Biochemistry and Microbiology for Paper Engineers

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The intensive efforts both into research within biochemistry and microbiology which have taken place all over the world during the last years have given rise to a new general pool of knowledge which ought to be used by the forest industries for their scientific and technologic development. This utilization of knowledge is certainly of interest within varying fields of the forest industry. One such field is paper production where a collision between the microorganisms and economical interest is at hand, since the microorganisms inhabiting the white water systems of the paper mills are causing slime problems which bring with them a decrease in the production.

He who has read the literature concerning the slime control in paper mills must have noticed that these problems have been discussed year after year without giving the paper maker any essential information about the physiology and biochemistry of the microorganisms. The intention of this article is to introduce the paper engineer to these fields, i.e., to give a glimpse of how the microorganisms function, how their cells are built up, which reactions are going on in the cells, which enzymes are in the cells, and finally to summarize how slimacides used in paper mills react with the cell and its functions. It must be considered important that the pulp and paper engineer has at least some knowledge about what is happening to microorganisms when a slimacide is added to a paper mill system. With this knowledge it ought to be easier to judge the slimacides already used and relate them to new ones suggested. The knowledge of the pulp and paper engineers is now often insufficient when it comes to critically evaluating the statements made by salesmen and to taking initiatives leading to a better slime control.

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THE CELL AND ITS COMPONENTS

The living cell is the basic unit in all living organisms. The evolution has created more and more differentiated cells. The difference between, for instance, brain cells and muscle cells is very big in both morphology and function. Some structures are, however, present in all cells; like the cell membrane, the cytoplasm and the cell nucleus. **Fig. 1** gives a schematic picture of the ideal cell. **The cell nucleus** or **the nucleus equivalent,** as the bacterial form of the cell nucleus is called, contains basically cromatin strings in which nearly all of the cell's deoxyribonucleic acid (DNA) is localised. The main and most specific aspect of the cell nucleus is its genetic information. The cell nucleus is particularly rich in nucleic acids. The nucleic acids are high molecular suubstances and can be divided into two groups, deoxyribonucleic acid (DNA) which exists predominately in the cell nuclei (recently DNA has also been discovered in mitocondria) and ribonucleic acid (RNA) which is mainly in the cytoplasm but also to a lesser extent in the cell nucleus. X-ray diffraction studies of DNA have confirmed the hypothesis that the DNA molecule consists of two strands wound around each other to form a double helix. Nowadays it is generally accepted that DNA is the substance that transfers the genetic properties from one generation to another. When one cell divides the two daughtercells get exactly the same group of chromosomes. This is made possible by a cleavage of the double helix in DNA. On each strand a complementary strand is built so that two double helixes are formed which are duplicates of the original. This duplication is possible because the DNA is made up of nitrogen bases which form hydrogen bonds with each other in a specific way. DNA is thus the chemical base for hereditary. The function of the DNA is to give the cell genetic information which regulates the activity of the cell via protein synthesis in the cytoplasm. You can conclude that the cell nucleus is the regulatory organel in the cell.

Mitocondria are the best investigated structural elements in the cell. They are found in all types of cells; varying from 50 to 5000 per cell. In the mitocondria you

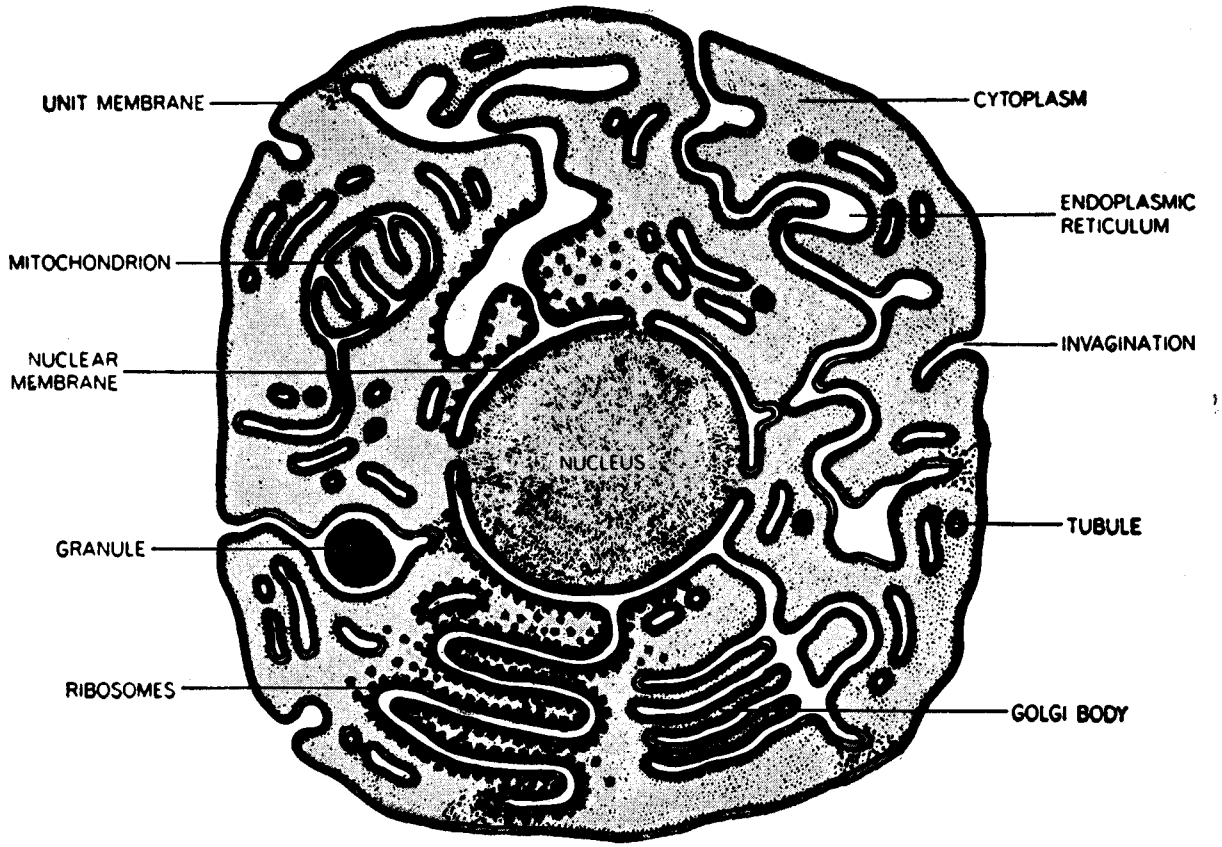

Fig. 1. Ideadized cell. *Countesy of J. D. Robertson and Academic Press.*

will find the enzymes of the citric acid cycle, of the fat oxidation and of the respiratory chain. These enzyme systems supply the cell with energy and the mitocondria are therefore called the motors of the cell.

The endoplasmatic reticulum is a membrane system which in the electron microscope can be seen as small tubes and vesicles to which different particles can be bound. These structures are best developed in the endoplasm of the cell. Upon fractionation of this part of the cytoplasm you will get among other things ribosomes.

The ribosomes are small bodies attached to the membranes mentioned above. The ribosomes contain RNA and protein and are thought to synthesize proteins from amino acids.

The cytoplasm usually is divided into ectoplasm which is the region closest to the cell membrane and the endoplasm which is located inside the former layer.

The cell membrane enclosing the cell, contains lipids, polysaccharides and proteins. It acts as a semipermeable barrier and is involved in active transport. In this way the cell membrane can retain the high molecular parts of the cell and regulate the flow out of lesser molecules between the cells and their surroundings.

INHIBITION OF CELL REACTIONS

In living cells there are a number of highly complex chemical reactions which are carried out by enzymes. These reactions create the energy necessary for the varying functions of the cell and synthesize the material that enables the cells to develop and multiply.

Enzymes are proteins having the power to catalyse chemical reactions, i.e., to influence the reactions without being used up themselves. Their effect is often decisive and dramatic. Reactions which will not take place at any measureable speed at biological temperatures can be tremendously fast after the addition of micro-amounts of enzymes.

Modern enzyme chemistry which is not more than about 50 years old (and is still in the beginning

of its development), has none the less discovered a great deal about the structure of proteins. It is known that enzymes are proteins built up of chains of amino acids in a determined number and order. The number can vary from a hundred up to several thousand amino acids per molecule. Since more than twenty different amino acids participate in the synthesis of the proteins it is easy to understand what an enormous richness of possibilities there are for structural variations. Not only the number and order of the amino acids in an enzyme are of importance for the enzymic activity but also the three dimensional structure of the amino acid chains. This structure is normally highly labile and is often irreversibly affected by inhibitors, changes in pH, ionic strength, temperature, etc. Unsuitable conditions during the isolation of the enzyme or during the study of an enzymic reaction can therefore easily cause a partial or even total inactivation of the enzymic activity.

It has been shown that the part of the enzyme molecule which possesses the activity often has a special sequence of few amino acids and in some cases it has been possible to split off a considerable part of the molecule without a complete loss of the enzymic activity. It therefore seems reasonable to ask if the enzymes need to be so complicated in their structure also if simpler structures could have catalytic activity. Part of the answer is that it is necessary that the proteins differ enough from each other so that each organism can recognize his own proteins and react towards strange proteins. This immunological demand, which must be met so that different individuals can exist, requires that the proteins have a certain degree of complexity in their structure. There are also other factors, too numerous to discuss here which are important in this connection. The complex structure of the proteins is thus not only due to their function as catalysts or as building materials in the cells. Enzymes with the same function but from different individuals or even from different organs in one and the same individual can show variations in their chemical structure. The differences are often very small but normally big enough to allow separation of the different types of enzymes and measurements of their relative amounts with the modern separation methods that now exist.

Organic chemists use group specific reagents to be able to identify groups in unknown compounds. In the same way, the use of chemical substances, which specifically react with different protein groups, offer one way to characterize the chemical pattern of the surface of enzymes. This is particularly the case if these substances influence the catalytic activity. By testing which poisons are effective you can sometimes get an indication of which groups are part of the catalytic center of the enzyme. The active center is that region of the enzyme which acts on the substrate, i.e., carries out the catalytic reaction. Such procedures have often been sucessful in investigating the configuration and topography of these regions of enzymes, and can in this way help us understand the mechanisms by which biologically important compounds are transformed. The biochemist thus often adds inhibitors to study isolated and purified enzymes as a part of the characterization program and this is of course a fascinating aspect of the biology on the molecular level. You can, no doubt, look with confidence into the not too distant future when enough will be known about active centers of enzymes and about the enzyme substrate bond to predict which type of substances that will inhibit a certain enzyme as is now the case with drugs which are tailor made for particular reasons based on what is known about the functions of the cells at different levels.

It is tempting to try to divide inhibitors systematically into groups based on the nature of their activity. Unfortunately there are too many cases where we do not know which reaction causes the cells to die. The action of inhibitors on the living cell can, however, often best be interpreted in terms of a modification of the flow of energy. There are many ways in which the direction of flow of energy in a cell can be changed and in turn influence those functions of the cell which are necessary for the life of the total organism. An inhibitor can for instance:

1) Influence the ability of a nutrient to penetrate the surface of the cell. By such changes in the permeability of the cell membrane the transport mechanism in the cell can be depressed. The inhibitor thus brings about a reduction in the total energy potential which is available for the cell.
2) Influence the formation and use of energy-rich phosphate bonds.
3) Influence the synthetic reactions important for the formation of cytoplasmatic components.

It must, however, be remembered that most inhibitors seldom are specific enough to influence only one simple metabolic way of synthesis in the cell but normally interfere with several. The delay between the addition of the inhibitor and the metabolic or functional response of the cell normally passes through three different phases.

a) The inhibitor must first reach the cell and penetrate the cell membrane.
b) Then it inhibits one or more enzymes.
c) The cell then reacts to the loss of this enzymic activity.

The speed at which the inhibition takes place is often completely determined by the speed with which the inhibitor penetrates the cell membrane and has then very little to do with the speed of reaction between the inhibitor and the enzymes. That the cell membrane can considerably decrease the speed of reaction between an extracellular substance and an intracellular cell component even in situations where you would expect rapid diffusion to take place is shown by the fact that the reaction between hemoglobin in red blood cells and oxygen is about 18 times slower than when the hemoglobin is in free solution. If the speed of diffusion of a small neutral molecule like oxygen is reduced by the cell memberane it is apparent that most inhibitors have relatively slow access to the cells and that therefore the reaction between an inhibitor and an enzyme would need to be very slow to the rate limiting. The reactions between the inhibitor and non-enzymatic substances in the cell will often decrease the effective concentration of an inhibitor and thus also the speed of the inhibition. A substance which can react with sulphhydrylic groups (SH-groups) and change enzymatic, metabolic or functional processes is normally called a sulphhydrylic reagent. Such substances represent a very important group of inhibitors and have often been used to determine if enzymic or metabolic reactions depend directly upon intact sulhphydrylic groups. Reactions between the most important SH-reagents have normally been divided into four different groups, namely :

1) Oxidation of SH-groups
2) Formation of mercaptides
3) Alkylation
4) Addition to double bonds

Examples of inhibitors which have been used as slimacides and can be considered to cause oxidation of SH-groups, are bi-sulphite and chlorine. Organic mercury compounds, arsenic and heavy metal compounds like copper, lead, tin and silver are examples of slimacides causing formation of mercaptides. To the third group of SH-reagents, i.e., reagents which cause alkylation of SH-groups belongs, for instance, the aliphatic bromine compound 1-acetoxy-1, 4-dibromebut-2-en. Acrolein, for instance, belongs to group four; inhibitors which cause addition of SH-groups to double bonds. The examples of inhibitors which have been mentioned above have all been used as slimacides in the paper industry. Acrolein has been used in the United States but has never been a success in Sweden.

The name sulphhydrylic group enzymes has previously been used to denote these enzymes whose activity was thought to depend upon the participation of the SH-groups in the reaction itself. As far as is known to the author it has not yet been demonstrated in one single enzyme that SH-groups definitely belong to the active center of the enzyme. The inhibition of an enzyme by SH-reagents certainly does not prove that these groups are functional. At present a more practical definition of sulphhydrylic group enzymes is therefore that these are enzymes which show loss of activity when some or all of their SH-groups have been modified by inhibitors.

Cellular molecules containing SH-groups can be grouped into three different categories : 1. Sulphur compounds with low molecular weight, e.g., co-factors like lipoate, coenzyme A and glutation. 2. Non-enzymatic proteins, e.g. most of the cytoplasmatic proteins. 3. Enzymes of all types.

Modification or reaction of SH-groups in these compounds with an inhibitor can directly or indirectly change the cellular metabolism and the function of the cell. Even a reaction of the SH-groups in non-enzymatic proteins can disturb the cellular metabolism as the role that such proteins play in the structural organization of the metabolic units is very important. In addition to the free SH-groups many proteins and enzymes contain disulphur groups which function as bridges between peptide chains in the protein molecule and are then important for the structural stability of the protein. These disulphur groups can under certain circumstances be reductively cleaved so that they form free SH-groups. This often leads to a change of the three dimensional structure of the enzyme or the protein.

THE HISTORY AND FUNCTION OF SLIMACIDES

What we colloquially call a slimacide is a compound that should prevent formation of slime or nasty smelling compounds in the white water system of the forest industries. It is well known that far from all slime is of microbiological origin. In this paper, however, the slimacides will be discussed only with respect to their action against microorganisms.

The slimacides need not necessarily have a killing effect on the microorganisms. It is enough if they prevent organisms from developing. Such compounds are said to have a static action. The fungistatic compound thus prevents the development of fungi, a bacteriastatic compound prevents development of bacteria. Compounds killing fungi and bacteria, respectively, are fungicides and bacteriacides. It follows from this that the fungicide or bacteriacide always has a static action, i.e., applied in the right concentration. If you study the literature concerning the microbiological slime in the pulp and paper industry you will find that in Europe the role of the fungi has been underlined

while in the United States mostly bacteria are discussed. These differences can depend upon the methods of investigation but can also depend upon the temperature in the systems in the factories. It is to be expected that at high temperatures bacteria will be by the majority since many bacteria have a higher temperature optimum for their growth than have fungi.

If you make a systematic grouping of those poisons which are used as slimacides they can be divided into five different groups, namely;

1) Simple organic and inorganic compounds
2) Substances with heavy metal atoms
3) Organic sulphur derivatives
4) Halogen-containing compounds
5) Other types of poisons

This categorization is in line with the real development in the field. **Chlorine** is probably the slimacide which has been used for the longest time in the paper industry. It was used already in 1910. Chlorine is effective as a bacteria-killing substance. It is an extraordinary compound when it comes to the treatment of raw-water and also to the killing of potential slimeforming bacteria. The raw-water should be treated with chlorine before it comes into the mill. Chlorine has certain limitations. It is not entirely effective against spore-forming bacteria or against fungi and it is too reactive. It reacts for example with fibres and is destroyed. When you chlorinate the water you have to be sure that you have an overdose of the chlorine, i.e., you add more than what is used up by the organic compounds that are already in the water. It is the additional chlorine which is active against the microorganisms. If the water is too dirty you cannot use chlorine. The amount of impurities in the water can be determined by the so called permanganate-number. If the permanganate-number is higher than a certain value it is not economical to chlorinate the water.

Chlorine as a slimacide can be used in many different forms. It can be added directly to the water by the aid of special chlorinating apparatuses or it can be used in combination with ammonia to produce chloramine. Chlorine can also be used in the form of hypochlorite. The effect of chlorine as a bacteriacide depends upon the concentration, the time of reaction, temperature and the pH. The pH-value should be rather low as hypochlorite acid is the most reactive compound. The time it takes for chlorine to kill bacteria is reduced by approximately 50 per cent for each 10-degree increase in temperature between 20 and 50°C.

It is pointless here to go into the physiological effect and biochemical reactions of the chlorine. Chlorine is such a reactive compound that it reacts very unspecifically. First of all it acts as an oxidant. That it, for instance, oxidizes SH-groups is beyond doubt. Other physiologically important groups, in for instance proteins, are certainly oxidized too. One drawback is that chlorine reacts with organic substances such as lignin and fibres which are to be found in the paper mill system.

To overcome these drawbacks **chloramines** have been used. Chloramines are less reactive and have a more specific antibiological effect. Treatment with chloramines has been shown to be particularly effective for such pulps as groundwood pulp, semichemical pulp and craft pulp. Chloramines are, however, not so well suited as chlorine to treat raw water.

After chlorine as slimacides came the **chlorinated phenols.** The most used is pentachlorophenol. Chlorinated phenols are very poisonous for bacteria, fungi and algae and they have been a success when it comes to the elimination of the most microorganisms. Polychlorophenols have many advantages over the chlorine. In the first place they are not consumed as is chlorine by organic material and they can therefore be added directly to the paper mill system. Another advantage is that the pentachlorophenols are particularly active against fungi, even to the extent that they can be looked upon as fungicides. They are also efficient specially against spore forming bacteria, such as Bacillus subtilis. On the other hand the drawback with them is that they need a longer time to sterilize a system than, for instance, chlorine needs. They are not particularly effective against coliform bacteria or against Monilia, Chladosporium and Oidium. It has been reported that certain bacteria develop resistence to chlorinated phenols under laboratory conditions and it has been shown that bacteria can grow in media containing about 9 times the lethal normal dose. These results are of course interesting since they illustrate the power of the microorganisms to adapt to conditions which are unfavourable. Such an adaptation to pentachlorophenols which is described above is, however, hardly normal to find under practical conditions. (Concerning adaptation and resistence, see below).

The physiological effect of pentachlorophenols is first of all that they act by disrupting oxidative phosphorylation. These processes take place in the mitocondria and are really important for life since the energy creating these reactions drives the whole machinery of the cell. Another compound having the same physiological effect is 2, 4-dinitrophenol. This compound is expensive to produce and, due to its tremendously poisonous capacity, hard to handle. Thus it has not

been used as a slimacide. As a curiosity it can be mentioned that even in nature there exist compounds with the same physical activity as pentachlorophenol and 2, 4-dinitrophenol. The stilbene compounds pinosylvine and pinosylvinemonomethylether, which are produced in the heartwood of pine, disturb the oxidative phosphorylation of microorganisms attacking this tree. This is thus the primary defence mechanism which the pine uses against wood-degrading microorganisms. These microorganisms have inturn adapted to these stilbene compounds. They produce the enzyme laccase which oxidizes the stilbene compounds of the pine as well as the poisonous phenolic lignin monomers produced during biological degradation of the lignin.

After the chlorinated phenols **the mercury compounds** came to be used as slimacides. The use of mercury compounds in different fields can be traced 3000 years back in time. Their modern therapeutic use started with the discovery of the diuretic action of mercury chloride in 1849. Since then this action has been rediscovered several times. The anticeptic action of mercury chloride was demonstrated by Koch in 1884. Oganic mercury compounds for diuresis, antiseptic and other chemoterapeutic uses were introduced from 1900-1920. The marked poisonous activity of organic mercury was known already in ancient times and became a critical problem 400 years ago when mercury came to be used in several different handycraft processes. A lot of speculation concerning the toxicity of mercury compounds took place already between 1900-1940 but relatively little of this is relevant today with the knowledge we now have. As a result of the mercury debate, which has taken place during the last few years, we have become aware of the problems created by the use of mercury. The problems have arisen partially because of the use of mercury compounds as slimacides and their subsequent contamination of waters and streams. Mercury compounds and chlorinated phenols were added to the white water system of the paper mills, most of the time by chockdosing, i.e., by periodically adding a large amount of poison. One of the drawbacks with mercury compounds was their high retention to paper-fibres which caused them to be rapidly taken out of the white water system. This meant that slime-defeating with mercury compounds was expensive as well as damaging to the milieu. As I have already said mercury like all heavy metals reacts with SH-groups. **Organic tin compounds** are another type of heavy metal compound which was periodically introduced into the slime-defeating market for a long time. Tributyltinoxide was the most used. There is not the slightest doubt that these compounds are strong poisons. As a matter of fact so strong that they can no longer be accepted from a milieu point of view.

Since the milieu questions have been more and more important it has become desirable not to slime-defeating agents of the same type as the chlorinated phenols and heavy metal compounds. Among the compounds used as slimacides after the heavy metals, **organic sulphur compounds** were initially the most important. They were used because of their low human toxicity and because they were relatively harmless from a milieu point of view. These compounds are often added continuously or semi-continuously to white water systems for perhaps 6 hours. The organic sulphur compounds which were developed and used as slimacid were shown to reduce microbiological slime and control normally appearing bacteria. Added in the right way organic sulphur compounds seem to have a tendency not to be absorbed by fibres and to be more concentrated in the white water. A slime-defeating program which was very common with the organic sulphur compounds was to apply them for two days followed by penta-chlorophenol on the third day.

Among the most recent slimacides are **the organic bromocompounds.** One of these compounds, i-acetoxy-i, 4-dibrombut-2-en, has a large market. Its most probable reaction, alkylation of SH-groups, has been discussed above. This type of organic bromocompounds is without doubt a step in the right direction when it comes to slimacides since they combine a good activity against a broad spectrum of micro-organisms with the advantage of being harmless from a milieu point of view. The mentioned bromocompound, for instance, is relatively instable and cannot cause unwanted reactions when the compound comes out into the water.

THE RESISTENCE OF MICROORGANISMS

The increased tempo in our efforts to triumph over the surroundings is resulting in the invention of a lot of substances, physical and chemical. Each of these products has the aim to overcome an obstacle for our well-being or for our progress. The use of antibiotics and synthetic and synthetic antibiotic drugs is one of the most fantastic fields within this area of development. To be successful in the use of these drugs you must, however, undertake special precautions so that the organisms cannot adapt themselves to the chemicals which are used to defeat them. When the sulphonamide preparations were started to be used in the middle of the thirties they gave rise to an enormous enthusiasm about the possibilities for defeating streptococci and other pathogenic organisms. The disappointment was therefore correspondingly high when bacteria resistent to these drugs started to appear.

The optimism concerning the possibilities of defeating staffylococci was even higher when the discovery of penicillin was made but soon it turned out that when penicillin-sensitive staffylococci were eliminated they were followed by penicillin-resistent strains and it became clear that an uncritical use of penicillin, particularly in the hospitals, was a great tactical blunder. The use of broad-spectrum antibiotic gave rise to another problem since the elimination of all antibiotic sensitive bacteria from organs like the intestine can result in the normal bacterial flora becoming replaced by drug resistent bacteria or fungi which can be pathogenic and very disasterous if present.

Transferable resistence against drugs is an excellent example of the disaster caused by an uncritical use of antibiotic substances. The discovery that bacteria could transfer resistence against an antibiotic from one bacterium to another was made in Japan in 1957. It was demonstrated that E. coli bacteria which had developed resistence against different broad-spectrum antibiotics could transfer this resistence to a bacterium of another type which was sensitive to the antibiotics. Contact between donor and receiver cell was necessary to transfer the resistenc. Cell-free preparations of the donor cells lacked the power to transfer resistence. The transferable resistence can develop against a simple antibiotic but it usually develops against a broad spectrum of antibiotics. At present this transferable resistence has hitherto been found in entero-bacteria, i.e., bacteria of the type Shigellae, Salmonella, E. coli, Klebsiella, Proteus and other closely related organisms isolated from the intestines of human beings or animals.

The resistence against antimicrobial drugs has been of interest in the past mainly for two different reasons. First that it apparently is a serious problem in the treatment of infectious diseases which even today continues to worry designers of germicides. The resistence against drugs has also been a question of fundamental interest for modern biology, namely which role, if any, does the preparation itself play when it comes to giving the microorganisms inherited resistence. An elegant and incisive technique to answer this question has now been available for about ten years. These experiments have shown that mutations producing resistant strains are random events which are not effected by the drug itself. The drug acts only as a selective agent favouring the survival of resistent strains over sensitive strains once the genetical change has taken place. Since it was first discovered the drug resistence has been identified as a problem in enzymatic and other bio-chemical mechanisms. Nevertheless drug resistence has experimentally been verified in relatively limited scale. The discussion of resistence will here be limited to these cases where the inhibitors have one single site for their action. A single mutation which produces resistence against metabolic inhibitors at several points is hard to analyse. We thus lack definite studies of the biochemical basis for resistence against these substances.

Apart from these points of view on the drugs themselves it seems as if it should be most beneficial to discuss resistence on the basis of reaction mechanisms rather than on the basis of different kinds of drugs. It is evident that some knowledge or at least speculation about the activity of antimicrobial drugs must be available to allow the suggestion of resistence mechanisms. In an early attempt to suggest the most likely bio-chemical mechanisms leading to drug resistence the following possibilities have been suggested :

a) Decreased permeability of the fungal or bacterial membrane to the drug

b) Increased degradation of the drug

c) Increased concentration of the metabolite antagonizing the drug

d) Increased concentration of an enzyme utilizing this metabolite

e) Decreased quantitative requirements for a product of a metabolite

f) Alternative metabolic pathways which bypass the point of inhibition

g) Enzyme with decreased affinity to the drug compared to the newly formed metabolite

Five of these mechanisms are still believed to lead to resistence. Only two of the suggestions, a lower requirement or demand for the product of an inhibited reaction and the use of an alternative metabolic pathway do not seem to be mechanisms by which mutation produces resistant microorganisms. In addition to the fact that we have failed to demonstrate the next to the last suggestion; an alternative metabolic pathway as a mechanism for resistence, there is a theoretical basis to ask if this can take place at all. The development of an alternative metabolic pathway should require the formation of a new enzyme system which is not normally present. If the organism already had the necessary genetic information in a masked form, a normal mutation might allow the production of one new enzyme system. But if the large pool of necessary genetic information was lacking it is highly improbable that it can be inherited by a simple mutation or even by a series of mutations. In spite of the fact that alternative metabolic pathways are not believed to be involved in bringing about resistence they can nevertheless have an effect upon the ability of a special drug to perform a certain kind of inhibition. You would for instance expect that a facultative anaerobe which can get energy either by fermentation or oxidative proces-

ses will be less sensitive than a strict aerobe to inhibitors of oxidative phosphorylation. Decreased power to take up drugs often follows drug resistence. Such decrease can be the result of a decrease in affinity or activity of the intracellular receptors of the drug. On the other hand it can also be a consequence of the loss of a stereo-specific mechanism which is necessary for the drug to penetrate or in another way pass through the otherwise unpermeable cell membrane. It is obvious that the power of the microorganisms to develop resistence has been studied most intensively when it comes to resistence against antibiotics. The mechanisms of resistence which have been suggested for these chemicals can, however, also be applied to slimacides. Mercury is one of the few slimacides for which the ability of the microorganisms to develop resistence has been studied.

Most types of microorganisms seem to be able to adapt themselves to the presence of mercury but usually they cannot adapt as rapidly or to the same degree as they can to arsenicals, sulphonamides or antibiotics. Since we do not exactly know the mechanisms by which mercury compounds suppress the growth of the microorganisms it is immediately clear that we cannot postulate the logical mechanisms for the developed resistence. However, a few interesting observations can possibly contribute to casting some light over the existing conditions. The resistence apparently does not depend upon the reduced permeability of cell membrances to mercury compounds as is the case with arsenicals. It has been found that staffylococci tolerant against mercury grow when they have taken up much more mercury than necessary to prevent growth of normal strains. When it comes to organisms resistent against thiol-reagents the situation is somewhat confused. It has thus been shown that E. coli tolerant against phenylmercuric acetate has less SH-groups than normal but on the other hand it has also been found that mercury tolerant candida yeast contains 6 times more SH-groups than the normal yeast. It has also been shown that mercury adapted yeast produced more H_2S and it has been thought that this is what inactivates mercury. Normally yeast produces hydrogen sulphide only from sulphite while adapted strains of yeast produce it also from sulphate and hyposulphate, however, not from cystein or glutathione. If metabolic changes take place while an organism is adapting itself to mercury it is not particularly marked. It has thus been found that the respiration of Aerobacter aerogenes initially is suppresed by the addition of mercury but after a few hours the normal level is reached again. The adaptation to mercury compounds is apparently specific in most cases and it has been found that salmonella tolerant against mercury is not tolerant against copper or vice versa.

It would of course be desirable to be able to deal with the power of the microorganisms to adapt themselves to different slimacids more generally. This is, however, an extraordinary difficult task since in the first place the microorganisms behave differently against every slimacide, in the second place you have to consider the influence of the environment on both the organisms and the slimacides. The best way to solve this problem is to study the microflora in the white-water system continuously and study its tolerance against the slimacide in current use.