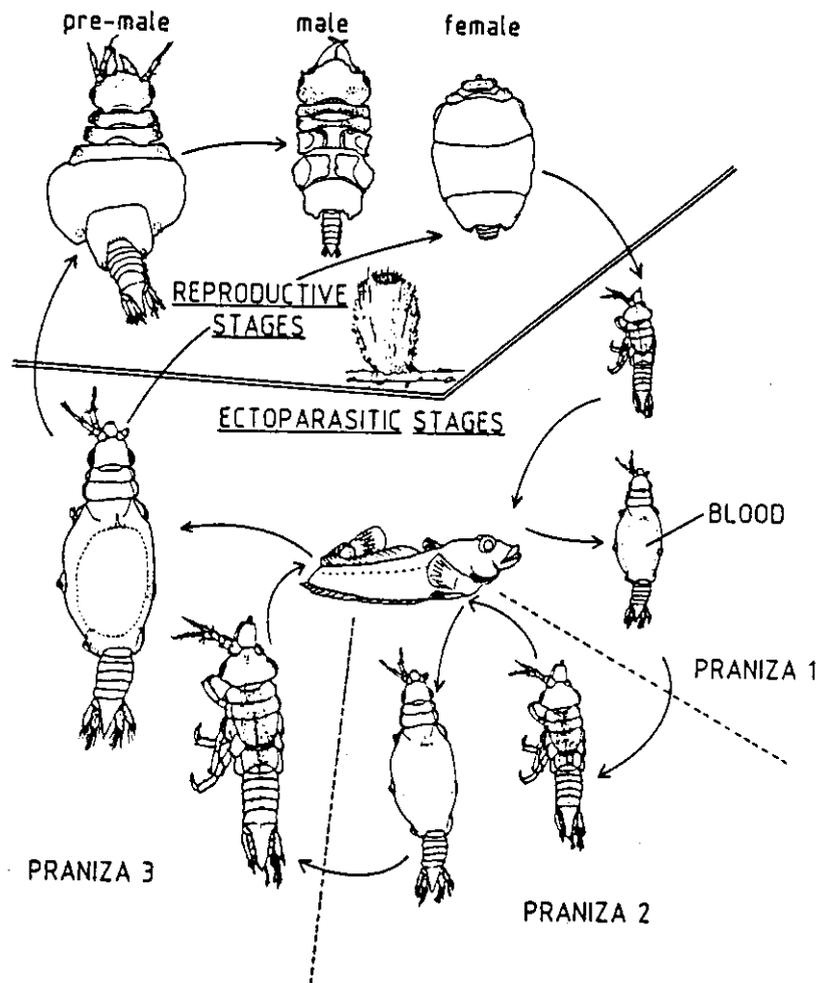


Fig. 3: Life cycle of the Antarctic fish parasite *Gnathia calva* (after Wägele 1988).

It can be expected that low temperatures induce a retardation of all biological processes. The study of life cycles is logistically difficult, but some data support the suspicion that life in cold water is slow. While isopods of the North Sea usually live for 1 year, summer generations only for few months, the life cycles of Antarctic species take much longer.

The large valviferan *Glyptonotus antarcticus* is probably sexually mature after 5 or more years; females of *Paraserolis polita* produce the first brood after 28 months, while the larger serolid *C. trilobitoides* needs 3.5 to 4 years, *Gnathia calva* 3 - 4 years (Fig. 3), *Aega antarctica* more than 10 years to mature and spawn (Wägele 1987, 1990). In view of the comparatively lower number of eggs it is not surprising that the production of eggs per minimum generation time is very low in Antarctic species (Table 1). Slow growth and low fecundity indicate that Antarctic populations must have a much lower regenerative potential than populations of related species in warmer seas.

In this context it is interesting that metabolic adaptations to cold water seem to be absent, at least according to respiration measurements (e.g. Luxmoore 1984). Anti-freeze protection has not been studied.

In view of this slow life cycles the gigantism of many species is paradoxical. Faster reproduction should be possible when maturity is reached at a smaller size, cold-water species should have a selective advantage with a reduced body size. Obviously the ecosystem favours K-selection: concurrence is in a stable environment more important a factor than the reproductive rate. Many Antarctic species have the typical features of K-selected organisms: longevity, large body size, low reproductive rates (see e.g. Pearse 1969, Ralph & Everson 1972, Arntz & Gorny 1991). Slowness as a physiological effect of low temperatures can not easily be discerned experimentally from evolutionary adaptations to a stable environment.

Genetic adaptations need time to evolve. This time was available in the Southern Ocean. Polar conditions exist for at least 25 m.y. since the formation of the circumpolar current, which isolated Antarctica thermally (and faunistically) (Kennett 1977, Barker et al. 1988). Fluctuations of the extension of the ice sheet must have had an effect on the availability of food and space, but not on water temperature. It is obvious that the species diversity, the peculiarities of morphology and life cycles are the result of a long period of evolution in a polar environment. Temperature stress in the sense of factors that reduce fitness within this ecosystem has not been detected; those species that survived the cooling of the Southern Ocean evolved to a highly specialized, adapted fauna.

That such a speciation really took place has recently been shown (Wägele, in press). A phylogenetic - biogeographic

analysis revealed that the Antarctic Serolidae evolved in situ, isolated of populations of the neighbouring continents (South America and Australia). Offsprings of an older Gondwana fauna still survive in a disjunct area of cold-temperate climate (tips of southern continents), a distribution that must be explained with vicariance by continental drift.

Table 1 Average number of eggs produced per day of minimum generation time by 1000 females
(**bold** = Antarctic species, *italics* = stygobionts)
(for references see Wägele 1987, 1988, 1990)

<u>species</u>	<u>eggs, day</u>
Asellus hilgendorffi (25°C)	780
Idotea baltica (Sommer)	666
Idotea baltica	580
Idotea baltica (Winter)	403
Idotea chelipes (Sommer)	400
Asellus hilgendorffi (15°C)	310
Asellus aquaticus	290-300
Idotea chelipes (Winter)	282
Idotea chelipes (Sommer)	259
Glyptonotus antarcticus	210
Idotea chelipes (Winter)	177
Sphaeroma hookeri	160
Asellus aquaticus	150-160
Saduria entomon	132
Dynamene bidentata	120-130
Asellus aquaticus	82
Idotea pelagica	80
Exciorolana braziliensis (Sommer)	79
Gnathia calva	71
Eurydice pulchra (Winter)	61
Limnoria lignorum	55
Cyathura carinata	55
Exciorolana braziliensis (Winter)	52
Gnorimosphaeroma insulare	44
<i>Proasellus cavaticus</i>	21- 30
Serolis polita	28
Ceratoserolis trilobitoides	11
Aega antarctica	9
<i>Stenasellus virei</i>	7

References

- Arntz, W. E., Gorny, M. (1991): Shrimp (Decapoda, Natantia) occurrence and distribution in the eastern Weddell Sea, Antarctica. *Polar Biol.* 11: 169-177.
- Barker, P. F., Kennett, J. P., Scientific Party (1988): Weddell Sea palaeoceanography: preliminary results of ODP leg 113. *Palaeogeogr. Palaeoclimat. Palaeoecol.* 67: 75-102.
- Bodungen, B. von, Smetacek, V. S., Tilzer, M. M. (1986): Primary production and sedimentation during spring in the Antarctic Peninsula region. *Deep-Sea Res.* 33: 177-194.
- Brandt, A. (1991): Zur Besiedlungsgeschichte des antarktischen Schelfes am Beispiel der Isopoda (Crustacea, Malacostraca). *Ber. Polarforsch.* 91: 1-240.
- Coleman, O. (1989): On the nutrition of two Antarctic Acanthonotozomatidae (Crustacea Amphipoda). Gut contents and functional morphology of mouthparts. *Polar Biol.* 9: 287-294.
- Coleman, O. (1991): Comparative fore-gut morphology of Antarctic Amphipoda (Crustacea) adapted to different food sources. *Hydrobiologia* 223: 1-9.
- Dayton, P. K., Robilliard, G. A., Paine, R. T. (1974): Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecol. Monogr.* 44: 105-128.
- Hellmer, H., Bersch, M. (1975): The Southern Ocean. *Ber. Polarforsch.* 26: 1-114.
- Hempel, G. (1985): On the biology of polar seas, particularly the Southern Ocean. In: Gray, J.S., Christiansen, M.E.: *Marine Biology of Polar Regions and Effects of Stress on Marine Organisms*; John Wiley & Sons Ltd.: 3-33.
- Jazdzewski, K., Arnaud, P. M., Presler, P., Sicinski, J. (1987): Faunistic, zoogeographic and biocenotic remarks on benthic fauna of the Admiralty Bay (King George Island, South Shetland Islands, Antarctica). *Przegl. Zool.* 31: 127-137.
- Kennett, J. P. (1977): Cenozoic history of the circumantarctic current. Dunbar, M. J. (ed) *Polar Oceans, Arctic Inst. N. Am., Calgary*: 575.
- Knox, G. A., Lowry, J. K. (1977): A comparison between the benthos of the Southern Ocean and the North Polar Ocean with special reference to the Amphipoda and

- the Polychaeta. In: Dunbar, M.J. (ed), Polar Oceans, Antarctic Inst. North America, Calgary: 423-462.
- Lipps, J. H., Hickman, C. S. (1982): Origin, age, and evolution of antarctic and deep-sea fauns. In: Ernst, W.G., Morin, J.G. (eds.), The environment of the deep-sea 2: 324-354.
- Luxmoore, R. A. (1984): A comparison of the respiration rate of some Antarctic isopods with species from lower latitudes. Br. Antarct. Surv. Bull. 62: 53-66.
- Pearse, J. S. (1969): Antarctic sea star. Austr. nat. Hist. 16: 234-238.
- Presler, P. (1986): Necrophagous invertebrates of the Admiralty Bay of King George Island (South Shetland Islands, Antarctica). Pol. Polar Res. 7: 25-61.
- Ralph, R., Everson, I. (1972): Some observations on the growth of *Kidderia bicolor* (Martens) (Mollusca: Lamellibranchiata) at South Georgia. Br. Antarct. Surv. Bull.: 31: 51-54.
- Thorson, G. (1936): The larval development, growth, and metabolism of Arctic marine Bottom invertebrates. Meddr. Grönland 100(6); 1-155.
- Thorson, G. (1950): Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1-45.
- Voß, J. (1988): Zoogeography and community analysis of macrozoobenthos of the Weddell Sea (Antarctica). Ber. Polarforsch. 45: 3-144.
- Wägele, H. (1989): Diet of some Antarctic nudibranchs (Gastropoda, Opisthobranchia, Nudibranchia). Mar. Biol. 100: 439-441.
- Wägele, H. (1990): Revision of the Antarctic genus *Notaeolidia* (Gastropoda, Nudibranchia), with a description of a new species. Zool. Scr. 19: 309-330.
- Wägele, J. W. (1986): Polymorphism and distribution of *Ceratoserolis trilobitoides* (Eights, 1833) (Crustacea, Isopoda) in the Weddell Sea and synonymy with *C. cornuta* (Studer, 1879). Polar Biol. 6: 127-137.
- Wägele, J. W. (1987): On the reproductive biology of *Ceratoserolis trilobitoides* (Crustacea: Isopoda): Latitudinal variation of fecundity and embryonic development. Polar Biol. 7: 11-24.

- Wägele, J. W. (1988): Aspects of the life-cycle of the Antarctic fish parasite *Gnathia calva* Vanhöffen (Crustacea: Isopoda). *Polar Biol.* 8: 287-291.
- Wägele, J. W. (1990): Growth in captivity and aspects of reproductive biology of the Antarctic fish parasite *Aega antarctica* (Crustacea, Isopoda). *Polar Biol.* 10: 521-527.
- Wägele, J. W. (199.): Notes on Antarctic and South American Serolidae (Crustacea, Isopoda) with remarks on the phylogenetic biogeography and a description of new genera. *Zool. Jb. Abt. Syst.* (in press).-

El Niño : effects of environmental stress on pinniped populations.

Fritz Trillmich, Bielefeld

Abstract

The 1982/83 El Niño event in the eastern Pacific, is one of the best documented events where severe environmental stress affected a whole ecosystem. El Niño is a meteorological and oceanographic phenomenon which occurs at irregular intervals. The 1982/83 event was particularly drastic, but smaller events seem to occur quite regularly, though unpredictably. Such events are part of the environmental variance to which most animals are exposed during their lifetime and they may have significant consequences for social and population processes or structure. El Niños can also serve as models of the potential reaction of top predators to human induced global changes.

During the 1982/83 El Niño, social patterns changed in response to reduced space and food competition in the declining pinniped populations. Mother-yong interactions and interactions among both females and males were altered, and animals redistributed over the available habitat. Dispersal, mortality, and fertility changed in the impacted populations. The magnitude of the effects on sexes, cohorts, and (sub-) populations depended on the timing of the event relative to the annual life cycle of the animals.

Rare events may influence differentiation among populations and reduce the longterm mean carrying capacity of the environment and/or the probability of reaching the carrying capacity. They also may create bottlenecks thereby reducing population heterozygosity. The split-up of populations into more or less separated sub-populations decreases the probability of extinction. Density-dependent factors are known to influence pinniped population dynamics, but environmental stress as a density-independent, stochastic process influences the population dynamics of mammals as large as pinnipeds far more than is generally appreciated.

Relevant Literature:

TRILLMICH, F. (1991). El Niño in the Galapagos Islands; a natural experiment. p.3-31; in MOONEY, H.A. et al. (eds.): Ecosystem Experiments. SCOPE 45, John Wiley & Sons, Chichester

TRILLMICH, F., LIMBERGER, D. (1985). Drastic effects of El Niño on Galapagos pinnipeds. *Oecologia* 67: 19-22

TRILLMICH, F., ONO, K.A. (eds.) (1991). Pinnipeds and El Niño: Responses to environmental stress. Springer/Heidelberg

ON SOCIAL STRESS IN MAMMALS

Hubert Hendrichs, Bielefeld

We are engaged in research into social structures and psycho-social processes in higher, mostly non-primate mammals - ungulates, carnivores, ungulate type caviomorph rodents -, and in marsupials. Investigations are carried out in the field - including desert areas, in larger enclosures, and in the laboratory. Although in some studies we assess physiological correlates, our main method is the long term observation of well-known individuals in varying social contexts.¹ Social stress and the compensatory coping reactions are therefore central issues in our studies. For many years I took it for granted - somewhat naively - that the concept of stress had been especially designed for mammals and, in its full and proper sense, required a mammalian type of organization, i. e. organisms with an antagonistic system of sympathetic and para-sympathic vegetative enervation, with adrenal cortex and medulla - as addressed in this workshop by the speakers on stress in humans² - which can be activated in a specific syndrome induced in an unspecific way by a great number of different factors, such as heat and cold, enemies and sexual partners.

Only in recent years I learned that already SELYE, developing the concept of stress, did not intend to restrict its use to organisms with nervous systems,³ and that today the concept of stress is used in a wide range of biological systems from cells to ecosystems. In looking further for possible analogies of stress processes, we invariably get to the domain in which the terminology used in stress theory originated: the mechanics of technical constructions, like bridges or cars, aspects of stability and elasticity of constructive elements under strain: the building up of tensions, the changing of forms, and depending on normal or excessive use, the retaining or losing of elasticity, deforming irreversibly, and finally breaking or tearing. The mathematical description of such processes has become highly developed, associated with names as HOOKE, MARIOTTE, BERNOULLI, EULER, COULOMB.⁴

Generally speaking, stress theory is concerned with the reactions of elements in an environment, with stress being a state of the element in relation to the environment, not a quality of the environment. The element activates its possibilities in an effort to retain its elasticity, flexibility, the control of its potential, or even its "well-being". An important question, which cannot be left unanswered, is to what extent such a wide use of the term and the conception of stress combines homological and analogical aspects with metaphorical heuristics. Some clarification of these points could be a valuable contribution to stress theory by a workshop like this. The projected publication containing the papers of this workshop should therefore, I suggest, contain a chapter, possibly written jointly by several of the participants, that takes these questions.⁵

In this paper I will not try to answer such questions, but propose four functional contexts of social stress that could be used as complex examples in a comparative evaluation of these questions. The four functional contexts in which social stress and coping reactions are of importance, are contexts that, although also present in other vertebrates, are especially relevant in mammals:

- I. The regulation of competitive agonistics and aggressiveness in social groups, fight and flight reactions in a social context, producing order, i. e. a social organization of relative stability.
- II. The regulation of reproduction in populations providing mechanisms of density regulation.
- III. The regulation of mental states in individuals, producing in ontogeny an individual mentality, with modifications in the areas of possible stimulation and of possible tactics for coping.
- IV. The support of a phylogenetic development of cognitive intelligence, of awareness and consciousness - by exploring its mental space in stressing situations, detecting or producing cognitive niches for coping.

Thus, there are four levels of organization: individual, group, population, and species. It is, in this view, always individual animals that are stressed - not groups or higher-level systems, but the functional consequences of this also have an impact on these other levels: the organization of groups, the reproduction in populations, the mentality of individual animals, and the cognitive capacity achieved in species.

I. On maintaining a cohesive organization in social groups of mammals

The social life of mammals can be organized in different ways.⁶ The individuals of many species live on their own, with only passing closer contacts to conspecifics. The individuals of other species live permanently together in closely knit groups with division of labor and highly differentiated social relations. The goats and ibexes described yesterday⁷ are somewhere in between: not living solitarily, but in groups of aggregating individuals, not permanently attached to each other.

It is a complex task to integrate several competing adult individuals of the same sex into one closed cooperative unit. In a number of species - like wolves, dwarf mongooses and marmosets - this has been achieved by restricting reproduction to only one female and one male in the group, while complex behavioral and physiological mechanisms prevent the reproduction of the other adult subdominant animals. In many species, especially in ungulates and primates, it has been achieved that more than one of the group's females can breed regularly. Only in very few species - like lions, or some baboon species - it has been achieved that more than one male can breed regularly. This can be considered the most complex organization in mammals living in permanently bonded groups.

The actions of animals living in permanent groups are restricted in many ways. This is obvious for the subdominant individuals. They cannot show certain behavior, they cannot use certain places, they may not be able to contribute to the decisions leading to the group's actions. Their attention is mainly directed toward group members. In stressing agonistic encounters the subdominant individuals learn their dominance position. They also learn to find places and situations that allow them to avoid stressful encounters. They also learn to accept their social situation. That is a necessary condition for cooperative group performance. The fact that the sub-dominant animals are obviously restricted does not imply, however, that they are the individuals of the group subjected to the highest levels of stress. Dominant animals can be highly absorbed and stressed by events outside the group, such as the approach of another, competing group, or of predators. The subdominant animals are less stressed by such events, keeping their attention on the dominant ones, ready to follow their "evaluation" of the danger and their "decisions" to attack or to flee - these terms are used here without necessarily assuming states of awareness in the acting animals.

The point I want to make is that each animal in a cohesive group of mammals has to adjust itself to a specific situation, holding a specific potential for discomfort and stress, but also a specific potential for reassurance and for coping. For the latter it can develop individual relations to places that provide security and to other group members that can be approached for reassurance and support. The same bush or the same rock or ground, may for one animal possess a quality of providing security and reassurance and for another of the same group (in relation to its specific experiences) a quality of inducing nervousness or fear. Adapting to their specific situations, the animals produce individual profiles in relation to the perception of their environment and concerning their responsiveness to events in their environment. This individual profile enables the animal to generate the activations and the states of arousal that are helpful in gaining and maintaining the states of well-being, as far as these are attainable in its specific situation.

II. On density dependent regulation of reproduction in populations of mammals

Regulation of reproduction is not the only way to regulate population density, and it is not specific to mammals.⁸ But there are in mammals specific mechanisms connected with the close and long-lasting relationship between mother and young and with the long-lasting dependency of young animals on the presence of older conspecifics. High social stress in a population can lower the rate of conception or even suppress estrous in females of breeding age. Pregnant animals may not carry until birth, mothers may not produce enough milk, the young may not get a chance to find the milk, or have enough time to suckle. Even when social stress is lower and consequences are less severe, it still is of importance for the development of the young. In later ontogeny the young encounter in their groups or populations a different social environment in relation to the levels of social stress in the adult animals. They may develop different physiological and mental qualities, for example specific avoidance reactions, specific sensibilities or insensibilities, or specific forms of aggressiveness.

III. On the generation of individual mentalities in mammals

In relation to differences in their ontogenetical development, whether stress-induced or not, mammals can produce differences in their emotional and cognitive mentality.⁹ With specific coping tactics individual mental profiles are developed, providing specific environmental structures with specific potentials for stimulation. Regulating its stress-stimulated states of arousal in its specific situation, the mammal can develop a specific cognitive and emotional mentality. By producing profiles of subjective relevance in this process, it shapes its own environment so to speak, and at the same time some of the conditions of its well-being. With increasing complexity of the psychosocial organization of the species, the influence of individually acquired profiles changes in terms of quality and proportion. SELYE distinguished the processes of stress reactions from those of CANNON'S homeostatic regulation.¹⁰ This is an important distinction, although both processes are closely interrelated, especially at the physiological level. There may be an additional relation between stress and homeostasis at the psychosocial level. Stress reaction can become functional in supporting mental homeostasis, adapting the individual's mentality to its specific environment, and maintaining the regulation of this adaptation.

IV. On the phylogenetic development of cognitive intelligence

Equipped with an individual mentality, the mammal can develop specific tactics in its specific situation, e. g. in searching for the stimulations required to maintain adequate activation, and for possibilities of coping with excessive stimulation. Choosing among possible stimulations and among possibilities of coping, regulating its states of arousal, does not necessarily imply any awareness in the choosing animal, but it does promote a recognition of the situation, and an evaluation of the possibilities for adequate action in relation to appropriate stimulation, selecting specific stressors and avoiding others.¹¹ I propose that this context of tactical evaluation and decision making - exploring, in different states of activation and of emotional arousal, the spaces of mental and of environmental possibilities for coping tactics and strategies, including more distant goals and occurrences distant in space and in time - contains important conditions for the evolutionary development of cognitive performance in mammals, and of their awareness, possibly up to human moral consciousness.¹²

A complex social structure is neither a sufficient nor necessary condition for the development of a complex cognitive ability. The same social structure can be found in species with modest cognitive abilities, and in others with high cognitive capacities. The same social structure in one species can be maintained without, and in another with, complex mental performances. A social structure can be maintained and used with both low and high cognitive differentiation. The mechanisms involved in efficient performances can be of simple or high complexity. Structures and rules may be perceived or not, intended or not. The (evolutionary) development of increasing complexity of the mechanisms maintaining and using a complex social structure can be seen as one important thread in the differentiation of perception and orientation in a differentiating environment with widening spatial and temporal horizons. But the differentiation of social structures has to be considered separately from the differentiation of the mechanisms used to establish and maintain the structures. And the differentiation of these mechanisms has, in turn, to be considered separately from the differentiation of the perceptual and cognitive processes involved. The differentiation of these perceptual and cognitive performances has then to be considered separately from the differentiation of the awareness accompanying the processes. The differentiation of social and nonsocial environments brings new ways of being stressed and new possibilities to cope with stress. Changing phases of "hot" and "cold" stress, each followed by phases of specific forms of relaxation, provide ample opportunities for mental development, for a differentiation of representations and cognitive abilities. Individual mental representations, as developed in complex socially transmitted environments, can imply individual causes of fear and of reassurance, individual ways of being stressed and individual ways of coping with stress. With the differentiation of perception and cognition, and with an increasing differentiation of mental representations, an increasing demand can be assumed for mechanisms generating and stabilizing decisions in situations with several possible alternatives for effective action, mechanisms for evaluating alternatives in regard to possible goals. The demand is for the differentiation of the possibilities of communication, both inside the individual - planning, "thinking" -, and between individuals - "language". And it is also a demand for the development and stabilization of identity, a self that has to, and can, retain its original decisions against changing actual impulses, that can persistently adhere to a goal against actual difficulties.

Notes

1. Recent publications include:

SACHSER, N.: Different forms of social organization at high and low population densities in guinea pigs. *Behavior* 97, 235-272, 1986.

STAHNKE, A., and HENDRICH, H.: Social variability in male guinea pigs: Different dominance quality resulting from early social experience. *Zoologische Beiträge N.F.* 29, 413-435, 1986.

STEFANSKI, V., HENDRICH, H., and RUPPEL, H. G.: Social stress and activity of the immune system in guinea pigs. *Naturwissenschaften* 76, 225-226, 1989.

THYEN, Y., and HENDRICH, H.: Differences in behavior and social organization of female guinea pigs as a function of the presence of a male. *Ethology* 85, 25-34, 1990.

SCHWEDE, G., HÖLZENBEIN, S., and HENDRICH, H.: Sparring in White-tailed deer (*Odocoileus virginianus*). *Zeitschrift für Säugetierkunde* 55, 331-339, 1990.

DRESSEN, W., GRUEN, H., and HENDRICH, H.: Radio telemetry of heart-rate in male tammar wallabies (Marsupialia: Macropodidae): Temporal variations and behavioral correlates. *Australian Journal of Zoology* 38, 89-103, 1990.

KORZ, V.: Social relations and individual coping reactions in a captive group of Central American Agoutis (*Dasyprocta punctata*). *Zeitschrift für Säugetierkunde* 56, 207-218, 1991.

SCHWEDE, G., HENDRICH, H., and WEMMER, C.: Activity and movement patterns of young White-tailed deer fawns. In: BROWN, R. D., Ed.: *The biology of deer*. Springer, New York -Berlin-Heidelberg 1992.

DRESSEN, W., and HENDRICH, H.: Social behaviour and heart rate in tammar wallabies (Macropodidae: *Macropus eugenii*). *Journal of Zoology* 227, 299-317, 1992.
2. Cf. the four papers of ZIMMERMANN, STOCK, TEUCHERT-NOODT, and DAWIRS, this volume.
3. SELYE, H.: *The stress of life*. p. 53 f., Mc Graw-Hill, New York-Toronto-London 1956.
4. Cf. SZABO, I.: *Geschichte der mechanischen Prinzipien und ihrer wichtigsten Anwendungen (IV. Geschichte der linearen Elastizitätstheorie)*. Eds. ZIMMERMANN, P., and FELLMANN, E.A., Birkhäuser, Basel-Boston-Stuttgart 1983.
5. Cf. conclusions by BRECKLE, MANNESMANN, and WAISEL, this volume.
6. Cf. HENDRICH, H.: *Die soziale Organisation von Säugetierpopulationen*. *Säugetierkundliche Mitteilungen* 26, 81-116, 1978.
7. Cf. the paper of SHKOLNIK, A., this volume.
8. Cf. THIESSEN, D.: *Population density and behavior. A review of theoretical and physiological contributions*. *Texas Reports on Biology and Medicine* 22, 266-314, 1964.
COHEN, M. N., MALPASS, R. S., and KLEIN, H. G., Eds.: *Biosocial mechanisms of population regulation*. Yale University Press, New Haven-London 1980.
9. Cf. HENDRICH, H.: *Die individuelle Selbstgestaltung von Umwelt und Wohlbefinden bei Säugetieren*. In: NIEGEL, W., and MOLZBERGER, P., (Eds.): *Aspekte der Selbstorganisation*, p. 27-40, Springer, Berlin-Heidelberg-New York 1992.
HENDRICH, H.: *Individual psychosocial structures in mammals*. In: KHALIL, E. L., and BOULDING, K. E., Eds.: *Social and natural complexity*. Cambridge University Press, in press.

10. CANNON, W.: The wisdom of the body. Norton, New York, 1932.
SELYE, H.: Stress. Bewältigung und Lebensgewinn, Piper, München 1974.
11. We touched the relevance of stress for processes of perception and learning in the discussion of the papers of TEUCHERT-NOODT and DAWIRS (this volume).
12. Cf. HENDRICHS, H.: Individual psychosocial structures in higher mammals: possible requirements for the realization of human mind and morality. In: SCHMITZ-MOORMANN, K., Ed.: Origins, Time and Complexity. In prep.

Conclusions - What is Stress ?

A Common Phenomenon in Organisms

S.-W. BRECKLE, R. MANNESMANN & Y. WAISEL

People who have to do too many things, or those who face great challenges, are stressed. Bacteria which are exposed to a flush of hot water develop an apparent heat-stress (RON, see p. 7 ff.). Mice that are confined to a closed territory, with too many of their colleagues around, exhibit specific phenomena of social stress (HENDRICHS, see p. 105 ff.). Plants that grow on saline soils exhibit structural adaptations (BRECKLE, see p. 39 ff.) and develop salt stress responses: their enzymes function at suboptimal levels, their growth is reduced, their senescence starts earlier etc. (WAISEL 1972).

What is in common to all these examples?

Stress can be defined as the response of an organism to a threat or a pressure of an unusual factor. A similar definition from the zoological view-point is given in the introduction paragraph by AR (p.61). Such stress responses may reflect pathological reactions, but they may also represent an adaptive change that enables the stressed organism to withstand the new, stressful situation. Dangerous levels of abiotic factors, can be termed "stress factors" (LEVITT, 1980): too high a temperature (heat stress), too low a temperature (cold stress), too much water (flooding stress or oxygen stress), too little water (desiccation stress), etc. (Table 1).

Table 1 : Stress-effects in plants caused by exogenous factors (partly from LEVITT 1980)

Abiotic Stress	
temperature	high (heat)
	low (cold)
	chilling
	freezing (frost)
water	deficit (drought)
	excess (flooding)
radiation	visible light (high / low)
	UV (high)
	IR
	Ionizing (X-, γ -, α -, β -)
chemical	osmotic
	salts, ions, heavy metals, etc.
	pH (high / low)
	organics (herbicides)
	gases (SO ₂ , NO _x , O ₃ , etc., but also hypoxia)
	fire (combined chemical and mechanical effects)
mechanical	wind, pressure, etc.
Biotic Stress	
competition	interspecific
	intraspecific
	herbivores (grazing, frugivores etc.)
pests	parasites
	diseases
	flowering / fruiting

Evaluation of stress conditions should consider the magnitude and the diversity of the stressing factors. In some cases, only the influences of the upper extremes of certain parameters can inflict stresses e.g., wind, salinity, etc. In other cases e.g. nutritional stress etc. both the upper limit and the lower limit will induce stress phenomena.

Biotic factors are much more complex, and interrelationships that end in the dominance of one partner induce stresses in the others. The effects of parasites and herbivores on plants (Table 1), or the effect of parasites on animals, can be seen as examples for that. This is especially important where high population densities are involved.

The effect of environmental stress factors may be synergistic. For example, the European forest decline is a result of such a combined stress (KULL, 1992), where acid rain, heavy metals and changes in the soil microflora have joined in the kill.

In the long run evolution has been forced by many stress events, and progressed via the influences of abiotic environmental parameters, either in a chaotic or in a directed non-linear way (KULL, 1992).

Stress effects are manifested in a multitude of adaptive responses (on the structural and/or functional level). Those enable organisms to overcome the effects of the abiotic or biotic constituents of the environment.

Stress responses can develop at all levels of biological organization (Table 2 A-E). They can develop on the molecular level, i.e., induction of changes in biochemical or biophysical reactions. Stress may affect higher levels of organization, i.e., the regulation of genes, or the formative changes in organelles, cells and tissues (WAISEL, 1972). Stress can be reflected by modifications of structure and function of whole organisms, by alterations of the competitive equilibria among various constituents of a specific ecosystem, and by that the moderation of social equilibria at the society level.

Organisms tolerate certain limits of stressing conditions. At the lower level they exhibit a remarkable or even astonishing buffering capability. However, intensification of such factors would induce detrimental stresses. Such a situation may take two courses: it may eventually lead to hardening and improved tolerance or it may cause damage and death. Thus, whenever stress effects are discussed, the intensity of the inducing factor, its time course and its duration, should be considered in order to reach the essence of the specific stress responses (LARCHER, 1987; BECK and LÜTTGE, 1990).

In the present symposium several instructive examples for, "stress at all levels", were presented and discussed. A matrix of the most common stress factors and of the level of organization which were discussed in the symposium, were summarized in Table 2.

Stress responses are mediated by several genes (VIERLING and KIMPEL, 1992). However, the elucidation of signal transduction or the definition of transcriptional and post transcriptional regulatory mechanisms are only the beginning of the process. This is followed by a series of biochemical reactions, and up the order of biological organization up to the biome level. Many of the stress responses of organisms -- or of their various constituents -- overlap. The reasons for that stem, in animals, from the similarities in the physiological changes that such organisms exhibit and in their hormonal balance.

Table 2 : Matrix of stress factors (examples and stress response) in various groups of organisms; by number 1 - 15 is indicated the relevant paper of this "stress-workshop", in brackets: [the relevant starting page]; by letter A - E is indicated the regarded level of stress effect (xxxx : range of levels)

- A : biochemical, physiological level (membranes, organelles)
- B : cellular level
- C : tissue and organs level, formative effects
- D : organisms
- E : populations, ecosystems

organism:	Bacteria	Plants	Animals	Humans
examples of stress factors	A B C D E	A B C D E	A B C D E	A B C D E
temperature	1x [7]		13 [93] 14 [103]	
water		4xx [23] 5xx [33]		
radiation/light			7x [53] 8x [61]	
salinity osmotic stress		6xxxx [39] 6xxxx [39]		
chemical factors O ₂ CO ₂ high altitude	2xx [11]	3x [15] 4xx [23] 5x [33]		11xx [81] 12xx [87]
mechanical factors				
intraspecif. competit. (social stress)			15 [105]	
interspec. competition			7x [53]	9xxx [69] 10... [71]

It is relatively easy to describe the effects of abiotic factors on stress induction in plants, because some of the stresses (e.g., drought stress, cold stress and salt stress) involve a single factor, like low water availability. This conceals an advantage: because of such an overlap of responses, exposure of a plant to one type of a stressing factor changes its tolerance also to other types.

In animals, the sensory system is probably even more complex, and in many of them, not only the direct environmental influences but also learning of it can affect the organism's responses.

Though it is obvious that stress-factors play an important role in the development of whole and intact organisms, it is still possible to outline many of their specific effects of the lower levels of organization, the molecular, membranal, organelle or cellular levels. Hence, adaptation of whole organisms as well as their stress avoidance, should be regarded as a complex sequence of events or as a network of reactions. Nevertheless, such reactions are meaningful only when considered as components of the whole and not as isolated events. This is also true for all levels of organization: for *E.coli*, for elephants and for an ecosystem. Stress in the later is expressed only through modification of the individual organisms. However, the effective levels of complexity are not easy to define; in some cases the interactions among organisms may reflect a unity, e.g. as in the case of symbionts, whereas in others, each organism runs for its life.

Thus, it can be concluded that in most cases stress effects can not be segregated into levels of organization, and the understanding of stress phenomena should cover all aspects of life, from the tiny molecules of an enzyme and up to the huge and complex biomes.

Citations

BECK, E. & U. LÖTTGE 1990. Streß bei Pflanzen.- Biol.in unserer Zeit 20: 237-244

KULL, U. 1992. Luftschadstoffwirkungen auf Pflanzen als Stresseffekte und das Chaos in der Ökologie.- in FREY,H.D. (ed.): Unser Planet Erde - Umweltwissen - fächerübergreifend. Herbsttagung VDBIOL und MNU in Stuttgart, p.67-76

LARCHER, W. 1987. Streß bei Pflanzen.- Naturwiss. 74: 158-167

LEVITT, J. 1980. Responses of plants to environmental stresses.- Vol. I, II, 497, 606pp.; Acad.Press NY, London

VIERLING, E. & J.A. KIMPEL 1992. Plant responses to environmental stress.- Current Opinion in Biotechnology 3: 164-170

WASEL, Y. 1972. Biology of halophytes.- Acad. Press, New York and London. 395 pp.

Epilogue

Yoav WAISEL, Department of Botany,
The George S. Wise Faculty of Life Sciences,
Tel Aviv University, Tel Aviv, ISRAEL.

Two rationales constitute the basis of cooperation between scientists: the scientific aspect and the human one. A scientist can get a lot of information from the published literature. However, one must be exposed to an environment of learning, and to be part of an active discussion, in order to develop a genuine intellectual dialogue. Thus, the idea that fostered the organization of this joint symposium, was to engage the scientists of our two universities in a discussion of a common subject, to trigger fruitful dialogues between them, to promote personal acquaintances and to stimulate future cooperation in research and teaching.

"Stress" was chosen as the topic of the first symposium, because it is a controversial topic, it is very little understood, and often it has different meanings, in different scientific categories. Stress is something that is invoked in an organism, which are exposed to an environmental challenge. Still, conditions that are stressing for one organism may be an ideal environment for others. We know that stress is one of the driving forces of nature, and that without it, evolution would have not happened. In spite of that, our knowledge of how stress situations develop, or what are their direct causes, are very limited. Consequently, only one thing was unanimously agreed upon by the participants, i.e., the fact that stress imposes a difficult academic challenge to scientists. No doubt that this was a good reason to try and cope with it.

Though scientists are typically dealing with theoretical problems, they are not detached from the real world. Therefore, in view of reemergence of Nazism, of increased racism, and of the replacement of reason by fanaticism and violence, we consider this joint symposium as being of special importance. No doubt that all enlightened people should be distressed by the occurrence of such phenomena. However, this is especially stressing for the people of our two countries, where the present, and the future, have to overcome the atrocious experiences of the past. It is frustrating to realize that after all the efforts that were done to overcome the dark ages, and to amend the relationships, such phenomena still reappear.

The establishment of understanding among people, can be achieved only if it is based on continuous and solid personal relations. In this respect, our joint cooperation programme represents the humble contribution of the two groups of biologists, to science, but also to humanity.

Scientists should never remain satisfied with their past achievements, and should always look forward to new ones. The plans for the next joint symposium are already on their way. We also look forward to the establishment of joint research projects between members of the two faculties. One of our goals was the initiation of an exchange programme for students. Unfortunately this important part of the programme is not yet realised (... was it ignored by the city of Bielefeld ? ...). Nevertheless, we do not give up. We shall continue to nourish the seed that we have planted, until it develops into a fully grown tree, bearing fruits of knowledge, of scientific cooperation and of friendship.

**Kurzbericht zum
deutsch-israelischen workshop**

"Stress auf allen Ebenen"

Unter diesem etwas zweideutigen Titel fand am 2. und 3. Juli im ZiF (Zentrum für Interdisziplinäre Forschung der Universität Bielefeld) von der Fakultät für Biologie veranstaltet ein workshop besonderer Art statt. Der Untertitel "**Anpassungen an Stress, von der molekularen Ebene bis zum Ökosystem**" gibt über den biologischen Inhalt des Themas genauere Auskunft. Dieser workshop war aber vor allem deshalb von besonderer Bedeutung, weil er erstmals im Rahmen der Universitäts-Partnerschaft zwischen der Universität Tel Aviv in Israel und der Bielefelder Universität eine wissenschaftliche Begegnung ermöglichte, die über die bisherige Kooperation der "Brückenköpfe" Prof. Breckle / Bielefeld und Prof. Waisel / Tel Aviv weit hinausging. Die Partnerschaft wurde 1991 formell geschlossen. Ihr war eine bereits etwa 15-jährige Kooperation der oben erwähnten Partner vorausgegangen. Die Partnerschaft setzt sich zum Ziel in Zukunft nicht nur eine engere wissenschaftliche Zusammenarbeit zwischen der Abteilung Ökologie in Bielefeld und dem Department of Botany in Tel Aviv weiterzuführen, sondern die jeweiligen Fakultäten insgesamt in engere Kooperation zu bringen, und zwar auf möglichst verschiedenen Ebenen. So soll in Zukunft auch ein kurz- und langzeitiger Dozentenaustausch stattfinden, es wird angestrebt pro Jahr 1 - 2 Studenten an der Partnerfakultät im Austausch zu fördern, und die jeweiligen Kurse sollen gegenseitig anerkannt werden. Hierfür sind noch Förderungsmöglichkeiten zu schaffen. Gemeinsame Exkursionen und Geländekurse könnten ebenfalls durchgeführt werden. Jährliche gemeinsame Seminare sollen die Möglichkeiten intensiver Diskussionen erweitern. Neben den schon bisher gemeinsam angestrebten Forschungsarbeiten und Publikationen sollen aber auch verstärkt größere Forschungsprojekte gemeinsam in Angriff genommen werden. Es ist also eine **weite Palette der Zusammenarbeit** angestrebt.

Das gemeinsame **Seminar über "Stress"** in Bielefeld war die erste Möglichkeit des besseren Kennenlernens. Sechs israelische Gäste konnten hierzu dank der Mithilfe verschiedener Institutionen der Fakultät und der Universität eingeladen werden. Einer der besonderen Nebeneffekte einer solchen Veranstaltung soll hervorgehoben werden: auch die deutschen Teilnehmer hatten Gelegenheit einmal genauer zu hören, was eigentlich ihre Kollegen an der eigenen Fakultät so machen. Das gleiche betonten die Israelis für ihre Seite. Daß Stress nicht nur bei höher organisierten Tieren eine Rolle spielen kann, sondern bei Bakterien und Pflanzen ebenso von Bedeutung ist und daß dabei ähnliche Phänomene ablaufen, war ein interessantes Ergebnis dieses Seminars. Auf Einzelheiten kann hier nicht eingegangen werden, doch sollen einige Themen stichwortartig abgekürzt genannt werden: Sozialer Stress bei Säugetieren (HENDRICHS) - Das El-Niño-Phänomen und die Anpassungen bei Robben (TRILLMICH) - Anpassung an Kaltwasser bei Krustentieren (Wägle) - Anpassungen beim Menschen an größere Meereshöhen (ZIMMERMANN, STOCK) - Veränderungen im Gehirn des Menschen bei Stress (TEUCHERT-NOODT, DAWIRS) - Anpassungen an unterirdische Lebensweise (AMOS AR) - Antibiotika-Synthese bei Streptomyces unter Stress-Bedingungen (AHARONOWITZ) - Anpassungen von Bakterien an hohe Temperaturen (RON) - Stress durch giftige Spurenelemente und Einfluß auf Baumwachstum (HAGEMEYER) - Salzstress und Absalzung bei Pflanzen (BRECKLE) - Wie erhält eine Pflanze genügend Kohlendioxid unter Stress? (WAISEL) - Produktion unter extremen Bedingungen (SKOLNIK) - Wurzeln haben Sensoren für Trockenstress (ESHEL). Aus diesen Stichworten der Themen

geht die breite Palette der Themenbereiche hervor. Die meisten Beiträge werden zusammengefaßt publiziert in einem Band der "Bielefelder Ökologischen Beiträge" (BÖB6).

Das Stichwort **Stress** erwies sich als ein sehr tragfähiges Diskussionsfundament für zahlreiche Phänomene von der Ebene des Bakteriums bis zum Menschen. Da das Programm reichlich Zeit für Diskussion eingeplant hatte, war nach Meinung aller Teilnehmer dies ein besonders nachahmenswerter Punkt für zukünftige Seminare. Ein Empfang des Rektors der Universität einerseits, durch die Frau Bürgermeisterin der Stadt andererseits, als auch ein Spaziergänge in der Stadt, ein Ausflug nach Lemgo rundeten den Aufenthalt für die Israelis ab.

Aus israelischer Feder liest sich die **Bedeutung der Partnerschaft** wie folgt: "Die Zusammenarbeit zwischen den beiden betreffenden Universitäten wird auf den beiden wichtigen Grundlagen fußen: Kontakte zur wissenschaftlichen Zusammenarbeit und menschliche Gemeinsamkeiten und Freundschaften. Man kann zwar sehr viel Information und Wissen aus der immensen Fülle an Literatur herausholen, aber man braucht eine geeignete Umwelt, um Informationen und Wissen lernend zu verarbeiten und dies in einer ausreichend langen Zeit. Nur dann kann man neben der **Ausbildung** auch eine echte **Bildung** erwarten. Ähnliches gilt für die menschlichen Beziehungen, die im Rahmen der Partnerschaft mit dem bereits bisher durchgeführten Programm aufgebaut wurden und weiter ausgebaut werden sollen. Die Entwicklung von Freundschaften und das gegenseitige Verständnis der Leute kann nur entwickelt werden, wenn es auf ständigen und festen persönlichen Kontakten beruht. Dies gilt sicher ganz besonders für die Beziehungen zwischen Israelis und Deutschen. Es könnte auch Vorbildfunktion sein für eine im Umbruch befindliche Völkergemeinde, wo sich bisher vieles irrational entwickelt. Wie kann man das gegenseitige Angewöhnen, Kennenlernen zwischen den Jüngeren erleichtern? Wie kann man besser ausgebildeten wissenschaftlichen Nachwuchs heranziehen und das zukünftige Leben in beiden Gemeinschaften verbessern? Es gibt sicher keine einfache Antwort zur Bewältigung einer solch komplexen Aufgabe. Geschlossene Freundschaften und eine gut ausgebildete Jugend bleiben eine Garantie für individuelle Kontakte und können nicht durch Ideologien fehlgeleitet werden. Die Inauguration eines erweiterten und verbesserten Austauschprogrammes könnte 1993 erfolgen, wenn weitere Kooperationsmaßnahmen zum Tragen kommen könnten und Begegnungen zustandekämen. Dies ist zwar derzeit noch nicht konkret zu planen. Die israelische Seite wird alles versuchen, 1993 ein solches Begegnungsprogramm auf die Beine zu stellen und dazu deutsche Wissenschaftler und vielleicht auch andere Repräsentanten einzuladen. Im Rahmen einer solchen intensivierten Zusammenarbeit könnte der Austausch je eines Studenten von jeder Stadt vorgesehen werden. Würde sich dabei die Bielefelder Wirtschaft beteiligen, wäre dies auch Ausdruck einer außerordentlich dankenswerten und weitsichtigen, aber auch moralischen Haltung. Sie würde die Kooperation zwischen den beiden Institutionen gezielt fördern und ausweiten und das gegenseitige Verständnis der Bevölkerung unserer beiden Städte einander wesentlich näherbringen."

Die Bielefelder Universität hat viele Partnerschaften mit anderen Universitäten. Die **Partnerschaft mit Tel Aviv** ist im Gegensatz zu manchen anderen, vielleicht einseitigen "Papier"-Partnerschaften, eine echte gegenseitige Verpflichtung und Herausforderung, eine aktive Partnerschaft, die sicher nicht immer "stressfrei" ablaufen wird. Wir haben gelernt, die richtige Dosis an Stress wirkt fördernd.

Allen Beteiligten, die die Kooperation mit Rat und Tat gefördert haben und fördern, sei an dieser Stelle herzlich gedankt.

Prof.Dr.S.-W.Breckle

In der Reihe "Bielefelder Ökologische Beiträge" (BÖB) sind bisher die folgenden Bände erschienen. Sie können bezogen werden über:

Universität Bielefeld
Fakultät für Biologie
Abteilung Ökologie
Postfach 100131
D-4800 Bielefeld
Fax: 0521-106-2963

- Band 1 (1985) : Beiträge des Symposiums "Schwermetalle und Saure Depositionen" (hrsg. S.-W.Breckle & H. Kahle)
- Band 2 (1986) : Beiträge der Abteilung Ökologie zur Dendroökologie (hrsg. S.-W.-Breckle & J. Hagemeyer)
- Band 3 (1988) : Beiträge der Abteilung Ökologie zur Ökologie der Halophyten (hrsg. S.-W. Breckle & I. Wattenberg)
- Band 4 (1989) : Beiträge aus dem Symposium "Ecology and Evolution of the Mediterranean Vegetation and Flora" (hrsg. S.-W. Breckle)
- Band 5 (1989) : Beiträge des Symposiums "Komplexschäden an Buchen - Ursachenforschung zum Baumsterben" (hrsg. S.-W.Breckle & H. Kahle)
- Band 6 (1992) : Beiträge aus dem workshop "Stress - auf allen Ebenen / from the molecular to the ecosystems level" im Rahmen der Deutsch-Israelischen Universitätspartnerschaft, Tel Aviv/Bielefeld, im ZiF in Bielefeld, Juli 1992

Weitere Bände sind in Vorbereitung. Sie erscheinen in lockerer Folge.