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REMARKS

Concluding remarks

Uriel Bachrach

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Introduction

I was asked to submit concluding remarks for this Conference. This is a very difficult task, as 41 lectures were given and 22 oral posters were presented. It is impossible to evaluate each paper. Yet, I will try to summarize some of the highlights. As the main topics of the Conference were Cancer and Diseases, I will concentrate on them. I hope that you bear with me and excuse me, if I fail to mention some important contributions. Not that I regard them as trivial, it is simply due to the massive material presented and the lack of time for my remarks.

This Conference is an excellent one and its organization was superb. All this was done single-handed by Professor Enzo Agostinelli.

You may have seen from the program, that many new scientists from various countries attend this Conference.

Table 1 shows that most of the speakers are well known in polyamine research. However, new scientists from South Africa, Portugal, and Turkey are an important addition to the list of speakers; many speakers came from the United States and from Italy (Table 1). It may also be seen (Table 1) that new scientists, contributed much to the poster session. These scientists have recently joined the group of polyamine researchers, and did not participate in previous International Conferences dealing with polyamines. They were invited by organizers of the Conference with the hope that they will continue to work in the field of polyamines.

U. Bachrach (✉)
Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel
e-mail: urielb@ekmd.huji.ac.il

Polyamine research started about 50 years ago with the first generation of scientists (Table 2). Among them were: N. Seiler from France, D. Morgan from the United Kingdom, H. G. Williams-Ashman and C. J. Bacchi from the United States, and I. D. Algranati from Argentina and others listed in Table 2. When they retired, the second generation replaced them. The members of the second group are now reaching the age of retirement and it is essential to recruit new brilliant and devoted young scientists to ensure the progress and continuation of this important field. The presence of young new scientists at this Conference and the presentations of their interesting posters is the first indication of the emergence of a new generation of young polyamine scientists.

Cancer

The topics of this Conference are: "Role of Polyamines and their Analogs in Cancer and other Diseases". Indeed, cancer is the major topic of the lectures presented (more than 10 lectures), next come parasitic diseases.

The growth of cancer cells and the proliferation of parasites like *Plasmodium falciparum* can be inhibited in vitro by blocking polyamine biosynthesis, by compounds like difluoromethylornithine (DFMO). However, this approach failed when experiments were repeated in vivo, when drugs were administered to rodents or to patients. In the human body, the block of polyamine biosynthesis is overcome by the supply of polyamines by food or by their synthesis by intestinal bacteria. Moulinoux suggested to use polyamine-poor nutrients and then to apply an inhibitor for polyamine biosynthesis. These steps should prevent the exogenous supply of polyamines and deplete polyamines by inhibitory drugs.

Table 1 Participants from different countries

Speakers from different countries		Oral posters different countries	
USA	12	Italy	7 new
Italy	9	Japan	2 out of 3 new
Japan	7	Turkey	2 new
UK	3	South Africa	2 new
Spain	2	USA	2 new
South Africa	1 new	France	1 new
France	2	Portugal	1 new
Israel	1		
Russia	1	Russia	1 new
Germany	1	Sweden	1 new
Denmark	1	Taiwan	1 new
Turkey	1 new	UK	1 new
	41		22

Table 2 Polyamine researches—different generations

Italy
Moruzzi, G., Caldarera, C.M., Mondovi, B., Grillo, M.A. and Bagni, N. Agostinalli, E.[a], Beninati, S.[a] and Serafini Fracassini, D.[a]
United States
Cohen, S., Russell, D.H., Tabor, H., Canellakis, E. and Herbst, E, J.
Pegg, A.[a], Porter, C.[a], Marton, L.J.[a], Park, M.H.[a] and Casero, R.A.[a]
Japan
Hayashi, S.
Igarashi, K.[a], Oshima, T.[a] and Oka, T.[a]
Finland
Raina, A., Hōltta, E. and Jänne, J.
Alhonen-Hongisto, L.[a]

[a] Second generation of "Polyaminers"

Another approach is based on the inhibition of polyamine uptake, so that in the presence of an agent like DFMO, cells will be deprived of polyamines.

Depletion of polyamines in cancer cells

Cipolla (L35) is a member of Moulinoux's group. They reduced cellular polyamines by using a polyamine poor diet. This diet alone reduced the growth of prostate carcinoma cells by 40%. As DFMO was not available for clinical trials in Europe, the author used docetaxel as a drug. The big advantage of the study was the application of the concept to 28 patients of prostate cancer. By doing this, toxicities and side-effects were determined and the decrease in prostate-specific antigene (PSA) was established. It has been concluded that the side-effects were minimal and that the success of the treatment was confirmed by a decrease in PSA level.

It thus appears that this approach has a significant therapeutic potential and is an impressive example that a new concept has been applied successfully to treat cancer patients.

The second approach, namely to reduce polyamine uptake and the application of combined therapy has been proposed by various investigators.

Wallace (L37), who is a leader of polyamine research in UK, published numerous papers on polyamines and cancer. In this Conference, she described the effect of polyamine-anthracene conjugates on polyamine uptake by cultured human chronic myeloid leukemia cells, in which polyamine uptake is higher than in the normal controls.

However 2 questions may be asked:

1. Is there any adverse effect after prolonged administration of the anthracene conjugates to animals or to human patients?
2. Is the inhibition of polyamine uptake by the conjugate-a general phenomenon, how would this affect normal growth and differential processes?

Synthesis of polyamine analogs

Blagbrough (L36, P31) is a well-known organic chemist with a biological orientation. He attached compounds like guanidine to spermine or its analogs. Some of these are toxic, resembling spider toxins. It remains to be established whether such conjugates could be used as a selective anticancer drug.

Khomutov (L03) is an excellent organic chemist, who for years has been synthesizing polyamine analogs. Now, he synthesized spermine trine, which down regulated ornithine decarboxylase (ODC) and decreased polyamine levels. It was not clear whether the experiments were also conducted in vitro and whether toxicity was tested.

Polyamine metabolism

Agostinelli (L33) carried out extensive studies on the biological activities of the amino-aldehydes and hydrogen peroxide, produced by the oxidation of polyamines by bovine serum amine oxidase. In his lecture, he described the inhibition of growth of cultured human adenocarcinoma and melanoma cells by oxidized spermine. Unexpectedly, multi-drug resistant cells were more sensitive compared to the wild-type counterparts. This is a very

interesting observation, as means to combat drug resistant cells are very limited. This opens the way to new therapeutic strategies.

Combined therapy has been used to increase cytotoxicity and an anti-malarial drug has been selected (P26). The mechanisms of the anti-cancer activities of spermine oxidation products have been studied extensively. However, the final conclusion as to the therapeutic potential of this approach has to await clinical studies, in which delivery systems may be used (see P27).

Igarashi (PL 01) gave a very impressive plenary lecture, in which he reported that cell damage can be caused by reactive oxygen species. He found that acrolein is even more toxic than the reactive oxygen species. These two cytotoxic factors can induce thrombosis and brain infraction in a mouse model. Apparently, acrolein induces damage in RNA, which leads to the release of polyamines. These polyamines are subsequently oxidized to form more acrolein. These interesting studies may explain the causes and mechanisms of brain infraction, but do not have a direct relevance to cancer therapy or diagnosis (but see also P10).

Shantz (L19) continued her previous studies and maintained that ODC induction is both necessary and sufficient for the promotion of skin cancer. Now, she reported that ODC mRNA is stabilized fourfold during carcinogenesis. This stabilization has been explained by the binding of mRNA to a protein HuR, which is localized in the nucleus. It thus appears that the increase in ODC activity in cancer cells is due to the stabilization of its mRNA and not only to enhanced synthesis.

Casero (PL02) presented an interesting plenary lecture in which he described two important enzymes. One was spermine oxidase a-FAD dependent enzyme, which catalyzes the oxidation of polyamines to yield aminopropanal and hydrogen peroxide. Spermine oxidase induction leads to the formation of reactive oxygen species which are an early event in inflammation associated carcinogenesis. The second enzyme was a lysine specific demethylase, which demethylases mono and dimethyl lysine in histone 3. Its activity is associated with transcriptional repression and thus is important for epigenetic regulation of gene expression. The deregulation of spermine oxidase leads to the production of reactive oxygen species which trigger inflammation and carcinogenesis. The inhibition of spermine oxidase or lysine demethylase activities can prevent inflammation, reduce tumor formation and therefore can be considered as chemo preventive agents.

Diagnosis of cancer

Kawakita (L41) reported that N^1-N^{12}-diacetylspermine is frequently increased in the urine of colon, breast and lung cancer patients. It has been suggested that this spermine derivative could be used as a tumor marker, since its urinary levels are elevated in patients at early stages of cancer. Recent studies demonstrated that diacetylspermine can also be detected in colorectal-cancer tissues, but not in adenoma tissues. Diacetyl spermine is not taken up by growing tissues unlike the monoacetyl derivative. It has been suggested that diacetyl spermine is synthesized in some cancer cells and then excreted into the urine.

Moulinoux (L04) proposed to use polyamines bound to erythrocytes as a marker of malignancies. It is not a specific marker: false negative-breast cancer and melanoma. False positive: Parkinson disease and heart transplantation. Several questions may be asked:

1. What is the advantage of using an invasive method instead of using urine analyses, in which false positive results are not common?
2. Polyamines are excreted from cancer cells as acetyl derivatives. What is the source of the free polyamines which are bound to erythrocyte membranes?

Parasites

The headline of this Conference contains the terms—cancer and other diseases. Parasitic diseases are certainly included in this category.

Parasitic diseases affect African and South American countries. *Plasmodium falciparum*, which causes malaria, infects more than 600 million patients, resulting in several million deaths, mainly children. In the past malaria could have been controlled by drugs like quinine. The development the vector Anopheles was inhibited by spraying with dichlorodiphenyltrichloroethane (DDT). This is not possible today; the Plasmodia became resistant to the drugs and DDT is not active anymore. The continual development of resistance of Plasmodia to current antimalarial drugs indicates the pressing need for the discovery of novel chemotherapeutic approaches. Malaria parasites are essentially dependent on the biosynthesis of putrescine and spermidine.

In vitro studies demonstrated that drugs like DFMO can exert a cytostatic effect on the infected red blood cell. In vivo experiments, in which rodents were infected with *P. bergehi*, failed. Like in cancer, the rodent contains enough polyamines which could overcome polyamine biosynthesis disruption through the uptake of exogenous polyamines.

On the other hand, DFMO could be used to treat patients suffering from African sleeping sickness.

What could be done?

1. Go back to traditional medicine. The best example is artemesinin.
2. Synthesize new drugs.
3. Block polyamine uptake.
4. Produce vaccines (so far failed).

New drugs

Woster (L38) synthesized guanidine and biguanide polyamine derivatives and demonstrated their activities against Leishmania and Plasmodia. It was not clear whether toxicities were also determined.

Verlinden (P16) from South Africa tested antimalarial activities of (bis) urea and (bis) thiourea polyamine derivatives. In vitro studies demonstrated antimalarial activity against chloroquine-resistant parasites. No data are given whether in vivo studies were carried out and whether the drugs inflict toxic side effects.

Inhibition of polyamine uptake

Birkholtz This is the first time that we see a "polyaminer" from South Africa participating in a European International Polyamine Conference. As tropical diseases, mainly malaria, are so widespread in African continent. It is not surprising that this topic was investigated in South Africa.

As mentioned above, blocking polyamine uptake could promote the activities of anticancer or antimalarial drugs.

Birkholtz (L29) used putrescine-anthracene conjugates and demonstrated the inhibition of polyamine uptake by plasmodia-infected erythrocytes. Obviously, the toxicity of this conjugate has to be determined in mice infected with *P. berghei.*

Kaiser (L17) studied the molecular biology of *Plasmodium falciparum* and concentrated on deoxyhypusine synthase, which catalyzes the first step in hypusine biosynthesis of the eukaryotic initiation factor (eIF-5A). CNI-1493 is a pro-inflammatory cytokine inhibitor, by reducing eIF-5A concentrations. Kaiser used elegant knock-down studies in mice infected with *Plasmodium berghei* and demonstrated an absolute requirement of eIF-5A and the disappearance of deoxyhypusine. It should be noted that this is one of the exceptional studies in which a rodent model was used.

Microbiology

Only a relative small number of bacteria have been isolated and their physiological properties have been elucidated. Emphasis was given to pathogenic bacteria which cause disease in patients and animals. These bacteria could be grown in vitro and could be identified by biochemical and immunological methods. Intestinal bacteria like Salmonellae or Shigellae are good examples. Sometimes, more sophisticated methods had to be used to cultivate anaerobes (like Clostridia) or the so-called fastidious bacteria. Even if some bacteria could be isolated, their physiological functions remained unsolved. In many cases, synergism exists and the adhesion of oral bacteria like fusiform bacteria is still a mystery.

Many intestinal and soil bacteria are unknown. More recently, an innocent bacterium, *Helicobacter pylori*, which was recognized as a non-pathogenic organism, was shown to induce ulcers and gastric cancer. Obviously, this opened new possibilities for cancer prevention and the therapy of ulcers.

Michael (L09) demonstrated two unusual transitions. He left UK and moved to the United States. Then he left the field of plant molecular biology, in which he had impressive results and moved to Microbiology. He decided to go into a mission, who may be regarded as a mission impossible, and to identify and explore the nature of unknown bacteria. In the human body, more than 10^{14} microorganisms exist. Luckily, he did not forget polyamines and decided to map out polyamine biosynthetic strategies and also identify enzymes that covalently modify polyamines.

This is certainly a very difficult task, but Michael is brave and we wish him success in achieving these goals.

Wilson (L34) studied the physiological properties of *Helicobacter pylori* and suggested a new approach for the therapeutic intervention for curing and preventing ulcer and gastric cancer. He found that macrophage ODC is up- regulated by *H. pylori* and causes apoptosis. This step requires spermine oxidase, which metabolized spermine and generates hydrogen peroxide, toxic aldehydes and thus produces an oxidative stress. DFMO, which inhibits polyamine biosynthesis, reduces gastric inflammation and DNA damage, and thus opens new possibility for disease prevention and cure.

Oshima (L10) continued his impressive studies on the properties and structure of enzymes involved in the synthesis of polyamines in thermophiles. He found that ODC does not exist in thermophiles and that polyamines (long and branched chains) are produced from arginine. These polyamines are required for protein synthesis and for the integrity of nucleic acids. 3D structures of some of the enzymes was determined by X-ray crystallographic techniques and revealed that in the enzyme, the active site is kept open and can accept larger substrates than putrescine.

Transglutaminase

Covalent binding of polyamines on protein surfaces is catalyzed by various transglutaminases, a large family of

enzymes that cross-link proteins by transferring ε-amino groups of lysines or polyamines on one protein to the γ-carboxamide group of glutamic acid residues on a different protein. This enzyme has been linked to blood coagulation and preserving the integrity of the retina.

Extensive studies on this subject are carried out in Italy, mainly due to Simone Beninati, who also formed WHAT, a special group interested in this subject.

Beninati (L27) studied the role of transglutaminase on differentiation therapy, which is a new approach to treat advanced malignancies. He found that theophylline and flavonoids induce differentiation in cultured melanoma cells. More studies are required to substantiate this approach.

Serafini-Fracassini (L22, L26) carried out extensive studies on the role of polyamines in plants, some of which were done in collaboration with her late husband Nello Bagni. Now, she turned to a new interesting subject—environment and air pollution. She concentrated on the role of pollen (from apple and hazelnut) in causing allergy. She showed that transglutaminase is necessary to allow the germination of the apple pollen. Moreover, the expression of transglutaminase increases when pollens are exposed to stressful conditions such as high humidity. This is an interesting study combining plant polyamines and environment.

Hypusine

Hypusine is an unusual amino acid found in all eukaryotes. The only known protein containing hypusine is eukaryotic translation initiation factor 5A (eIF5A). There are two reactions and two enzymes involved: deoxyhypusine synthase catalyzes the cleavage of the polyamine spermidine and transfer of its 4-aminobutyl moiety to the ε-amino group of one specific lysine residue of the eIF-5A precursor to form deoxyhypusine and 1, 3-diaminopropane. Deoxyhypusine hydroxylase mediates the formation of hypusine by addition of a hydroxyl group to the deoxyhypusine residue. Hypusine is involved in protein biosynthesis and promotes the formation of the first peptide bond. Thus, hypusine and eIF-5A appear to be vital for the viability and proliferation of eukaryotic cells.

Park (L14) continued her extensive studies (started with the late J. Volk) on hypusine and the elongation factor eIF-5A, which is found in eukaryotic cells. In bacteria, neither eIF-5A nor deoxyhypusine hydroxylase or spermidine could be detected. On the other hand, a similar elongation factor-EF-P which does not contain spermidine was described.

Kahana (L15) tried to learn more about the function of polyamines. He found that polyamine depletion inhibits translation initiation prior to the inhibition of DNA synthesis. He studied the role of polyamines on the activity of eIF-5A, which is a translation initiation factor and is modified by spermidine. The modified protein is essential for cell growth and viability. Two inhibitors, DFMO and N^1-guanyl-diaminoheptane (GC7) reduced cellular polyamines and inhibited eIF-5A hypusination.

See also Kaiser "Inhibition of polyamine uptake" above.

Antizymes

In 1997, E.S. Canellakis described a low-molecular protein, which specifically binds to ODC and inactivates it. The paper, which was published in PNAS, was received with skepticism and he had problems in receiving support and grants. He was discouraged and left his position at Yale University and moved back to Greece, where he lived in a small island and kept himself busy by fishing and growing grapes. Today, his name does not appear in the list of polyaminers composed by Woster.

Later on, his findings were confirmed by other investigators and several types of antizymes were described. Moreover, anti-antizymes were also discovered. A unique pathway for the translation of antizyme mRNA, namely, a-frame shift was described by Kahana and by Japanese investigators. Mitchell reported that antizymes stimulated polyamine uptake. Today, active research on the physiological functions of antizymes is carried out all over the world and some of them are reported in this Conference.

Penafiel (L05) studied the occurrence and physiological functions of ornithine decarboxylase antizyme inhibitors AZI1 and AZI2. These proteins which are widely found in testis and in brain interact with antizymes and regulate intracellular polyamine levels by affecting ODC activity and polyamine uptake. The effect of different biguanides on ODC and diamine uptake was also studied.

Ray (L07) studied the effect of amino acids on ODC activity and found that asparagine had a stimulatory action. This amino acid prevented the accumulation of antizyme inhibitor 1. In contrast, lysine and valine induced antizyme inhibitor expression and decreased ODC activity.

Ohkido (L20) used antizyme 1 (AZ 1) knock out embryos and found that they suffer from anemia. The hematopoietic disturbances were prevented when putrescine synthesis was inhibited by DFMO. Hematopoiesis was also disturbed in antizyme 1 knock out experiments, when in adult mice were used. It has been concluded that this inhibitory effect is not limited to embryos.

Oka (L16) studied the etiology and the pathogenesis of diabetes mellitus using pancreatic tumor-derived cells. During differentiation, the cellular concentration of

putrescine decreased to low levels, whereas the levels of both spermidine and spermine remained constant. It has been reported that antizyme was necessary for glucagon production. Therefore, it has been concluded that polyamines and antizymes are functional in the differentiation of pancreatic endocrine cells.

Others

In this Conference many interesting papers dealing with the metabolism and function of polyamine biosynthetic systems were presented. **Palavan-Unsal** (L39) presented very interesting results concerning the modulation of polyamine metabolism and cyclin dependent kinases. She and her associates are becoming a new active group in polyamine research. They even plan to organize a Polyamine Conference in Istanbul in 2012. **Sanches-Jimenez** presented a very interesting paper.

The success of the Conference was mainly due to Profs. Agostinelli and Igarashi who invested more energy and vision in organizing and planning this successful meeting. Prof Agostinelli was assisted by young coworkers from his Department, who contributed much to the organization of the Conference. It is our hope that they and the other young and new participants of the Conference will continue their contribution to polyamine research and thus become a new active generation.

With the hope that all of you absorbed the interesting material presented and will have time to digest it. Soon, you will leave this beautiful hotel carrying your suitcases. But more important, you will carry with you new information, some criticism and many new friends.

Now I will end my concluding remarks, wish you all young and old, new and experienced, good luck and pray that you will continue your devotion to polyamine research.

Many thanks to you all!!!