The effect of airborne sulphur and nitrogen deposition on aquatic and terrestrial heathland vegetation

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Summary. As research on the effects of acidification on heathlands is scarce, the data discussed mainly concern western Europe and particularly the Netherlands. The change from heather into grass dominated heathlands appeared to be caused by the high atmospheric nitrogen enrichment. Acidification of oligotrophic, poorly buffered pools in heathland areas finally results in the disappearance of all submerged plant species, caused by the too low CO_2 levels in the water layer. The vitality of the pine-forests decreases; apart from the premature shedding of needles as a result of nutrient deficiences, they become more susceptible to secondary stress factors such as NH_3, O_3, drought and fungal diseases.

Key words. Acidification; nitrogen enrichment; nutrient leaching; heathland vegetation; afforestation.

Introduction

Heathlands and peatlands are often neglected areas in acid rain research[9], probably due to the low economic value of these areas. In western Europe, heathlands occur mainly on oligotrophic, weakly buffered sandy soils. Oligotrophic, poorly buffered ecosystems are characterized by plant communities consisting of persistent, slowly growing species[25]; in water these are mainly the tough, isoetid *Littorellion* species such as *Littorella uniflora* (L.) Aschers. and *Lobelia dortmanna* L., on the poor, oligotrophic sandy soils typical heathland species such as *Calluna vulgaris* (L.) Hull and *Erica tetralix* L. In former days part of the heathlands were planted with coniferous trees, particularly *Pinus sylvestris* L. (Scotch Pine), and regionally also *Pinus nigra* var. *maritima* (Ait.) Melville (Corsican Pine), *Picea abies* (L.) Karst. (Norway spruce), *Picea sitchensis* (Bong.) Karr. (Sitka spruce) and *Pseudotsuga menziesii* (Mirb.) Franco (Douglas Fir).

Mainly during the last decades dramatic changes have taken place in the plant communities of these areas. The number of moorland pools and small lakes in which *Littorellion* species occur has declined drastically[24], a lot of heathlands have turned into grasslands and particularly in areas with intensive stockbreeding the condition of the pine forests is bad[13, 15].

The change of heathlands into grasslands

The most obvious phenomenon in many heathlands during the last decades is the changing from heathland into grassland[11, 12, 25]. Particularly *Molinia caerulea* (L.) Moench and *Deschampsia flexuosa* (L.) Trin. expand strongly, at the expense of *Calluna vulgaris* and other heathland species. In order to estimate whether this phenomenon is related to changes in the physical-chemical environment, 70 grass-dominated and heather-dominated heathlands have been investigated[25]. Many parameters, such as the pH, showed hardly any differences. However, the nitrogen levels in grass-dominated heathlands appeared to be much higher (table 1). Both in grass-dominated and heather-dominated heathlands the ammonium levels were 10 to 20 times higher than the nitrate levels.

Investigations clearly show that a major part of the nitrogen originates from atmospheric deposition. In clean air this atmospheric nitrogen deposition is only a few kg ha^{-1} yr^{-1}. At the present time in the Netherlands the wet deposition (rain) alone is already on average 15 kg N ha^{-1} yr^{-1} and in areas with intensive stockbreeding 20–60 kg N ha^{-1} yr^{-1}; 75–90% of the nitrogen is deposited as ammonium sulphate. In raindrops and on wet surfaces ammonia reacts with sulphur dioxide, forming $(NH_4)_2SO_4$. As a result of the alkalinization by NH_3 the solubility of SO_2 increases considerably. The total nitrogen deposition, however, is considerably higher than the above values, because there is also dry deposition on soil and plants[1, 2, 7].

Impact of ammonium on heathland vegetation

1. Soil acidification. Although heathland soils are mostly acidic by nature, there are often certain spots where, due to natural causes (loamy places or upwelling deeper ground water) or to human activities (digging, paths, cattle drinking-places) the soil has become slightly buffered and thus less acidic[25]. Here plant species occur which are restricted to these slightly buffered, less acidic sediments. In the course of time a heterogeneous environment with a rich plant community has developed. The deposited ammonium at these slightly buffered locations is transformed into nitrate very quickly by nitrification, which causes acidification of the soil[6, 7, 25].

Laboratory experiments[25] with artificially buffered heathland soils show that nitrification stops or is strongly inhibited in this type of soil at pH 4.1. This appeared also to be the case for the average pH-value in both grass-dominated and heather-dominated heathlands, which indicates that the pH in heathlands is probably determined by the nitrification limit. The final result of NH_4-deposition is that the differences in pH disappear and thus also the plant species of the slightly buffered locations. A poor plant community remains, consisting of only a few acid-resistant species.

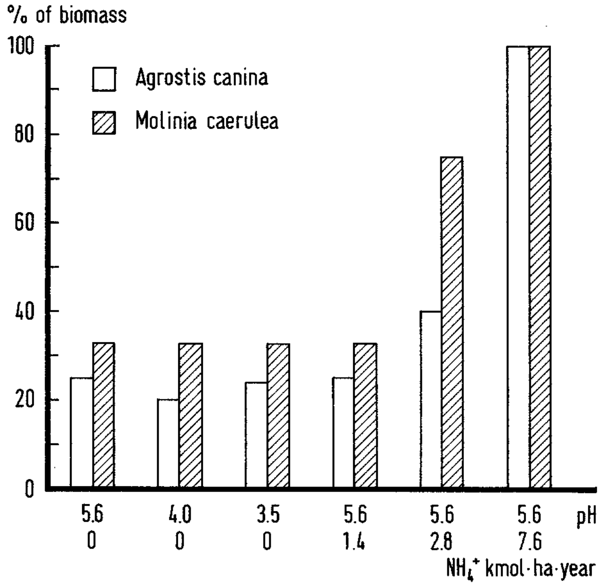
Table 1. The pH (H_2O) and average nutrient concentrations in the soil-solution of 70 investigated heathlands

Species	Coverage	pH (H_2O)	NH_4^+ µmoles kg^{-1}	NO_3^-	PO_4^{3-}	K^+
Erica tetralix	> 60%	4.1	55	0.0	4.0	37
Calluna vulgaris	> 60%	4.1	84	1.4	4.4	46
Molinia caerulea	> 60%	4.2	248	17.2	4.7	88
Deschampsia flexuosa	> 60%	4.1	429	29.0	6.0	182

2. Nitrogen enrichment. If the soil on which ammonium is deposited is acidic, a strong accumulation of nitrogen occurs in the upper soil layer, because ammonium is bound much more strongly to the soil absorption complex than nitrate. When there is competition between heather species such as *Erica tetralix* and *Calluna vulgaris* and grasses such as *Molinia caerulea* the grasses profit from these higher nitrogen levels[4,11,12,25,32]. Field fertilization experiments have shown that nitrogen enrichment indeed stimulates the development of grasses in heathlands[11,12]. However, the problem with these field fertilization experiments is that the high atmospheric nitrogen deposition was not taken into account. For this reason, experiments were carried out in a greenhouse. A number of small heathlands were created, using undisturbed, natural heathland soils. Precipitation experiments during one year showed that the biomass development of the grasses *Agrostis canina* L. and *Molinia caerulea* is not influenced by the acidity of the precipitation (fig.). If the precipitation contained ammonium sulphate, a strong increase in biomass with increasing NH_4^+ deposition was observed. The chosen annual ammonium deposition was comparable with the real field deposition. The highest deposition was comparable with the field deposition in areas with very intensive stockbreeding. The results of these experiments show that the NH_4^+ deposition level in cleaner areas of the Netherlands can already cause a marked increase in biomass of the two grass species. For this reason it can be concluded that the high atmospheric nitrogen enrichment is a main cause for changes from heather dominated into grass dominated heathlands.

Aquatic vegetation

In oligotrophic, poorly buffered pools and lakes in heathland areas of western Europe a plant community commonly occurs which belongs to the phytosociological alliance *Littorellion*[31,39]. The stands of this community are often characterized by the presence of isoetids such as *Littorella uniflora, Lobelia dortmanna* and *Isoetes* sp.[24]. Field investigations in the Netherlands[24] showed, that the waters in which *Littorella uniflora* was one of the dominant species appeared to be poorly buffered (alkalinity on an average 0.5 meq l^{-1}) and had a circumneutral pH (around 6.5), and the nutrient levels in the water layer were low (table 2), as were the CO_2 levels in the water layer (6–40 µmoles l^{-1}). The nutrient levels in the soil solution were 10–100 times higher.



The relative biomass development of *Agrostis canina* and *Molinia caerulea* on natural heathland soil during a one year treatment with precipitation with different pH and ammonium concentrations in a greenhouse.

Isoetid plant species, such as *Littorella uniflora, Isoetes* sp. and *Lobelia dortmanna,* have several morphological and biochemical adaptations to make successful growth possible under these conditions.

- *Lobelia* and *Littorella* are able to absorb free CO_2 by the roots[34,40].
- *Littorella* and *Lobelia,* plants with extensive lacunal systems, can recapture a considerable part of the photorespired CO_2, in this way contributing to a more efficient assimilation[33].
- The underground biomasses of these plant species depend on the nutrient levels of the sediment[30].
- *Littorella* and many species of the genus *Isoetes* have developed a diurnal acidification – deacidification cycle very similar to the Crassulacean Acid Metabolism (C.A.M.)[16,17].
- Isoetid plant species have a high oxygen release from the roots[27,29].

Acidification of the water

Field investigations in the Netherlands showed that over 80% of all naturally poorly-buffered pools and lakes in heathland areas have been strongly acidified during the last decades (pH at present on average 3.8). In most cases the original vegetation has been replaced by submerged *Juncus bulbosus* L. and/or *Sphagnum* species[8,24]. In these locations the CO_2 levels in the soil solution and the water layer were strongly increased (tables 2 and 3)[24,25,27]. Several authors also mention a strong development of *Juncus bulbosus* and/or *Sphagnum* sp. in acidifying Scandinavian waters[10,23,28].

Ecophysiological experiments have proved that *Juncus bulbosus* lacks the typical adaptations which enable suc-

Table 2. The average chemical composition of water and soil-solution on locations with *Littorella uniflora, Juncus bulbosus* and *Sphagnum* sp. (µM)

	L. uniflora	*J. bulbosus*	*Sphagnum*
Water			
n	33	107	59
pH	6.5	3.9	3.8
HCO_3^-	500	20	10
NH_4^+	5	40	46
NO_3^-	10	7	7
PO_4^{3-}	0.16	0.21	0.19
Soil solution			
n	9	19	9
HCO_3^-	1020	420	780
CO_2	900	3330	5010
NH_4^+	267	510	564
NO_3^-	90	60	51
PO_4^{3-}	5.1	5.4	5.4

Table 3. The CO_2-level of the water 10 cm above the soil at some acidified locations with differences in macrophyte development

Location	Macrophyte vegetation	pH (water)	CO_2 (µmoles l^{-1})
Staalbergven	No submerged macrophytes	4.0	66
Ven near Schayk	No submerged macrophytes	3.7	55
Vogelvijvers	No submerged macrophytes	4.7	60
Vogelvijvers	*Juncus bulbosus*	4.0	150
Rouwkuilen	*Juncus bulbosus*	4.1	170
Ven near Schayk	*J. bulbosus* + *Sphagnum cuspidatum*	3.9	840
Mariaveen	*Sphagnum cuspidatum*	3.6	870

cessful growth in poorly buffered, oligotrophic waters[27]. Under all conditions the root biomass of *Juncus* was much lower than that of *Littorella uniflora,* and *Juncus bulbosus* had a very low oxygen release by the roots, even when it had a relatively high root biomass. When only the roots of the plants were provided with carbon dioxide, *Juncus bulbosus* showed a very low photosynthetic response, even at very high CO_2 levels, whereas *Littorella uniflora* showed a relatively high photosynthetic response. When the leaves of the plants were provided with CO_2, *Juncus bulbosus* showed saturated photosynthesis at a concentration of 200 µmoles CO_2 l^{-1}, whereas *Littorella uniflora* showed no saturation until a concentration of 2000 µmoles CO_2 l^{-1} was reached.

Nitrogen uptake experiments demonstrated that the two plant species, when obtained from waters containing both nitrate and ammonium, used ammonium as their sole nitrogen source. Only after a few days of NH_4^+ starvation did they also use some nitrate. The nitrogen uptake rate increased when the incubation period in the ammonium-free culture medium was longer; however, this increase was much stronger for *Littorella uniflora,* particularly with respect to nitrate uptake. When ammonium was used, *Littorella* showed a net potassium release, whereas *Juncus bulbosus* showed a positive K uptake.

Culture experiments during ten weeks under fully controlled conditions showed that *Juncus bulbosus* only had a strong increase in biomass on sediments containing little carbonate when the pH of the water was low. When the pH of the water was higher, a marked decrease in biomass occurred. *Littorella uniflora,* however, showed an increase in biomass at each pH of the water. In carbonate-free sediments, *Juncus bulbosus* showed a strong decrease in biomass at each pH of the water, whereas *Littorella* showed a decrease in biomass at a low pH and an increase when the water was poorly buffered. *Juncus bulbosus* and *Sphagnum cuspidatum* Hoffm. had the strongest increase in biomass at high ammonium levels in the water, whereas *Littorella* had the highest biomass in ammonium free water containing nitrate.

It can be concluded that acidification of a water above a sediment which is not yet acidified leads to luxuriant growth of *Juncus bulbosus* and *Sphagnum* spec. as a result of increased carbon dioxide and ammonium levels in the water layer (tables 2 and 3). These plant species can utilize these nutrients much more efficiently[27,30,37,38]. The increased carbon dioxide level is a result of the strongly increased CO_2 level in the sediment[24], caused by the acid water layer acting upon the HCO_3^- buffering system and the microbial activities still present. The diffusion of CO_2 from the sediment causes a strong over-saturation with CO_2 in the deeper water layers (tables 2 and 3). The increased NH_4^+ and decreased NO_3^- levels can be ascribed to airborne ammonium sulphate deposition and reduced nitrification. After acidification of the sediment the CO_2 level in the sediment decreases strongly as a result of the reduced microbial activity and the absence of carbonates. The diffusion of CO_2 to the water layer also decreases strongly, resulting in the disappearance of all submerged plant species, caused by the too low CO_2 levels in the water layer.

Afforestation

In the past, mainly at the beginning of this century, large parts of the heathlands in western Europe were planted with coniferous trees. Species often used are Scotch Pine *(Pinus sylvestris)* and Norway Spruce *(Picea abies)* and regionally also Corsican Pine *(Pinus nigra* var. *maritima),* Douglas Fir *(Pseudotsuga menziesii),* Sitka Spruce *(Picea sitchensis),* Giant Fir *(Abies grandis* Lindl.) and others.

In the Netherlands more than 60% of the present forests are situated on former heathland soils[3]. The condition particularly of coniferous forests is often poor[5]. A nationwide investigation, carried out by the Dutch State Forest Service[5] in 1983, showed that 7–38% of all trees in Scotch Pine forests had less than one complete year-class of needles. In Douglas Fir forests 29–70% of the trees had less than 50% of the leaves considered normal for this climatic zone. 64% of the unhealthy Scotch Pine forests and 41% of the unhealthy Douglas Fir forests showed grey-green or yellow-green discolored needles. A comparable investigation in 1984 showed a further decline with respect to 1983[3].

In Germany the vitality of the forests has also decreased strongly. Air pollution is generally believed to be the main cause. Ulrich[36] ascribes the phenomena mainly to soil acidification as a result of acid or acidifying substances from the atmosphere. The high Al/Ca ratio, resulting from the dissolving of aluminium by mineral acids and from leaching of calcium, is harmful to the root system.

Other authors[19], however, believe that the observed nutrient deficiencies and concomitant damage to the trees can be ascribed mainly to leaching as a result of ozone damage to the leaves. In the forests on former heathlands in the Netherlands, however, there is no correlation between the mean ozone concentration in the atmosphere and the condition of the forest[2,3], but there is a clear correlation between the ammonia emission and the condition of the forest[1,3].

Some authors mention a relationship between intensive stockbreeding and the condition of pine trees[13,15]. Van Breemen et al.[6,7] state that ammonia from manure reacts with sulphur dioxide from the atmosphere, causing wet and dry deposition of large amounts of ammonium sulphate on trees and soil[35]. Nitrification of the ammonium[7,18] caused a marked pH-decrease of the soil (temporarily down to pH 2.8).

Whether deposition of ammonium sulphate on acidic forest soils will result in strong acidification depends on the type of forest and soil. Kriebitzsch[20], who conducted

Table 4. pH and chemical composition of soil-distilled water extracts (1:3) of A) healthy, B) moderately damaged and C) severely damaged *Pinus nigra* and *Pseudotsuga menziesii* forests (µmoles kg^{-1} dry soil)

		pH (H_2O)			NH_4^+	NH_4^+(KCl)*	NO_3^-	K^+	Mg^{2+}	Ca^{2+}	Al^{3+}
	n	mean	min.	max.	mean	mean	mean	mean	mean	mean	mean
Pinus nigra											
A)	20	4.1	3.5	4.6	334	687	271	137	77	153	191
B)	16	4.0	3.4	4.9	384	751	130	47	45	128	158
C)	20	4.1	3.7	4.4	509	1346	117	60	26	43	183
Pseudotsuga menziesii											
A)	10	4.1	3.9	4.4	245	499	164	89	60	106	214
B)	10	4.1	3.8	4.3	562	733	153	67	48	69	211
C)	11	4.3	4.0	4.6	692	1240	157	67	22	36	211

*0.5 M KCl extract.

nitrification experiments in many types of acidic forest soils, divided them into four groups: A, B, C and D. In the soils belonging to group A there was no nitrification and ammonium was the only nitrogen source. In the soils belonging to groups B and C there was partial nitrification and in those belonging to group D there was total nitrification. The investigations in this study showed that heathland and *Pinus* soils mainly belong to group A. Field studies in the Netherlands in *Pinus nigra*[26] and *Pseudotsuga menziesii* forests on former heathland soils showed indeed only partial or no nitrification. The nitrate levels were low, whereas the ammonium levels were high (table 4). The soils of healthy, moderately damaged and severely damaged forests had on an average a pH (H_2O) of 4.1, which indicates that also in this type of forest soil the pH is determined by the nitrification limit. Analysis of precipitation in open plots and canopy throughfall in a *Pinus nigra* var. *maritima* forest in an area with intensive stockbreeding showed that NH_4^+ and SO_4^{2-} are quantitatively by far the most important ions[26]. In open plots SO_4^{2-} is fully compensated by NH_4^+; however, in canopy throughfall SO_4^{2-} is only partially compensated by NH_4^+ (table 5). Some authors mention foliar uptake of NH_4^+[21,26].

Ecophysiological experiments with needles of *Pinus nigra* var. *maritima* indeed showed uptake of ammonia, which was compensated by excretion of potassium, magnesium and calcium. Needle analyses in healthy and damaged *Pinus nigra* forests showed that premature shedding of the needles in damaged forests was related to K and/or Mg deficiencies in the needles[26]. As a result of the NH^+ uptake the nitrogen levels were significantly higher in the needles of damaged forests. Whether the observed cation excretion will result in disturbed nutrient budgets depends mainly on the soil condition. A high ammonium sulphate deposition leads to accumulation of NH_4^+ and leaching of K, Mg and Ca from the soil. Field investigations in *Pinus nigra* var. *maritima* and *Pseudotsuga menziesii* forests demonstrated that the NH_4^+ levels were significantly higher and the Mg and Ca levels significantly lower in soils of severely damaged forests (table 6). As a result the NH_4/K, NH_4/Mg and the Al/Ca ratios increased. It is well-known that increased NH_4/K and NH_4/Mg ratios inhibit K^+ and Mg^{2+} uptake[14,22], while an increased Al/Ca ratio can damage the root system. Ulrich[36] mentions an Al/Ca ratio $\geq$ 5 in the soil solution as very critical for root damage. In both *Pseudotsuga menziesii* and *Pinus nigra* forests the NH_4/K and NH_4/Mg ratios are relatively low in healthy forests and significantly higher in damaged forests, while the average Al/Ca ratio is significantly higher in severely damaged forests and, particularly in *Pseudotsuga* forests, far above the critical value (table 6).

Apart from the premature shedding of needles as a result of nutrient deficiencies, the forests become more susceptible to secondary stress factors such as NH_3, O_3, drought and fungal diseases. In some regions of the Netherlands the condition of *Pinus nigra* and *Pinus sylvestris* rapidly decreases as a result of a certain fungal disease (*Diplodia pinea* (Desm.) Kickx). Needle analyses in infected and non-infected forests demonstrated that the nitrogen levels were significantly higher in *Diplodia*-infected forests[26].

Table 5. The average chemical composition of precipitation in a) open plots and b) throughfall in a *Pinus nigra* forest in an area with intensive stockbreeding (µmoles l^{-1})

	NH_4^+	NO_3^-	K^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Na^+	Cl^-	H^+
a)	597	71	53	35	71	318	170	197	1.7
b)	2283	147	196	94	150	1379	346	462	0.6

Table 6. The ratios of some nutrients in soil extracts of A) healthy, B) moderately damaged and C) severely damaged *Pinus nigra* and *Pseudotsuga menziesii* forests (mol/mol)

		NH_4^+/K^+			NH_4^+/Mg^{2+}			Al^{3+}/Ca^{2+}		
	n	mean	min.	max.	mean	min.	max.	mean	min.	max.
Pinus nigra										
A)	21	4.7	0.5	14.0	6.4	1.1	24.3	2.0	0.4	5.6
B)	17	9.2	0.8	36.8	10.0	1.8	26.3	1.3	0.2	2.8
C)	21	11.3	1.9	51.8	22.1	1.6	57.2	5.5	1.7	16.7
Pseudotsuga menziesii										
A)	10	3.8	0.5	11.8	4.5	0.6	10.0	6.6	0.8	40.9
B)	10	8.7	1.5	31.2	19.3	2.0	51.1	8.9	0.7	46.7
C)	11	18.2	3.8	64.5	47.6	7.9	118.0	15.6	1.4	54.0

Final conclusions

– Acidification of poorly buffered heathland soils as a result of acid or acidifying precipitation leads to a strong decline in the number of plant species.

– Acidification of a water body above a carbonate-free sediment leads to disappearance of all submerged macrophytes due to lack of carbon dioxide.

– Acidification of a water body above a sediment containing little carbonate, as a result of ammonium-containing acidifying precipitation, leads to suppression of the isoetid plant community by luxuriant growth of *Juncus bulbosus* and/or *Sphagnum* species as a result of increased carbon dioxide and ammonium levels in the water layer.

– Airborne ammonium deposition on acidic heathland soils leads to a succession from heather-dominated into grass-dominated heathlands.

– Ammonium sulphate deposition on pine forests leads to ammonium uptake, and excretion of essential nutrients such as potassium and magnesium by the needles. As a result of ammonium accumulation and cation leaching in the soil the uptake of K^+ and Mg^{2+} is also inhibited. This combination of effects leads to a disturbed nutrient budget in the trees, resulting in premature shedding of needles and increased susceptibility to secondary stress factors, which may finally lead to severely damaged forests.

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Reviews

Progress in molecular parasitology

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Summary. Substantial progress has been made in the last ten years in understanding the structural and functional organization of parasitic protozoa and helminths and the complex physiological relationships that exist between these organisms and their hosts. By employing the new powerful techniques of biochemistry, molecular biology and immunology the genomic organization in parasites, the molecular basis of parasite's variation in surface antigens and the biosynthesis, processing, transport and membrane anchoring of these and other surface proteins were extensively investigated. Significant advances have also been made in our knowledge of the specific and often peculiar strategies of intermediary metabolism, cell compartmentation, the role of oxygen for parasites and the mechanisms of antiparasitic drug action. Further major fields of interest are currently the complex processes which enables parasites to evade the host's immune defense system and other mechanisms which have resulted in the specific adaptations which enabled parasites to survive within their host environments. Various approaches in molecular and biochemical parasitology and in immunoparasitology have been proven to be of high potential for serodiagnosis, immunoprophylaxis and drug design.

Key words. Molecular parasitology; *Trypanosoma; Leishmania; Plasmodium;* helminths; antigenic variation; membrane proteins; kinetoplast DNA; metabolism; bioenergetics; tubulin; hemoglobin; anthelmintics.

Introduction

During the first half of this century the study of molecular aspects of parasitic organisms was a largely neglected research area which lagged far behind the biochemical knowledge about bacteria and vertebrates. During World War II the increasing public recognition of diseases caused by both protozoan parasites and helminths initiated a turning point in biochemical parasitology. A major goal of the increased and improved parasitological research was that a detailed understanding of the molecular differences between parasites and their hosts, and a comprehensive knowledge of the crucial molecular properties of parasites, would offer interesting opportunities for rational approaches to antiparasitic chemotherapy. As a result of these activities, significant advances were achieved over the next 25 years, in particular in the areas of intermediary metabolism, including the identification and characterization of enzymes and metabolic pathways, and of metabolic regulation, membrane transport, the role of oxygen for parasites and the mechanisms of antiparasitic drug action (see von Brand[113]). Although the potential chemotherapeutic significance of these studies remained to be demonstrated the associated increase in knowledge helped molecular parasitology to emerge from its infancy.

A further change in parasitology has occurred in the last 10–15 years. On the one side, application of powerful and precise new techniques in biochemistry and biophysics has made possible more detailed studies in the established fields of parasite biochemistry, including work on intermediary metabolism, enzymology, bioenergetics and the structural and functional organization of membranes[5, 44]. Simultaneously, recent developments in molecular biology and immunology, such as the cloning of genes, their expression in bacteria and hybridoma techniques, have opened up a completely new molecular area in parasitology[2, 115]. By employing these technologies, and the ability to sequence both proteins and nucleic acids, we are now in a position to study the antigenicity of parasites in more detail, to isolate and characterize parasite genes and analyze their organization and expression, and thus increase the knowledge of molecular genetic aspects of parasitology. Monoclonal antibodies and other immunological techniques, because of their exquisite specificity, have proved to be invaluable tools for purifying and characterizing parasite proteins. It is now possible to localize parasite antigens precisely within cells, and antigen isola-