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des technologies de l'UNESCO

Division of the Ethics of Science
and Technology

Table des matières

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Table of Contents

CHAPITRE / CHAPTER 1
TABLE RONDE SUR « BIOETHIQUE ET DEBAT PUBLIC : INFORMATION, EDUCATION, PARTICIPATION » /
ROUND TABLE ON 'BIOETHICS AND PUBLIC DEBATE: INFORMATION, EDUCATION, PARTICIPATION'

Ethique et débat public : information, éducation, participation
Mme Nicole Questiaux 3

L'enseignement universitaire de la bioéthique : résultats et difficultés
Mme Habiba Chaabouni 7

Non-Governmental Organizations and Public Debate
Mr Francis Crawley 13

Involving Youth in the Public Debate on Bioethics
Ms Diane Gal 17

L'éthique biomédicale dans les pays en développement : l'expérience du Maroc
M. Farid Hakkou 23

Bioethics and the Public Debate
Mr Darryl Macer 29

Information et debat public
M. Alain Perez 35

CHAPITRE / CHAPTER 2
State-of-the-art on Research on Cloning of Whole Organisms
Mr Michel Revel 43

CHAPITRE / CHAPTER 3
Ethical Considerations Related to Xenotransplantation
Mr Fritz H. Bach and Ms Elizabeth A. McGregor 59

CHAPITRE / CHAPTER 4
State-of-the-art on Embryonic Stem Cell Research
Mr César Nombela 67

Chapitre / Chapter 1

TABLE RONDE SUR « BIOETHIQUE ET DEBAT PUBLIC : INFORMATION, EDUCATION, PARTICIPATION »

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ROUND TABLE ON 'BIOETHIQUE AND PUBLIC DEBATE : INFORMATION, EDUCATION, PARTICIPATION'

- **Mme Nicole Questiaux** (France)
- **Mme Habiba Chaabouni** (Tunisie/Tunisia)
- **Mr Francis Crawley** (Belgique/Belgium)
- **Ms Diane Gal** (Pays-Bas/The Netherlands)
- **M. Farid Hakkou** (Maroc/Morocco)
- **Mr Darryl Macer** (Nouvelle Zélande/New Zealand)
- **M. Alain Perez** (France)

ETHIQUE ET DÉBAT PUBLIC : INFORMATION, ÉDUCATION, PARTICIPATION

Nicole Questiaux (France),
Présidente de la Conférence permanente
européenne des comités nationaux d'éthique

La préoccupation éthique dans les sciences, et notamment chez les chercheurs qui se consacrent aux sciences de la vie, ne date pas d'aujourd'hui. Elle a toujours représenté dans les milieux scientifiques une facette des défis intellectuels que le chercheur tente de résoudre. Dans toute recherche d'envergure, elle apparaît bien souvent comme l'un des multiples problèmes qu'il faut résoudre pour avancer, et ceux qui la portent ont, sans nous le dire toujours, l'habitude de trancher, en leur âme et conscience, les questions que leurs investigations ont d'elles-mêmes soulevées. On en parle, entre pairs : l'éthique fait partie du quotidien, dès que l'être humain est le sujet de la recherche.

Malgré cela, l'histoire nous a appris qu'à certains moments, l'humanité pensante et instruite se fourvoie, que les plus grandes intelligences dérapent et que la science a pu servir de marchepied à des atteintes graves aux droits de l'homme et à la dignité humaine. De telles dérives sont insidieuses et on se prend à penser qu'elles auraient été impossibles ou, du moins, se seraient trouvées contrecarrées, si l'activité de recherche avait été à l'époque plus transparente.

C'est l'avertissement qu'il faut entendre dans le contexte triomphant de la science contemporaine. La période récente, les quelques décades que nous venons de traverser, connaissent à la fois une explosion scientifique, du côté des sciences de la vie, et l'affirmation d'un mouvement d'idées dont l'existence du Comité international de bioéthique (CIB) et l'intérêt de la communauté universelle pour son activité portent témoignage. La science sort de ses laboratoires et va à la rencontre des inquiétudes que son pouvoir suscite. Ce mouvement d'idées, qui donne consistance à la bioéthique, est, du moins je le pense, sans précédent dans l'histoire des sciences.

Il suscite la création d'instances de réflexion, de débat et de conseil - les comités d'éthique -, il cherche à formuler de nouveaux concepts, il voit mûrir une méthode de questionnement et de réponse qui tend à caractériser notre domaine. Dans sa composition, ses réalisations, sa méthode, le CIB est le reflet de ce qui se passe à une large échelle dans de nombreux pays. Il nous appartient, par la discussion ouverte dans cette table ronde, de mettre en lumière combien le rapport avec le public est une composante nécessaire de ce mouvement d'idées. Information, éducation, participation en sont l'ardente obligation ; elles en représentent aussi la principale difficulté et, comme nous allons le voir, mettent vraiment au défi nos capacités d'invention.

Ce que nous évoquerons, en quelques minutes, dans cette table ronde, serait vraiment matière à une recherche à part entière; mais nous ne l'avons pas derrière nous.

Pourtant, un peu partout de par le monde, des esprits très avertis, très variés, se sont mobilisés sur ces questions. Des instances existent. Ce sont ces comités d'éthique, créés le plus souvent au niveau national. Ils réunissent, comme une première et essentielle composante, des représentants de la communauté scientifique, une élite qui est à même d'identifier les problèmes sensibles et dont l'opinion pourra influencer le milieu scientifique dans son ensemble. Il leur est demandé de mettre en commun ce savoir avec des personnalités qui exprimeront le mieux possible les attentes morales et sociales d'une société.

Le comité d'éthique ne décide pas, il exerce par ses avis une magistrature d'influence. Presque par construction, il est élitiste, par le savoir et par la capacité à mettre en discussion les conséquences de ce savoir. Quels que soient les efforts faits pour que sa composition reflète la variété des points de vue qu'il convient de rapprocher, se pose dans tous les pays la question de sa représentativité et, donc, de la légitimité de son influence. Elle sera recherchée dans la plupart des cas en donnant au comité, dans le cadre de sa mission, des obligations à l'égard du public et même du grand public.

Une enquête réalisée en 1998 dans le réseau que constitue, sous l'égide du Conseil de l'Europe, la Conférence des comités d'éthique, donne une indication des moyens envisagés. La publication d'un rapport d'activité est la règle, la majorité organisent séminaires ou conférences; il peut y avoir, mais c'est moins courant, une obligation statutaire à l'exemple français d'organiser des journées ouvertes au grand public; elles sont l'occasion de contacts organisés en direction des jeunes et de préparation avec le corps professoral. Surtout, les avis eux-mêmes sont rendus publics, même si dans la plupart des cas les discussions qui en précèdent l'adoption ne sont pas ouvertes à la presse et au public.

Des concepts, ensuite, à diffuser et à faire connaître. L'originalité de la discipline bioéthique est qu'elle cherche à dégager des normes qui par définition n'ont pas encore été clairement posées dans le droit positif et qui doivent rendre compte du caractère évolutif d'un contexte marqué par les progrès de la recherche. Il en résulte des contraintes importantes dès que l'on cherche à les faire comprendre et à leur donner une publicité. Le corpus de l'information n'est pas disponible et doit être constitué. Une première étape est le devoir de donner une information solide sur l'état de la science, qui commande l'intelligence des difficultés éthiques. Sinon, l'opinion s'égare dans les craintes désordonnées qu'inspire la science-fiction. L'expérience des comités d'éthique a montré que l'un des premiers apports de leurs avis peut être un travail exemplaire d'explication scientifique, dissuadant définitivement l'exploitation des réactions liées à l'ignorance. L'analyse éthique ne peut pas non plus utiliser purement et simplement comme matériel d'information les documents qu'établissent les différentes parties prenantes d'un problème moral, religieux, sociologique ou politique; le fond dont il est question de parler lorsque l'on informe ou éduque est le plus souvent un compromis, parfois solennel, parfois pratique, toujours sujet à révision. Il faut donc trouver les mots et les personnes qui sont capables d'exprimer ce type de concepts et d'en faire un matériau d'information, d'enseignement, d'étude et de critique. Il n'est donc pas étonnant que les rapports avec la presse, les médias, les enseignants n'aillent pas de soi et demandent une forme d'investissement. A notre débat d'éclairer ce point.

A cet égard, le document d'explication et de commentaire que nous pourrions élaborer à propos de la Déclaration universelle sur le génome humain et les droits de l'homme peut être un instrument très utile, à la fois par l'exercice qui consisterait à le rédiger et par les bases qu'il donnerait aux personnes envisageant de diffuser l'information.

Une méthode de réflexion, une discipline de pensée et de discussion. C'est certainement l'une des raisons du succès des comités d'éthique et ceci explique pourquoi l'UNESCO, à propos de ses travaux sur le génome, et la Commission des droits de l'homme des Nations Unies préconisent la mise en place de ces comités consultatifs, pluridisciplinaires et indépendants. Ils se livrent à un travail original, fondé sur la franchise et la confiance réciproque; ils font le pari, très souvent réussi, qu'une démarche itérative, tirant pleinement parti de la pluridisciplinarité peut explorer les risques sans les exagérer, distinguer des règles de comportement que l'on doit mettre en commun et ce qui relève de la conscience de chaque chercheur, encadrer la créativité de la recherche sans la suspecter ou la freiner, donner des conseils qui soient compris et suivis.

Le défi de l'information, de l'éducation, de la participation, c'est que ce travail ne peut se faire de plein pied dans des enceintes trop nombreuses : par conséquent il faut s'organiser de telle manière que tous ceux qui sont concernés soient tout de même consultés largement, associés en temps utile, destinataires de toutes explications et puissent reprendre à leur compte le débat.

Ce n'est pas une tâche facile. Parmi les idées que nous pourrons évoquer dans cette table ronde, certaines s'adresseront au milieu des comités d'éthique et en particulier au CIB lui-même. D'autres s'adresseront aux acteurs du débat social, que cette table ronde entend représenter par les enseignants, les enseignés, la presse, les médias. Les uns et les autres vont s'exprimer dans un instant, pour nous dire en quoi cette affaire leur importe et les difficultés qu'ils rencontrent quand ils veulent se saisir de ces sujets. Aux comités d'éthique d'être particulièrement attentifs à ce type de message. Indépendants, responsables, ils commencent à trouver leur voie. Mais ils ont le devoir d'être compris et il y a encore beaucoup à faire pour clarifier les messages et pour que leur élaboration permette aux citoyens d'y prendre pleinement leur part.

L'ENSEIGNEMENT UNIVERSITAIRE DE LA BIOÉTHIQUE : RÉSULTATS ET DIFFICULTÉS

Habiba Chaabouni (Tunisie),
Département de génétique,
Faculté de médecine de Tunis

Avec le développement de la recherche médicale et de la génétique et des technologies médicales comme la greffe d'organe, l'assistance médicale à la procréation et la médecine prédictive, la formation en bioéthique est une nécessité qui se fait de plus en plus sentir.

L'enseignement de la bioéthique à l'université a commencé il y a près de vingt ans, son objectif étant de concilier l'évolution rapide et importante de la science et de la technologie appliquée au vivant, d'une part, et le respect de la dignité humaine, d'autre part.

L'enseignement de la bioéthique, destiné au départ particulièrement aux médecins, s'est étendu peu à peu à d'autres catégories professionnelles dans d'autres secteurs. C'est ainsi que la formation en bioéthique intéresse aujourd'hui les juristes, les philosophes, les sociologues et les psychologues, à côté des professionnels de la santé et des biologistes.

Même si nous nous intéressons uniquement à l'enseignement universitaire, plusieurs questions se posent.

Qui demande cette formation ?

A qui profite-t-elle ?

Quel type d'enseignement est-il assuré ?

Qui assure cette formation ?

Quels sont les diplômes délivrés ?

Quelles sont les retombées de cet enseignement ?

Qu'en est-il de la situation actuelle ?

Quelles sont les difficultés rencontrées ?

La place qu'occupe l'enseignement de la bioéthique dans les institutions universitaires est d'une importance variable selon les pays. Si, dans certains pays, l'enseignement de la bioéthique se limite aux écoles et facultés de médecine, de biologie et aux institutions de formation du personnel de la santé, ailleurs cet enseignement a lieu également dans les facultés de droit, de théologie, de psychologie...

L'enseignement de la bioéthique se fait de différentes manières :

- il fait partie de l'enseignement d'autres spécialités comme la pédiatrie, la médecine légale par exemple ;
- il s'agit d'une discipline enseignée sous forme de module de quelques heures au cours d'une formation de magister (licence), d'une formation doctorale (DEA)... ;
- il s'agit d'un enseignement spécialisé en bioéthique qui aboutit à l'obtention d'un diplôme ou d'un certificat (DEA, Certificat complémentaire).

Par conséquent, cet enseignement peut avoir lieu au premier, deuxième ou troisième cycle de l'enseignement supérieur.

L'enseignement est assuré sous la forme de cours magistraux, de conférences, de séminaires, d'ateliers, de dissertation ou de participation à la réflexion. Il peut aussi être dispenser dans le cadre de la pratique médicale quotidienne (pendant une consultation par exemple).

L'enseignement de la bioéthique est assuré par des enseignants de formations différentes et de profils différents :

- les spécialistes en bioéthique, qui ont eux même au préalable bénéficié d'une formation spécifique et qui sont diplômés en bioéthique ;
- les non-spécialistes, qui enseignent la bioéthique sur la base de leur expérience, de leur formation pratique et de leur aptitude à la réflexion sur les problèmes éthiques soulevés dans leur domaine d'activité.

Dans les facultés de médecine et de biologie ou de formation du personnel de santé, l'enseignement de la bioéthique représente dans la majorité des cas un volume horaire inclus dans l'enseignement de certaines spécialités comme la génétique, la thérapeutique, la réanimation médicale, etc.

Dans d'autres facultés les questions éthiques sont soulevées à l'occasion de chaque situation où elles se présentent : par exemple, les greffes d'organes ou l'assistance médicale à la procréation.

Une enquête réalisée dans les pays d'Amérique latine montre que, dans 70% des cas, l'enseignement de la bioéthique dure au total un mois dans le cursus des étudiants en médecine et la durée de cet enseignement peut parfois s'étendre jusqu'à 12 mois.

L'enseignement de la bioéthique se fait sous forme de modules dans les formations du troisième cycle, post-doctorales, par exemple dans le cadre du DEA en génétique humaine de la Faculté de médecine de Tunis : l'enseignement relatif à la bioéthique et aux législations concernant les recherches en génétique et leurs applications représente dix heures du cursus.

Enfin, la formation académique en bioéthique se présente, dans certaines universités, sous la forme d'un cursus spécialisé conférant un diplôme en la matière. Cette formation a lieu dans plusieurs pays, aux Etats-Unis d'Amérique, au Canada, en Europe (France, Belgique, Royaume-Uni, Italie, etc.), au Japon, en Chine, etc.

A côté de l'université, plusieurs centres de bioéthique, dans différents pays, ont vu le jour, dont l'un des objectifs est l'enseignement de la bioéthique.

Les résultats de cet enseignement

L'enseignement de la bioéthique a largement contribué à la prise de conscience, par les médecins et les professionnels de la santé, des problèmes éthiques relatifs à leur activité quotidienne. Désormais un spécialiste de la santé conscient de la dimension éthique de sa profession sera plus à l'aise et encore plus efficace dans une décision de choix thérapeutique, pour donner un conseil génétique ou pour proposer une greffe d'organe, par exemple.

Cet enseignement a également permis de pousser un plus grand nombre de professionnels de secteurs autres que celui de la santé dans la réflexion sur les questions bioéthiques. Cette réflexion intéresse aujourd'hui les philosophes, les juristes, les psychologues, les sociologues et les économistes à côté des médecins et d'autres professionnels de la santé. Une approche interdisciplinaire se dessine, au bénéfice de l'homme, bien sûr.

L'enseignement de la bioéthique dispensé dans les cursus de formation à la recherche permet de donner au futur chercheur une perception plus humaine de la rentabilité de sa recherche et ce, en le poussant à approfondir la réflexion sur les enjeux éthiques de la recherche dans le domaine de la santé.

La technologie médicale avancée envahit le marché tant au niveau des explorations qu'en matière de thérapeutique. Il est donc nécessaire d'assurer une formation des médecins en bioéthique si l'on veut éviter les abus d'utilisation de cette technologie.

Il faut aussi former des spécialistes en bioéthique (DESS, DEA, Doctorat...) qui à leur tour vont former les étudiants. Dans certains pays, ces spécialistes siègent dans les organes de décision qui traitent de problèmes éthiques et contribuent ainsi à faire respecter des principes éthiques. Leur rôle est également important dans les administrations chargées de la santé publique.

Le développement de la bioéthique et de son enseignement a engendré la nécessité de créer à un rythme de plus en plus accéléré des comités de bioéthique. Ces comités siégeant dans les institutions sanitaires et de recherche œuvrent à faire respecter la dignité humaine en imposant des règles éthiques dans les protocoles de recherche et dans la pratique médicale quotidienne.

Bref, une dynamique de la bioéthique s'est créée.

Difficultés de l'enseignement universitaire de la bioéthique

L'enseignement de la bioéthique au niveau universitaire n'est cependant pas généralisé à toutes les institutions biologiques et celles des sciences de la santé, encore moins aux établissements supérieurs de droit, de sociologie, etc.

En réalité, l'enseignement de la bioéthique au niveau universitaire n'est pas assuré dans tous les pays, ni dans toutes les institutions. Pourquoi ?

Il se heurte à certaines difficultés :

- ⇨ l'absence de conviction et le manque d'information des responsables de la formation universitaire ;
- ⇨ l'utilité de cet enseignement est discutée en cas d'absence de recherche dans le pays : la bioéthique est-elle une nécessité ou un luxe réservé aux pays avancés dans le domaine de la recherche scientifique, biologique et médicale ? Il s'agit là d'un faux problème, car les applications des recherches se font aussi dans les pays non producteurs de technologie ;

- ⇨ les programmes universitaires sont surchargés et par conséquent il n'y a pas de place pour l'enseignement de la bioéthique. Mais la formation en bioéthique et le savoir-faire qu'elle permet d'acquérir sont indispensables;
- ⇨ l'absence d'enseignants ayant la formation requise en bioéthique.

Que faire ?

- D'abord, développer les concepts d'éthique et de bioéthique, qui constituent de nos jours un besoin ressenti quotidiennement, et décider si l'enseignement de bioéthique doit être, ou non, assuré à l'université.
- Etablir un programme d'enseignement dans chaque institution supérieure ayant un rapport avec les sciences de la vie et, surtout, en fixer les objectifs.
- L'éducation en matière de bioéthique doit faire face aux nouveaux dilemmes posés par les avancées scientifiques et technologiques, qui sont en train de changer la relation médecin - malade et médecin patient sain, et même la relation citoyen - citoyen.
- Les systèmes de santé et l'évolution du système des assurances doivent nous pousser à généraliser l'enseignement de la bioéthique. C'est notamment le problème de la gratification des médecins qui réalisent des économies pour les assurances ; lorsque la liberté du médecin d'agir selon sa conscience est contrainte, il est moins utile qu'il fasse preuve de perspicacité dans les dilemmes éthiques posés par le traitement d'un patient.
- Réexaminer les questions de bioéthique enseignées à l'université à la lumière des considérations sociales, économiques et commerciales concernant la santé du citoyen.

En conclusion

Depuis quelques années, l'enseignement universitaire de la bioéthique connaît un développement notable dans certains pays. Cet enseignement concerne les étudiants des premier, deuxième et troisième cycles des facultés de médecine, de biologie et aussi de lettres, de droit et d'économie. Cependant cet enseignement est réduit ou encore inexistant dans la majorité des universités d'autres pays, en développement notamment. Pourtant, sa nécessité est grande dans les divers secteurs de formation, tant pour la recherche que pour la pratique quotidienne. En outre, il permet de renforcer la formation et l'information du public en matière de bioéthique.

NON-GOVERNMENTAL ORGANIZATIONS AND PUBLIC DEBATE

Francis P. Crawley (Belgium),
Chairman, Ethics Working Party
European Forum for Good Clinical Practice

The International Bioethics Committee of UNESCO has played an important role in promoting global discussion on ethical questions concerning scientific research, biomedical research, public health, and the environment. Since its inception, this Committee has been careful to give non-governmental organizations (NGOs) a seat at the table. This exemplary way of including all sectors in the discussion of bioethics has served to further the participation and the education of non-governmental organizations throughout the world with regard to the central importance of the ethics of science and health in our global village.

Non-governmental organizations have a valuable contribution to make to the public discussion on issues in bioethics. Similarly, they need to share in the responsibility of promoting the integrity and the ethics of the debate itself. NGOs are characterized by groups of people who have come together because they share an interest and a general concern. It is this shared interest and concern that gives them their identity and also their legitimacy. Often these organizations have the advantage of a certain 'freedom' or 'independence' with regard to governmental or intergovernmental organizations or institutions.

The knowledge and understanding they bring to the debate on bioethics is usually not structural or abstract. It is primarily rooted in experience and driven by practical concerns with an eye towards achieving concrete outcomes in the lives of others sharing similar concerns. Open and free public debate on issues in bioethics requires the organized contribution of those most affected by the concerns expressed in those issues. Three concrete examples can be provided here regarding the manner in which NGOs have contributed to the current global debate on bioethics.

The European Forum for Good Clinical Practice (EFGCP) established a place to meet for those concerned with the implementation of high scientific and ethical standards in biomedical research. It brings together members of academia, government, industry and patient organizations in a forum where their mutual interests and concerns in clinical trials can be discussed. The EFGCP has been successful in assisting in the dissemination of information on the science and ethics of biomedical research. It has also assisted national governments and the European Union in making decisions on legislation with regard to such areas as ethical review of research protocols, informed consent, and inspection of clinical trials.

The Cameroon Bioethics Society (CBS) has played an important role in Cameroon and Africa in promoting the discussion on bioethics within society. The CBS promotes debate on questions of bioethics as they affect the peoples of Africa. It was instrumental in drafting and advancing the 1996 Organization of African Unity Resolution on Bioethics. More recently, the CBS has been instrumental in advancing a framework for the establishment of ethical review committees in Africa and the continued education of their members in questions of science and bioethics.

Finally, in the area of AIDS we have learned today that addressing the concerns of those affected by the disease as well as developing appropriate research programmes for fighting the pandemic would be nearly impossible without the active contribution and support of NGOs. NGOs are not only essential for dealing with the needs and concerns of those suffering from this disease and their families - assisting in the care of AIDS patients, establishing support groups, contributing to the active response to the pandemic, locally and internationally - but NGOs have been further integral in contributing to our appreciation of the ethical issues surrounding this disease and the ways in which we can respond. I mention here only one example: the *Association marocaine de lutte contre le SIDA* (ALCS) led by Professor Himmich in Casablanca. The ALCS has made important contributions to understanding the ethical issues involved in research on populations at high risk for contracting or spreading AIDS.

These examples help to illustrate the valuable contribution NGOs around the world have made to public debate on bioethics. The Universal Declaration on the Human Genome and Human Rights (1997) refers to the key role States have in establishing appropriate measures for promoting public debate on the issues raised in the Declaration and, by extension, on all issues in bioethics that concern society. The International Bioethics Committee and the Intergovernmental Bioethics

Committee of UNESCO will have important roles to play in assisting governments in finding the appropriate means and ways to foster participation, information and education through public debate on issues in bioethics. NGOs will need to contribute to these debates and share in the responsibility for their success. Public debate on issues in bioethics will become an increasingly important part of our great human conversation.

INVOLVING YOUTH IN THE PUBLIC DEBATE ON BIOETHICS

Diane Gal (The Netherlands),
Chairperson of Information and Education 1999-2000,
International Pharmaceutical Students' Federation

I would like to thank UNESCO for this very important opportunity to share my views as a young person here today. My main goal will be to explain that young people should be involved in the public debate on bioethics and to give examples of how young people should become involved.

In an age when technology is seen as a means to the end of certain diseases and medical conditions, one is struck with pure awe at how such minute changes in one base-pair of our DNA, a small mutation, can drastically affect the outcome of our quality of life. One cannot help but applaud the success that scientists have had in helping to overcome certain diseases and medical conditions such as hemophilia, diabetes, hepatitis and infertility through the use of technology. Yet scientists and the public still face large controversies in view of these and other advances in technology.

I come here today as a recently graduated pharmacist and as a representative of the International Pharmaceutical Students' Federation. As you can already tell the focus of my presentation will lie on medical bioethics - as my bias/education is in the healthcare sciences.

I would like to suggest that there is a delicate balance between the human's need to seek out the truth and to hide from the fear of the unknown. In seeking the truth, one's morals and thoughts on how these truths will affect society in the future can become clouded by the pure scientific want to dissect something to it's most basic component, to analyze and understand it and to be able to apply that knowledge elsewhere. I might even venture to say that scientists are trying to conquer the challenge of finding the truth and gain an immense feeling of

achievement in the knowledge developed from that one small truth. The significance of these truths that are found might vary from making a minor alteration to a being's life to affecting the entire system and balance of life on this planet. It is my opinion that humans possess a strong fascination for and fear of the unknown and that we are more likely to fear the worst than to fear the best. The profound fear of the unknown, the lack of trust in the purpose of scientific exploration and experimentation and the lack of knowledge in the science that is being practiced, together cause people to fear the worst from new advances in technology. An old motto that I was once taught is 'Hope for the best, but prepare for the worst'. Our societies do hope for the best by contributing large sums of money and volunteers to scientific research to help cure diseases like cancer and AIDS, to allow for the development of science to be used with the best of intentions. How do we as a society prepare ourselves for the worst... we discuss how such minor changes in the knowledge gained through science will affect our societies and how to best avoid disastrous occurrences in the future. Now is the time for everyone to get involved in the public discussions on bioethics and on where our knowledge will take us in the future. I say discussions because, at this point, I feel that the public needs to become more informed about the subjects in technology that are at hand before well thought out debates can take place. The answers/debates may still be speculative of what will occur in the future but they must at least be founded on the truths in science that we know today and not based on the fantasies created by multimedia. As a society we can prepare ourselves by imposing structures that will lead to the use of scientific knowledge in appropriate ways.

The moral views attached to the subjects in which bioethics concerns itself range from one end of the spectrum to another. Are they founded on facts, religion, culture, politics? It is important that all views are expressed and pondered upon. So how in the sight of all of these views do we find a reasonable solution or common ground on which policies can be based? If every voice could be heard and tabulated into a computer, the outcome being the position of society as a whole balanced with the position of the individual and factored to the benefits versus the risks of the outcomes, an answer could be found ... a compromise made. The only problem is that most ethical viewpoints are not based on logic, or on mathematical equations, they are more emotive in nature and less factual. As I mentioned before, education is the first step towards finding a common ground on the issues involved in bioethics. Before opinions are formed, everyone, especially young people, must be educated of the

basics of the topic at hand in a factual/non-biased manner (if this is possible). Once all the facts are understood it is up to our societies to consider these facts within the realms of their moralistic views - whether they be religious, philosophical or political.

So why should young people become involved in these debates involving such complex issues ... the reasons are simple and obvious in my mind.

1) The decisions and policies that are made today can have an impact on the daily lives of young people today and in the future. Young people of today should be the best equipped with knowledge and insight to be able to effectively deal with the changes and challenges of tomorrow.

2) Young people are active in improving many aspects of their lives, including their education and bioethics is an educational topic that many students would like to discuss and learn more about.

3) Young people can also bring a different perspective to the debates. Young people can be more inclined to accept and look at the technological advances in a positive light. After all, technology is at our fingertips everyday and we have grown up with the rapid changes throughout the years. It is partly due to this acceptance of technology that young people can put forth new ideas and accept advances and changes readily in the future.

4) Most importantly, young people should be included in the public debate on bioethics because it is a great way for young people to learn from the experts or more informed people on these issues.

Now the challenge we face is getting young people involved. More young people must become involved because it is our future that we are discussing. Student organisations and NGO's should be considering the topic of bioethics and discussing it with their members and encouraging the education of their members, such as the discussions that have been taking place within IFMSA on the topic of medical ethics[1]. Groups of NGO's should be forming committees looking at the issues in bioethics, how they affect each group and how they affect the committee as a whole. An interdisciplinary committee will allow each group to see how a topic can affect the other groups leading to a wider understanding of the effects of the topic at hand.

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1. http://www.ifmsa.org/projects/frame.htm.

Education is the key. Everyone should be informed and educated on the different aspects involved in the topic at hand, including experts in the fields, professionals, spiritual leaders, teachers, young people. Through education, young people can be taught to think critically on these issues and review all aspects before making an informed decision and/or opinion on these matters.

It is curious to note that I feel there has been a lack of discussion on bioethical issues in my years of education and it is a feeling that is shared by many of my peers. As pharmacists and/or scientists these bioethical issues are issues that we will be or are facing in our professional lives - for example the administration of drugs that will cause abortions or for the purpose of euthanasia or the use of animals in experimentation. It is true that the basic ethical theories were covered in a few classes and some cases were studied and presented by groups of students - mostly concerning patient confidentiality ... but the issues which are discussed do not seem to encompass the wide range of bioethical topics that are facing society today. Students must be encouraged to evaluate their educational system and take the responsibility of initiating change. The topic of ethics in pharmacy education is touched upon in a book that has recently been published by IPSF and EPSA[2]. The book discusses the role that professionals and students have of improving higher education in pharmacy and with the topic of ethics in particular the need for formal training in ethical decision making to become part of our education systems. The AEGEE (*Association des états généraux des étudiants de l'Europe*) has also published a book on the future of higher education which contains statements concerning the need for proper education in research that is shaped and fed by the principles of scientific ethics[3]. Lastly, I would like to point out some of the research that was done by Dr Macer on bioethics in education. In the International Bioethics Survey, 90% of the people from all the countries surveyed supported that 'discussion of social issues associated with science and technology should be included in school, so that students can participate in contemporary debates'[4].

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2. International Pharmaceutical Students' Federation-European Pharmaceutical Students' Association. *Pharmacy Education: A Vision of the Future*. July 1999, p. 35.

3. AEGEE Europe. *The Future of Higher Education: A Student's Vision*. September 1998, p. 68-70.

4. Macer, D.R.J. *Bioethics for the People by the People*. Christchurch: Eubios Ethics Institute, 1994, pp. 242-248.

It is important to me, as a young person, to become involved in the discussions on bioethics because the topics discussed are relevant to every day life. I would encourage all young people to become more informed and to take part in shaping our future. I would like to leave you with a quote from Isaac Asimov that 'if knowledge can create problems, it is not through ignorance that we can solve them'.

I thank you for showing interest in a young person's point of view. I hope that this interest will continue to grow and I challenge all organisations and bioethical committees to involve young people in the public debates on bioethics.

L'ÉTHIQUE BIOMÉDICALE DANS LES PAYS EN DÉVELOPPEMENT : L'EXPÉRIENCE DU MAROC

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La situation de l'éthique médicale dans les pays en développement ne peut être tout à fait comparable à celle des pays industrialisés pour diverses raisons. D'abord, parce que la recherche de pointe dans le domaine des sciences de la vie, qui nécessite l'implication de l'éthique, comme la manipulation génétique, n'est pas encore à la portée du Tiers monde. Ensuite, parce que la situation politique et socio-économique exerce une influence : quand cette situation est préoccupante, elle ne peut favoriser un climat propice à l'examen prioritaire de questions et principes éthiques. Enfin, parce que la religion, la culture et les habitudes jouent un rôle suffisamment important pour donner des aspects particuliers ou spécifiques à l'éthique de chaque région ou de chaque pays. Cependant, on a tendance à dire que pour les pays en développement ayant un niveau économique comparable, c'est du niveau de l'éducation, de la démocratie et de la culture qui dépend le développement de la réflexion éthique.

La présente communication, qui porte sur la situation éthique au Maroc, tente de tenir compte de ces particularités.

Dans les pays en développement, les pratiques de la recherche médicale qui nécessitent l'implication de l'éthique concernent essentiellement les essais cliniques des médicaments et, dans une moindre mesure, les dons et greffes d'organes et l'insémination artificielle.

L'histoire de ces pratiques dans les pays industrialisés se répète au Maroc, car ces activités s'effectuent actuellement en l'absence de lois éthiques les régissant. Cette situation peut paraître inconcevable, mais avant de la juger, il faudrait peut-être tenter de l'analyser en la situant dans le temps et l'espace.

Une greffe de rein, une insémination artificielle, pratiquées dans un pays comme le nôtre, sont rares. De ce fait, elles constituent pour le moment des événements exceptionnels. Elles nécessitent, aux yeux de la population, une infrastructure lourde, sophistiquée et un savoir-faire remarquable. Aussi leur réalisation constitue-t-elle avant tout pour le pays une avancée, voire une réussite scientifique. Les médias locaux rapportent en grande pompe l'événement et vantent les mérites du ou des auteurs. Les sujets qui ont fait l'objet de ces recherches ne sont cités que comme sujets d'expérimentation ou comme témoins de la véracité et/ou de la réussite de l'événement. Ils sont aussi cités comme témoins pour louanger l'équipe qui a réalisé l'exploit. Ce qui est mis en lumière, c'est la nouveauté, l'originalité, le progrès réalisé et le fait que l'événement semble avoir réduit la dépendance du pays vis-à-vis de l'étranger.

A qui incombe la responsabilité de l'absence des questions éthiques que posent ces événements en ces moments « de gloire » ? Au pouvoir politique et administratif ? A l'ordre des médecins ? Aux différentes associations et académies ? Aux scientifiques chercheurs ? Aux philosophes ? Aux hommes de religion et à l'ensemble des intellectuels du pays ? A la fois à tout le monde et à personne.

A personne, parce que ces événements sont en nombre très limité. De ce fait, le pouvoir politico-administratif n'a pas encore saisi les implications éthiques que ces pratiques pourraient impliquer dans le futur. D'autre part, les préoccupations économiques (chômage important), sanitaires (mortalité infantile importante, couverture sociale faible) et éducatives (taux d'alphabétisation faible) sont très aiguës. Elles ne permettent pas aux décideurs de réaliser qu'il existe des sujets d'actualité mondiale, comme l'éthique, dont il faut se préoccuper sans attendre, sous peine de voir s'installer des pratiques peu recommandables.

L'absence de lois sur l'éthique est aussi imputable à tout le monde, car il s'agit là de l'application des instruments fondamentaux concernant les droits de l'homme, ainsi que des Déclarations d'Helsinki I et II concernant la pratique de la recherche médicale sur l'homme. Toutes les forces vives du pays (institutions politiques, services administratifs, structures associatives) ont le devoir de combler cette absence de loi-cadre sur l'éthique de la recherche biomédicale. L'application des principes énoncés dans ces instruments (qui revient au premier chef au pouvoir politico-administratif) refléterait l'intérêt concret que porte le pays à la protection de l'intégrité physique et de la dignité de ses citoyens. Il refléterait également une certaine évolution philosophique, culturelle et morale du pays.

L'application de ces principes au Maroc se justifie aussi par d'autres raisons. Notamment par le fait que l'industrie pharmaceutique propose la réalisation d'essais cliniques de plus en plus nombreux au fil des années. Et, aussi, par le fait que le développement scientifique de facultés et institutions à vocation de recherche et en progression constante, de plus en plus dans le domaine des sciences de la vie. D'autres faits importants justifient la nécessité d'une loi sur l'éthique au Maroc. D'abord, la Constitution stipule dans son préambule que le Royaume du Maroc adhère aux instruments fondamentaux concernant les droits de l'homme. Ensuite, il existe depuis plus de trois ans un comité consultatif national des droits de l'homme et, depuis un an, un ministère des droits de l'homme. Enfin, deux fondations de recherche médicale ont été créées au cours de l'année 1993, avec pour objectif principal d'encourager et de promouvoir la recherche médicale dans le pays, et un projet de création d'une académie des sciences est en cours.

On peut dire que l'histoire se répète en ce qui concerne l'éthique au Maroc. En effet, c'est sur l'initiative d'un groupe d'enseignants de la Faculté de médecine et de pharmacie de Casablanca et sous l'impulsion du service de pharmacologie clinique que le premier comité d'éthique pour la recherche biomédicale au Maroc a été constitué. Cette auto-constitution est un fait qui se retrouve dans l'histoire de l'éthique dans les pays industrialisés. Elle n'a reçu l'adhésion du décanat qu'en décembre 1989 au bout de pratiquement quatre ans de sensibilisation continue.

Le comité était conscient qu'il ne devait subir aucune forme de contrainte extérieure pour faire respecter les règles éthiques. De même, il était conscient que, pour être efficace, il ne devait ni constituer un obstacle à la recherche biomédicale, ni en retarder le démarrage.

Aucun budget n'était alloué au fonctionnement du comité et ses membres y travaillaient à titre bénévole. Une procédure de fonctionnement non contraignante a été mise en place. Chaque protocole soumis au comité était envoyé à trois de ses membres désignés par tirage au sort ; chacun d'entre eux devait donner son avis éthique sur une fiche préétablie, et ce, dans un délai de trois semaines. Si les trois avis ne soulevaient pas d'objection éthique, le Secrétaire général adressait à l'investigateur l'avis favorable du comité. Si, au contraire, il y avait au moins une objection, le comité se réunissait pour débattre du protocole et donner un avis. La nature de ces avis était de trois ordres : favorable, défavorable ou proposition de modifications. Le comité veillait, bien sûr, à ce que le protocole fournisse toutes les garanties éthiques et que toutes les mesures protégeant la sécurité des patients ou des sujets associés dans ces recherches soient prises.

Depuis sa création, il y a dix ans, le comité a traité 61 dossiers. La date et l'origine de la demande d'instruction sont présentées dans le tableau ci-dessous :

Années	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Nombre de dossiers	2	12	5	8	3	6	6	19	13	15	8
Origine	IP : 1 U : 1	IP : 8 U / 4	IP : 4 U : 1	IP : 7 U : 1	IP : 2 U : 1	IP : 4 U : 2	IP : 5 U : 1	IP : 18 U : 1	IP : 13 U : 0	IP : 14 U : 1	IP : 8 U : 0

Légende : IP : industrie pharmaceutique, U : université

Le nombre de 61 essais traités par le comité en dix ans paraît très limité. Mais ce chiffre ne reflète pas la totalité des essais effectués dans le pays. En effet, le passage par le comité d'éthique n'est pas obligatoire ; son existence n'est peut-être pas connue de toutes les institutions qui pourraient y avoir recours. Enfin, c'est un comité qui s'est constitué sur l'initiative d'un groupe de personnes : de ce fait, il n'a aucun statut administratif ou juridique.

L'évolution des demandes sur les dix années est intéressante. La demande a été importante dès la création du comité. Mais par la suite, une certaine réserve s'est manifestée. Peut être le comité a-t-il été jugé exigeant. En effet, parmi les suggestions du comité qui étaient le plus contestées figuraient l'exigence d'un consentement écrit et le refus des essais en « ouvert non comparatif ». Sur le premier point, le comité a évolué - bien qu'il fût conscient que le recueil du consentement écrit constitue un élément fondamental de l'éthique. Il a évolué en acceptant parfois des consentements oraux pour diverses raisons. D'abord, il existe une importante population analphabète, pour laquelle une signature ne signifie pas un engagement. Ensuite, dans le pays, les transactions d'affaires se font traditionnellement pour une bonne part par entente orale. Enfin, la qualité de l'individu se juge notamment par son respect de la parole donnée ; pour louer un individu, on dit souvent : « c'est un homme de parole ». Tous ces facteurs ont amené le comité à accepter des consentements oraux donnés devant deux témoins sans aucune relation avec le projet de recherche en question.

En ce qui concerne le deuxième point (les essais non comparatifs), il a été remarqué que certains laboratoires pharmaceutiques, lors du lancement d'un nouveau produit, mettaient en place des essais cliniques chez plusieurs prescripteurs en même temps. Pour faciliter cette mise en place et la réalisation de ces essais, leur méthodologie se présentait souvent « en ouvert non comparatif », bien que l'objectif stipulât « l'évaluation de l'efficacité et de la tolérance ». La méthodologie n'était évidemment pas adaptée à l'objectif. Par ailleurs, les résultats de tels essais ne peuvent faire avancer les connaissances sur la substance testée. De ce fait les investigations mises en œuvre pour l'évaluation du produit sont susceptibles de générer des risques non justifiés pour les patients testés. Ces arguments ont amené le comité à être intransigeant sur ce point.

Une autre remarque concerne les motivations de la saisine du comité. Elles diffèrent selon la nature du demandeur. L'universitaire cherche plutôt à se rassurer : il a besoin d'une certaine caution de ses pairs pour réaliser son expérimentation. Pour certains laboratoires pharmaceutiques, l'avis du comité est exigé par le règlement interne de la maison mère, dont le siège se trouve dans un pays où l'administration peut exiger un tel avis.

Dans le courant de l'année 1993, le comité d'éthique de Casablanca a organisé une journée de réflexion sur son fonctionnement et sur ses missions. Cette journée avait réuni des personnalités appartenant aux milieux médical, paramédical, juridique et religieux. Les débats furent extrêmement intéressants, courtois et sans passion. Au terme de cette réflexion, les recommandations suivantes ont été adoptées par les participants :

- il est nécessaire de rattacher le comité d'éthique de Casablanca à un cadre juridique ;
- le consentement des sujets associés aux projets de recherche doit être libre et éclairé, révocable à tout moment. Les modalités de demande du consentement doivent être écrites et présentées au comité d'éthique ;
- l'assurance doit être obligatoire pour les personnes incluses dans les essais et pour les investigateurs ;
- la saisine du comité par des entreprises de l'industrie pharmaceutique doit être payante ;
- la saisine du comité d'éthique ne peut émaner que de l'investigateur du projet de recherche et non du promoteur ;

- le *curriculum vitae* de l'investigateur et la description du lieu de la recherche doivent être mentionnés dans le dossier de demande présenté au comité ;
- le dossier de demande présenté au comité doit comporter, outre le protocole du projet de recherche, l'ensemble des données scientifiques concernant le produit expérimenté (médicaments ou matériels) ;
- le comité doit établir un programme d'information et de formation du corps médical au sujet des problèmes éthiques ;
- le comité aura la possibilité de demander l'avis d'un expert à chaque fois qu'il le jugera nécessaire, notamment concernant l'aspect scientifique ou l'aspect religieux du projet.

On envisage actuellement de créer un comité national d'éthique pour la recherche biomédicale. Cependant, cette idée a beaucoup tardé à voir le jour. Toute action visant à la concrétiser rapidement à l'aide de l'expérience d'autres pays doit donc être encouragée et soutenue.

Conclusions

Le Maroc n'a pas encore réservé à l'éthique de la recherche biomédicale la place qu'elle mérite.

Les raisons en sont multiples. D'une part, les services ou institutions qui devraient s'en occuper ont d'autres problèmes (notamment sanitaires ou éducatifs) plus préoccupants et plus urgents à résoudre. D'autre part, les responsables administratifs appartiennent en majorité à une génération qui n'est pas assez sensibilisée aux questions de l'éthique biomédicale pour les considérer comme une priorité. Enfin, la situation socio-économique et culturelle du pays ne favorise pas l'émergence de valeurs éthiques. Elle peut même s'y opposer - c'est ce que l'on constate dans le domaine des dons d'organes ou des essais cliniques des médicaments.

Aussi l'adoption et la diffusion de principes éthiques ne peuvent-elles se réaliser qu'avec l'aide de forces sociales et médicales internes qui, elles, génèrent et pratiquent ces principes éthiques de base, notamment grâce à leur accès aux connaissances des pays avancés dans ce domaine.

Au Maroc, il a fallu quatre ans pour que s'impose l'existence d'un comité d'éthique local au niveau de la Faculté de médecine et de pharmacie de Casablanca. Il fonctionne depuis neuf ans, mais il ne reçoit, dans la majorité des cas, que des protocoles qui exigent le passage par de tels comités. Des textes réglementant la recherche biomédicale et la mise en place d'un comité national d'éthique font encore défaut.

BIOETHICS AND THE PUBLIC DEBATE

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It is my pleasure to speak on bioethics and public debate, in Africa. It is significant that the Sixth Session of the International Bioethics Committee of UNESCO (IBC) is being held on the continent of Africa, which should be seen as a little step towards participation of people of Africa in the global bioethics debate. Actually, I came here to be educated, however I will share some of my thoughts on bioethics and the public.

I wrote my 1994 book, *Bioethics for the People by the People* (Macer, 1994), precisely because I saw the standing paradigm, that academics, philosophers and lawyers for example set the terms of bioethics as a contradiction to the message of bioethics. Bioethics is to empower people, both as individual or families, and the societies we construct to make better decisions.

I simply call bioethics as love of life (Macer, 1998), because I consider that this is the universally accepted message of our biological, social and spiritual heritage. However, to put love of life into practice we need to learn from each other, gather information and develop education.

Let me explain how we can approach bioethics. There are three ways to view bioethics:

1. 'descriptive bioethics' is the way people view life, their moral interactions and responsibilities with living organisms in their life. Everyone in this room has their own bioethics. I have mine and you, yours;
2. 'prescriptive bioethics' is to tell others what is good or bad, what principles are most important; or to say something/someone has rights, and others have duties to them. The Universal Declaration on the Human Genome and Human Rights is the most international attempt at prescriptive bioethics in our modern age;

3. 'interactive bioethics' is discussion and debate between people, groups within society and communities concerning paragraphs 1 and 2.

In all of these approaches, success for society can be lasting only if the view is from the people. In order to find the views we have, we cannot rely on assumptions of what people think. Rather, we should ask people what they value, and what principles they use to make decisions. There are several methods of descriptive bioethics. These include surveys, interviews and observations via field work. In surveys, the questions can be a fixed response between set options or open response questions. The studies of descriptive bioethics lead to better descriptions of what the public thinks. There is a range from quantitative to qualitative interpretation, but raw results depend on the interpretation of the questions by the respondents. Some key findings of the surveys conducted in different countries, and in particular the International Bioethics Survey (Macer, 1994) I coordinated in 1993, were:

1. the diversity of the responses to questions on bioethical dilemmas is wide within each group surveyed. For example, in each country a wide range of reasons was given to justify decisions;

2. the diversity within samples exceeds the differences between samples;

3. the types of response support the concept that the value sets people form cannot be predicted by simple demographic factors, such as gender, religion, education, etc. There may be some other psychological predictors, but there is no simple predictor of persons who tolerate risks, and those who do not.

My conclusion was that universal cross-cultural ethics should be developed to allow diverse views to be maintained within any single community, as well as throughout the world in the global community. It is not consistent with the data to use narrow nationalist views, for example, Japanese believe this; Moroccans believe that. Rather we see a diversity of views on questions of bioethics in all countries. Having recognized this diversity of responses, to issues like abortion of fetuses with different genetic diseases, genetic engineering of tomatoes, donation of organs for transplant, we need to devise education programs to allow people to exercise informed choices.

In order to develop these programs, we need to find a simple message. The first priority is to have a solid foundation of ethical principles. The four ethical principles that have been accepted in bioethics are: autonomy, justice, beneficence and do no harm. These are ideals so that their application has to be balanced when they clash.

I use an alternative vocabulary, based on my view that bioethics is love of life. Autonomy is self-love, too much is selfishness, but too little means we ignore the responsibility we have to ourselves to develop our potential. Justice derives from the love of others. Beneficence is loving good, which is an ethical ideal in all cultures. Do no harm stems from a love of life that we feel.

We may be able to supplement the values that we develop from these core principles, but they provide a basis for education. Education ranges from family, to school, to media. All these sources form our values and our very culture itself. There are several target groups for bioethics education that we have seen used.

People firstly consider schools. I have been developing a network of school teachers in Japan who discuss bioethics. The bioethics education network includes both biology and social studies teachers, who learn from each other, how to provide multidisciplinary bioethics education to students (Asada and Macer, 1998). The goals are usually one of two basic objectives:

1. to teach students to better respect life, or
2. to make students better decision makers.

Providing sound scientific and historical information are keystones of bioethics education. However, classes must be participatory, if students will fully benefit by applying the information to daily life. Bioethics can be part of Science, Technology, Society (STS) approach to education.

I target school teachers because I think university is too late. Only 30% or less go to the university. Of course I still teach bioethics to university students. Actually, for ten years I have taught bioethics to biology and science students, trying to make scientists think broadly. Basically, all third year undergraduates have a class once a week all year discussing science, society and ethics, and half chose an extra 30 hours optional courses specifically on bioethics. Postgraduates can optionally take bioethics as a subject, or even as full-time research as a M.Sc. or Ph.D. in bioethics, as a biology degree. It is gradually being accepted at a few other universities.

The media has a critical role, and we can see more programs now on television and articles in newspapers, discussing the different points of view people have. They also provide information from one group in society to another. Presenting different viewpoints is one way to enhance real participation of all in developing our society. While we always hope for better media, I can see improvements in the quality of information over the last two decades, and we have to ensure a free press on what are often controversial issues. The Internet is providing participation opportunities to more people than ever before (please visit the Eubios Ethics Institute to find more).

If we are really to see participation, both professionals and users need to be willing. Let us think about the communication, and the level of participants in relationships. Take the example of a visit to a doctor to seek treatment. Traditional societies use a paternalist doctor, telling a patient what to do. The Nuremberg Code and the Helsinki Declaration and modern bioethics tell us to consider doctor and patient on the same level. Also if the doctor prescribes a drug, the drug should be accompanied by information from the drug company. Ideally we can see a move to informed choice where the patient chooses what is best given their values and choices. We can take another example, the visit to a supermarket to buy food. Here there is a longer tradition of informed choice. If a retailer offers a product, it should be accompanied by information provided by the producer or food processor.

Actually in both cases the producers are represented by a middle person, the doctor or the shop. The quality of drugs and the food may be assessed by government regulatory agencies and the level of information provided to the consumer varies. There is still a lot to develop to have equal participation of all in bioethical decision making, but the role of education is central.

I hope more research will be conducted to ask what ordinary people think. I continue to use surveys and interviews to explore this. One emerging method is the use of focus groups, that are made up of representatives of society in general, and then given information to work on together. This year one such group I have been developing is called the 'Life and Bio Thinking Group'. We focussed on whether genetically modified (GM) food should be labeled. We found it possible to develop and agree on a statement, despite the fact the group included the most active proponents and opponents of GM food.

As a society, we need to develop together as partners, so that all can reach consensus on what we, as societies, should do when we face the difficult choices of life and how to use new science and technology. So, in conclusion I hope we will work together for a global society that involves all, in a frame of mind to learn from each other.

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INFORMATION ET DÉBAT PUBLIC

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Cela fait une quinzaine d'années que je suis journaliste scientifique. Une des premières choses qui m'a marquée en joignant ce petit club a été de découvrir que la recherche n'était ni un long fleuve tranquille, ni un îlot de sérénité. Le monde des chercheurs est traversé par des courants, des clans, des querelles d'écoles et des rivalités qu'on n'imagine pas quand on est extérieur à ce milieu. Je n'ai pas l'intention de dénigrer ce noble métier qui a rendu des services immenses à l'humanité. Je souhaite plutôt essayer de vous expliquer les problèmes d'éthique que peut rencontrer un journaliste scientifique qui travaille dans un quotidien économique.

La semaine dernière j'assistai à une réunion sur la recherche dans le domaine du Sida. Chacun sait que cette maladie est un véritable fléau mondial qui accable surtout les pays en développement. L'an prochain, près de 40 millions de personnes dans le monde pourraient être infectées, dont les trois quarts en Afrique sub-saharienne.

L'action du rétrovirus HIV est encore très mal comprise. Pour l'instant, seules les tri thérapies dont l'usage reste réservé aux pays riches permettent de soigner, mais pas encore de guérir les malades et aucun traitement préventif n'a démontré son efficacité. La mise au point d'un ou de plusieurs vaccins, qui serait une solution idéale du point de vue économique et sanitaire, s'avère extrêmement complexe et beaucoup plus difficile que prévue, malgré quelques déclarations retentissantes. Un des chercheurs que j'ai rencontrés n'hésitait pas à dire que le rétrovirus HIV possédait, je le cite, « une forme de génie pour déjouer les défenses immunitaires de l'organisme », ce qui donne à la fois le frisson et une idée du chemin qui reste à parcourir.

De très nombreuses équipes sont en compétition dans le monde pour trouver une parade à cette épidémie et les enjeux scientifiques et industriels sont évidemment très importants. Des lauriers sont en jeu pour les scientifiques et d'énormes jackpots potentiels sont à décrocher pour les industriels. Imaginez le retentissement médiatique mondial le jour où une équipe annoncera qu'elle a mis au point un vaccin contre le HIV et que la maladie est enfin vaincue. Cela lui vaudra peut être un prix Nobel et, en tout cas, une notoriété mondiale. Autant dire que la compétition est rude et sans pitié entre les laboratoires et les équipes, même si tous œuvrent pour l'accroissement des connaissances. Mais au cours de cette réunion sur l'état de la recherche, plusieurs scientifiques français se sont plaints d'un fait étonnant. Selon eux, la littérature scientifique mondiale est, je cite, « polluée par des communications approximatives ou insuffisamment vérifiées ».

En d'autres termes, les chercheurs du secteur public ou privé qui travaillent sur le Sida – ils sont des milliers dans le monde – sont parfois contraints d'aller un peu plus vite que la musique en annonçant des résultats de façon un peu hâtive, sans prendre toutes les précautions d'usage. Soit pour montrer à leurs autorités de tutelle ou leurs directions générales qu'ils sont efficaces et que les budgets qu'on leur alloue sont utilement dépensés, soit pour attirer l'attention d'industriels susceptibles de financer leurs travaux. Une fois encore, il ne m'appartient pas de porter un jugement sur ces pratiques. En général, les scientifiques ne sont d'ailleurs sensibles qu'au jugement de leurs pairs et ils ont sans doute raison. Mais ils doivent également rendre des comptes à la société sur le plan de l'éthique de leurs travaux et sur l'utilisation qu'ils font de l'argent public ou privé qu'on leur confie.

Mais un journaliste, même s'il connaît le fonctionnement de la communauté scientifique, a rarement les moyens, le temps ou les connaissances pour remettre en cause les déclarations d'un chercheur. Donc, quand l'un d'entre eux annonce qu'il a fait telle ou telle découverte, la presse va en général reprendre l'information, surtout quand il s'agit d'une première dans le domaine médical. Est-ce une faute contre l'éthique d'annoncer le succès d'un essai *in vitro*, alors que personne ne sait vraiment prévoir le passage de l'*in vitro* à l'*in vivo* ? Est-ce une faute contre l'éthique d'annoncer la découverte d'une nouvelle molécule qui produit un certain effet sur la souris quand on sait que la route est parfois très longue entre l'animal et l'homme ? Je ne sais pas bien répondre à ces questions et peut être les débats d'aujourd'hui m'aideront à trouver une solution. Une chose est sûre, la technique de « l'effet d'annonce »

assez courante dans le monde politique, touche désormais la science notamment dans le domaine de la santé. Donc les journalistes peuvent, tout comme le maïs ou le soja, être manipulés par les chercheurs.

Je voudrais à ce propos vous citer une mise en garde récente du biologiste français Axel Kahn que la plupart d'entre vous connaissent, car il intervient très souvent dans les médias. Dans un éditorial destiné au célèbre *New England Journal of Medecine*, il fait quelques remarques assez inquiétantes. Il estime notamment que, je cite, « les pressions exercées par les pouvoirs économiques, l'industrie pharmaceutique par exemple, se font de plus en plus grandes sur les chercheurs. Ces pressions extérieures touchent de plein fouet la recherche et son fonctionnement ». Axel Kahn redoute, je le cite, « que la fiabilité des résultats obtenus soit affectée, car c'est le système actuel de validation de tous les travaux qui est en danger. Comment pourrait-on accepter que des chercheurs publient des données qui ne soient pas exactes, honnêtes et validées. Il en va de l'avancée de la science et, pour ce qui a trait à la science médicale, de la santé publique », fin de citation. Le moins qu'on puisse dire c'est que cet avertissement est très sérieux, venant de la part d'un chercheur réputé qui est lui-même conseiller scientifique dans un grand groupe pharmaceutique.

Pour mieux comprendre ces risques, je voudrais vous rappeler quelques données sur le fonctionnement du système de financement des industriels des biotechnologies. Il y a actuellement aux Etats-Unis d'Amérique environ 1.500 entreprises qui travaillent dans les biotechnologies appliquées à la santé. A peu près 300 d'entre elles sont cotées sur les marchés financiers comme le fameux *Nasdaq* new-yorkais qui est un instrument électronique spécialisé dans ce qu'on appelle les valeurs de croissance, qui par parenthèse ne concernent pas seulement les biotechnologies.

L'Europe est partie avec du retard dans cette compétition, mais le vieux continent s'est aussi lancé dans cette course aux médicaments de l'an 2000 qui, pour la plupart, auront une forte composante génétique. C'est à dire qu'ils prennent en compte les causes génétiques des maladies. Ils soigneront donc, en théorie, les maladies à la source, en corrigeant les défauts qui se cachent dans l'ADN de nos cellules.

Il y a dans le monde industrialisé un peu plus de 2.000 *start-ups* de ce type. Mais, force est de constater que pour l'instant, elles ont surtout produit une montagne de dettes et à peine une dizaine d'entre elles gagnent actuellement de l'argent. Les autres vivent des sommes considérables que des investisseurs publics ou privés ont injectées dans

ce secteur. Non pas tellement dans le but de faire progresser la science, mais plutôt dans l'espoir de faire une bonne affaire financière. Quand ça marche, la mise initiale peut effectivement être multipliée par 50 ou 100.

Plusieurs dizaines de milliards de dollars ont été investies dans ce secteur depuis le début des années 90, essentiellement par des investisseurs américains dont certains ont pris des risques énormes. Mais tout récemment, le patron de *Genzyme*, un des leaders mondiaux, m'indiquait que le secteur des biotechnologies risquait d'avoir de gros problèmes de financement, compte tenu de deux difficultés majeures, qui sont le retard dans la commercialisation des produits, d'une part, et de la quantité d'inconnues scientifiques qui restaient à élucider, d'autre part. Ce dirigeant demandait d'ailleurs ouvertement l'aide des laboratoires de recherche publics américains pour résoudre certaines impasses scientifiques.

Ces problèmes sont confirmés par une récente analyse de la banque d'affaire américaine *Lehman Brothers*, qui indique que les premiers médicaments issus de la génomique ne seront pas disponibles au mieux avant 2003. Du coup, nombre d'investisseurs sont tentés de laisser tomber les biotechnologies au profit des technologies de l'information. Ce délai est la conséquence d'un phénomène que vous connaissez : la course d'obstacle que représente la mise au point d'un nouveau médicament. Une œuvre de longue haleine qui peut prendre une dizaine d'années entre la première idée et la mise sur le marché d'une nouvelle molécule. Cette course est ponctuée par une série de formalités obligatoires que sont les essais cliniques qui laissent sur le carreau nombre de postulants. Dans ces conditions, le taux de réussite est très faible et moins d'une molécule sur mille réussit ce parcours du combattant. Résultat, le coût de développement d'un nouveau médicament est en moyenne de 500 millions de dollars. Il faut donc avoir soit une trésorerie très solide, soit des actionnaires très patients ou philanthropes pour résister à ce marathon.

Quand on fait le bilan de ces sympathiques *start-ups* souvent dirigées par des chercheurs entreprenants et inventifs, on se rend compte qu'elles restent pour l'instant de formidables machines à dépenser de l'argent. Les analystes financiers utilisent d'ailleurs un terme très imagé pour décrire ce phénomène : le « *burn rate* ». Cet anglicisme mesure les quantités d'argent qu'une de ces sociétés dépense ou brûle tous les mois avant même d'avoir réalisé un centime de chiffre d'affaires. Typiquement, une entreprise de ce type peut ainsi consommer 8 à 10 millions de dollars par an, uniquement pour payer ses chercheurs, ses essais et ses

laboratoires, alors que son chiffre d'affaires résultant de la vente de produits peut être nul ou très faible. Si pour une raison ou pour une autre, comme par exemple un essai clinique raté, les investisseurs ont des doutes sur l'avenir de l'entreprise, le cours de l'action peut littéralement s'effondrer en quelques jours avec des conséquences qui peuvent être fatales pour l'entreprise.

Ces firmes très endettées sont donc contraintes de maintenir le suspense et l'intérêt des investisseurs, grâce à des annonces savamment distillées. Régulièrement elles annoncent donc des accords avec des grands groupes pharmaceutiques ou des découvertes dont certaines sont parfois contestables. Il s'agit souvent de la découverte d'un gène impliqué dans telle ou telle maladie avec à la clé un fabuleux marché de plusieurs milliards de dollars. Il y a deux ans, une firme britannique a été ainsi prise la main dans le sac, si j'ose dire, pour avoir arrangé certains résultats de recherche. Cette affaire a fait la une de tous les journaux britanniques. Outre que la valeur de la firme s'est écroulée, toutes les entreprises de biotechnologies européennes en ont subit le contrecoup, ce qui montre à quel point ce secteur est fragile et sensible aux mauvaises nouvelles.

Dans le même ordre d'idée, j'ai rencontré récemment le président d'une entreprise sur le point de s'introduire sur un marché financier, qui me disait assez naïvement qu'il aimerait qu'on parle de lui dans la presse économique pour « être visible » par les marchés financiers. On touche là à une des difficultés croissantes dans notre métier qui est l'amalgame de plus en plus fréquent entre communication et information. Dans certains cas les entreprises ont intérêt à faire parler d'elles dans le cadre d'une stratégie de communication financière et les journalistes ne sont pas toujours à même de faire la différence.

Je voudrais terminer en vous livrant quelques réflexions inspirées par la lecture d'un ouvrage récent du biologiste français Henri Atlan. La plupart d'entre vous ont entendu parler de ce biologiste connu pour ses travaux scientifiques, mais aussi parce qu'il est membre du Comité consultatif national d'éthique pour les sciences de la vie et de la santé de la France. Le titre de ce livre est en soi tout un programme, il s'appelle *La fin du tout génétique* et je voudrais reprendre quelques jugements qu'il contient.

Selon toutes probabilités la première étape du séquençage du génome humain sera terminée beaucoup plus tôt que prévu et certains experts annoncent la grande nouvelle pour mars de l'an 2000. Il ne s'agira que d'un « *working draft* », c'est-à-dire d'une espèce de brouillon qui devra ensuite être décodée base par base, et annoté comme disent

les spécialistes tout au moins pour la partie utile de l'ADN qui code pour les protéines. Cette nouvelle sera probablement un événement mondial qui va ouvrir une série de polémiques sur l'accès à l'information génétique et son utilisation. Un sujet au programme de vos travaux samedi matin. Cette information sera-t-elle suffisante pour percer les secrets de l'être humain ? Sans doute que non et plus personne ne croit désormais à ce déterminisme génétique tel qu'il était autrefois présenté.

Pour la plupart des experts l'ADN n'est pas le programme génétique à lui seul mais contient plutôt les données qui font partie du programme. « L'idée selon laquelle la totalité ou l'essentiel du développement et du fonctionnement des organismes vivants est déterminé par un programme génétique tend petit à petit à être remplacée par un modèle plus complexe, qui repose sur des notions d'interactions, d'effets réciproques entre la génétique, dont il ne s'agit pas de nier le rôle central, et l'épigénétique, dont on découvre progressivement l'importance », indique Henri Atlan.

Autrement dit, les gènes ne sont sûrement pas tout et les interactions avec les autres gènes, les protéines et d'autres composants de la cellule rendent le système autrement plus compliqué que ce qu'on attendait. Ce qui compte le plus n'est donc pas le gène mais ce qu'on appelle l'expression du gène qui peut varier considérablement d'un individu à l'autre, d'un tissu à l'autre, voire d'un moment à l'autre. Le système fonctionne donc avec une série de boucles de rétroactions successives d'une extrême complication. « Ce que l'on sait faire de plus facilement aujourd'hui c'est repérer ce qu'on appelle des déterminismes génétiques. Il ne se passe pas de semaine qu'on identifie de nouveaux gènes donnés pour responsables de l'apparition de caractères normaux ou pathologiques. Il y aurait évidemment beaucoup à dire sur le manque de rigueur qui entache trop souvent la manière dont cette identification est établie, en général à partir de corrélations statistiques approximatives sans qu'aucune relation causale ne soit démontrée. Mais c'est beaucoup plus facile à faire que d'analyser les déterminismes épigénétiques qui font appel nécessairement à une multiplicité de facteurs causaux intriqués les uns dans les autres », indique Henri Atlan.

Ce biologiste philosophe s'en prend également à toutes les dérives sémantiques attribuées au génome qui posséderait des propriétés mystérieuses voire magiques. Je cite : « Derrière la métaphore du programme apparaît alors l'essence de la vie, et celle ci est bien vite transformée en sanctuaire et en patrimoine. Le génome devient alors un fétiche source d'autant de peurs que de fascinations. Et comme tout

fétiche, celui ci se présente déjà comme une source de profits non négligeables à exploiter en jouant habilement de cette peur et de cette fascination. Autrement dit et comme toujours autour de tout fétiche, les marchands du temple ne sont pas loin ».

Je suis tout à fait conscient que ce discours pourra paraître iconoclaste. Mais, au risque de choquer certains d'entre vous je voudrais terminer par une dernière citation d'Henri Atlan quand il regrette que la divinisation du génétique soit devenue un paradigme dominant. Je le cite de nouveau, « puisqu'on nous a répété que tout est génétique, que tout est dans le programme, qu'il suffit d'avoir le listing du dit programme pour avoir tout compris, etc. etc.. Ou bien c'est l'inverse et le génétique est diabolisé, parce qu'un certain nombre de gens prennent peur et pensent que si tout est dans le programme génétique alors il ne faut surtout pas y toucher. Sans parler de la notion de patrimoine génétique qui est monstrueuse d'ambivalence et conduit à crisper les esprits sur des attitudes antagonistes. Car s'il s'agit d'un patrimoine, ou bien, il est sacré et on n'a pas le droit d'y toucher ou bien au contraire il faut le faire fructifier. L'utiliser au maximum pour améliorer l'espèce humaine. Toutes les dérives auxquelles on assiste dans un sens ou dans l'autre, divinisation ou diabolisation, procèdent finalement de cette mauvaise théorisation et non de l'outil génétique qu'il faut évidemment développer ». Voilà, Mesdames et Messieurs, quelques thèmes de réflexion que je vous propose pour démarrer cette sixième session du Comité international de bioéthique.

Chapitre / Chapter 2

STATE-OF-THE-ART ON RESEARCH ON CLONING OF WHOLE ORGANISMS

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The purpose of these state-of-the-art presentations is to bring to the attention of the International Bioethics Committee of UNESCO (IBC) the progress and recent developments in fields of research that are particularly problematic in term of their ethical implications. This is clearly the case for the field of reproductive cloning and the ongoing research on mammalian cloning. This presentation will focus on the results of animal cloning publicized during the last two years, on research avenues that may have therapeutical applications in human reproductive medicine, and on the subject of non-reproductive cloning for the purpose of obtaining transplantable tissues. It is primarily intended as a presentation of scientific observations.

Update on Mammalian Reproductive Cloning

The technology of nuclear transfer into enucleated oocytes (cytoplasts) was already successfully used in 1987 for reproduction of live bovine and murine offsprings (1, 2). However, embryo development could then be obtained only when the transplanted nucleus originated from blastomeres, i.e. cells from the first divisions of the preimplantation embryo development. These cells are still capable of totipotent differentiation, whereas cells from later stages have already differentiated functions and would have to undergo reprogramming (or erasing of chromatin changes linked to differentiation) in order to direct embryo development. That such reprogramming was possible in the oocyte cytoplasmic milieu became clear after the demonstration in 1996 that fertilization by quiescent nuclei of differentiated cells such as embryo fibroblasts could produce live cloned sheeps (3). But it is the birth in 1997 of the first sheep cloned from an adult mammary gland cell (4) that conveyed the full impact of the potential of reproductive cloning since now it could be done at any time during the life of an individual being.

The major drive for research on reproductive mammalian cloning remains to obtain and perpetuate animals with identical nuclear genetic characteristics offering some economic advantage, such as production of milk, meat or expression of a gene yielding a therapeutic protein. It is essential to study the phenotypic homogeneity of the cloned animals which are not identical due, among others, to environmental factors during pregnancy and life (for example the milk production can vary by 30% in cloned cows). Controlling the phenotypic variability factors and insuring mitochondrial genetic identity by using oocytes from cloned animals may improve phenotypic homogeneity in herds and facilitate registration by health authorities. Another aim of the research is to increase the still low rate of success, which remains at around 2% live offsprings per oocyte reconstituted after nuclear transfer. Many reconstructed oocytes do not achieve preimplantation development but the main problem is in the very low number that develop to full-term after implantation in a foster mother. There are chromosomal abnormalities (aneuploidy or polyploidy) and a large number of death and abnormalities (oversize) are seen during fetal and neonatal development. Studies reported since 1997, while confirming that reproductive cloning is feasible, have mainly addressed the question of which type and state of donor cells may give better results.

Cultures of embryonic and fetal cells would offer a consistent means for reproductive cloning of animals with economically valuable genetic characteristics. Cibelli et al. (5) showed in bovine that non-quiescent fetal fibroblasts can be used for nuclear transfer. The cells were transfected by marker genes (β-galactosidase and neomycin resistance) to demonstrate the feasibility of cloning transgenic animals. Blastocysts developed from transferred enucleated oocytes at a rate of 12% and after implantation 14% of these developed to term. Three calves remained alive and one died after birth of abnormal heart and vessels. A fetal somatic cell line was also employed by Baguisi et al. (6) to generate three transgenic female goats that express human antithrombin III in their milk, providing genetically identical animals in which expression of a therapeutic transgene can be compared. Cytoplasts from telophase oocytes gave an overall efficiency of about 2% offspring per nuclear transfer, higher than metaphase oocytes (0.7%). Recently, murine embryonic stem (ES) cell lines from long-term established cultures were used for nuclear transfer cloning of 26 mice, with an overall efficiency of 2.5% (7), providing another approach to clone large number of animals from a single cell source over an extended period of time.

The feasibility of using cells from adult mammals for reproductive cloning has also been confirmed, although with significant limitations. The sheep Dolly had been obtained with a quiescent G_0 cell from a serum-deprived culture of mammary cells, introduced by electrofusion in an enucleated oocyte, the efficiency being around 0.3% (4). Wakayama et al. (8) tested cells that are naturally quiescent and found that cells of the cumulus surrounding oocytes were adequate for reproductive cloning of mice. The cumulus cells were taken from the oviducts of female mice stimulated to hyper-ovulate, and the nuclei were microinjected into enucleated oocytes which were then subjected to a chemical activation step (instead of electrical activation). A high percentage of implanted embryos (50-70%) were obtained but only 5-16% developed into fetuses and full-term development was only around 2.5% of the implanted embryos. About 30 survivors, apparently healthy, were obtained and cumulus cells were again taken from some of the clones to produce 4 generations and a total of 80 cloned mice. Attempts were made also to employ Sertoli testicular cells and brain cells as a source of nuclei, but efficiency was lower (1 fetus/159 oocytes for Sertoli cells). An interest of this study, involving a relatively large number of cloned animals, is that it points out the high loss (95%) of cloned fetuses that die *in utero* possibly due to failure of genomic reprogramming or imprinting, or due to somatic mutations and other reasons (9). Having to use cumulus cells is also not the same as being able to clone from any cell in the body (10). In bovine, cumulus and oviduct cells from serum-deprived cultures were also successfully used (through electrofusion nuclear transfer) to give birth to 8 calves cloned from a single adult cow (11). The efficiency was remarkably high with 49% of the cumulus cells giving blastocysts (versus 23% for oviduct cells) and 8 of the 10 implanted blastocysts developed to full-term. Although larger series would be needed, monitoring the quality of the blastocyst may increase the efficiency. Four of the 8 calves died soon after birth, but environmental factors appeared to account for the deaths rather than abnormalities.

The long-term fate of cloned animals is still being carefully monitored. It was shown that Dolly as well as mice cloned from adult cumulus cells were able to reproduce naturally. But fear that reprogrammation of aged genomes may not be complete after transfer to enucleated oocytes, and that the cloned animals may be born with 'aged' genes (and aged bodies), was rekindled by the observation of telomeres shortening in Dolly's chromosomes (12). This report aroused much interest but may be just saying that Dolly indeed developed from an adult

cell genome. Long telomeres reduce cell senescence and maybe genomic instability. Senescence was reversed by nuclear transfer to bovine embryos (5). What Dolly's telomeres shortening means in term of her aging is still unclear: mice lacking telomerase gene, therefore having short telomeres, lived and reproduced and only at the third generation there were defects in wound repair and premature hair greying (13). A cloned animal reproducing normally would not transfer the short telomeres, and itself would probably not show aging problems.

Reproductive cloning for the production of domestic livestock with valuable genetic or transgenic characteristics, has definite attractive applications (14, 15). However, improvement in the technology of nuclear transfer, whether by electrofusion or by isolated nuclear injection (16), are clearly needed. Moreover, the low efficiency of full-term fetal development and the multiple genetic and anatomic abnormalities found in newborn cloned bovine, caprine and murine animals show that much has to be learned about the reprogramming process that the genome undergoes in the oocyte cytoplasm (17). Research should, therefore, be allowed to continue to resolve these tremendous biological, genetic and developmental questions.

From all this, it is abundantly clear that reproductive cloning is not a technology that would be anywhere near ready for applications in human medicine (but see below discussion of therapeutic cloning for human transplants). The major problems that emerged from research on animal cloning (very low efficiency, chromosomal and anatomic abnormalities) would first have to be resolved to even envisage therapeutic applications for human reproduction. Moreover, until now, the only primates born by reproductive cloning were obtained through nuclear transfer from blastomeres of early preimplantation embryos (18). Whether after these two cloned rhesus monkeys it will be possible to develop safe and efficient non-human primate cloning from adult cells remains to be seen (19).

Human Implications of Cloning Research

Such a look into the state of research to develop reproductive cloning of farm and laboratory animals for specific economic and scientific purposes should have at least the merit to defuse fears of immediate applications to humans. Unfortunately, the large press and a few individuals have, since the cloning of Dolly, exaggeratedly fantasized about cloning humans, giving the public the impression that cloning may become an alternative to sexual reproduction and endanger human

diversity. Understandably, this has driven governments and international organizations to issue bans on any use of reproductive cloning in humans. For example, the Universal Declaration on the Human Genome and Human Rights (drafted by the International Bioethics Committee and adopted by the General Conference of UNESCO in 1997 and later endorsed by the United Nations General Assembly) says, in Article 11: 'Practices which are contrary to human dignity, such as reproductive cloning of human beings, shall not be permitted'.

It is not the purpose of this state-of-the-art examination of the field of mammalian cloning research at the end of 1999 to enter into the ethical questions in any detail, but it would be incomplete without mentioning that cloning research still interests clinicians involved in reproductive medicine. At a recent joint meeting of the American Society for Reproductive Medicine and the Canadian Fertility and Andrology Society, held in Toronto on 25-30 September 1999, three presentations were made on cloning technology. The President's guest lecture was on 'Human Therapeutic Cloning: Opportunities and Challenges', there was a seminar on 'Cloning: Where do We Go from Here' and there was an ethical debate on cloning. George Annas argued that cloning should be prohibited by law, and John A. Robertson presented what could be the therapeutic applications assuming the method was safe. The strictly medical applications for which fertilization by nuclear transfer could be of interest are for infertile couples, with azoospermia or without oocytes. In the present assisted reproduction protocols such couples need extramarital gamete donation, usually from anonymous donors, which is not always accepted. Fertilization by the nucleus of a cell taken from one of the spouse could, if safe, become a new modality of *in vitro* fertilization (IVF). As in all IVF, a mother would give the baby birth. Genetically, the child would be a twin to the cell donor. This is viewed by some as unbearable determination but arguments have also been presented against genetic determinism and that having the same nuclear genome does not determine the identity of human beings on which education, environment and life conditions play such an important role (20, 21). Monozygotic twins are not identical, having only 50% correlation in cognitive and personality traits, and this should even be lower since the child will not be from the same womb and separated of his twin parent by many years. In this view, whether a strictly therapeutical application, with informed and free consent, be against human dignity will not be easy to decide. It seems important that the untrue illusion of making copies of humans, living or dead, be entirely discarded, but without condemning upfront all medical

applications of fertilization by nuclear transfer. Science only opens possibilities, bioethics should set guidelines ensuring respect of individual human dignity and precluding misuses of potential medical technologies.

Fertilization *in vitro* by diploid nuclear transfer may also have medical applications for families carrying lethal autosomal recessive diseases or genetic malformations. Healthy progeny could thereby be insured, which may one day be preferred to embryo selection. Mitochondrial DNA diseases may be avoided by use of enucleated oocytes from healthy donors. Nuclear transfer was reportedly investigated as a mean of achieving fertilization in aging women, by introducing the pronucleus of the infertile oocyte into the cytoplasm of a young oocyte, followed by IVF with sperm (22). The young oocyte may be mainly contributing mitochondria and direct injections of young mitochondria into aged oocytes could be envisaged.

An alternative way to produce oocytes is investigated by T. Takeuchi from Cornell Medical Center in New York, in which nuclei are transferred into immature oocytes which would then cause the haploidization of the diploid genome generating a gamete that could be fertilized by sperm. This would not be cloning, but shows the benefits that cloning research may lead to.

Also in September 1999, the first human ovarian transplantation was reported opening the way to freeze ovaries of patients before chemotherapy or irradiation, to later recover laboratory-grown oocytes for IVF. These examples of research on human reproduction, more than sporadic often unconfirmed rumors of nuclear transfer in infertile women, stress both the research activity in alternative IVF and the need for open ethical monitoring and debate. IVF, including intracytoplasmic sperm injection, has shown that instrumentalization of human reproduction is not incompatible with therapeutic medical intervention for treating infertility with respect of human autonomy and dignity.

Legislation in many countries prohibits the use of diploid nuclear transfer technologies for human reproduction. Other countries have preferred to have time-limited bans or moratorium. Since, as clear from this review, the reproductive cloning technology is nowhere near a state where its use in human medicine can be envisaged due to lack of safety and efficiency (hundreds of human oocyte would have to be wasted to have a chance of pregnancy), the difference between bans and moratorium is unimportant. However, a moratorium sends a message that medical and individual applications of new technologies are not condemned *a priori*. Research in this field is not condemned and the right

to benefit from scientific advancement is theoretically preserved. An example of such 'balanced' legislation was passed by the Israeli Parliament in December 1998. The law has two parts:

1) during 5 years there will be no intervention for the purpose of: a) cloning humans, and b) making humans with reproductive cells that have undergone germ-line gene modification;

2) an advisory commission will follow the progress of medicine in the field of reproductive genetics in human beings, and will submit a yearly report to the Health Minister. It will advise on whether the moratorium should remain in effect and on conditions that may allow a type of presently prohibited intervention.

Human Therapeutic Cloning for Transplantable Tissues

Therapeutic cloning is an avenue of research which is distinct from reproductive cloning, as it does not imply giving birth to a child conceived by diploid nuclear transfer. On this ground, it is generally considered not to fall under the definition of cloning humans and its prohibition. However, the ethical questions are no less momentous since therapeutic cloning would imply making use of preimplantation human embryos for deriving cells and tissues that would be valuable for replacing defective tissues in patients. The concept has been recently discussed (23, 24) and is based on the properties of embryonic stem (ES) cell lines that can be obtained by *in vitro* culturing of blastomeric inner cell mass (ICM) cells from IVF embryos at the blastocyst stage. Such ES cells can be propagated in undifferentiated state while retaining their pluripotent capacity to differentiate into various tissue cells including neural and glial cells, cardiac and skeletal muscle, vascular endothelium, blood cell precursors and even precursors of pancreatic islet β-cells (25, 26).

Several reports have indicated that murine ES cells can be a source of cells for transplantation and repair of pathologic damage to tissues and organs. Klug et al. (27) succeeded in obtaining almost pure cardiomyocytes, selected with marker genes from undifferentiated ES cells, and these formed stable intracardiac grafts when injected into the hearts of adult mice suffering of a cardiac dystrophy. The aim of repairing regions of the heart muscle damaged by myocardial infarction is still far away but there are ideas of building myocardial 'patches' on three-dimensional scaffolds with such cells (24). Populations of 37% pure oligodendrocytes and astrocytes were obtained from murine ES cell

cultures induced with various growth factors, which after transplantation to myelin-deficient rat by injection in the spinal cord and cerebral ventricules showed evidence of nerve remyelination (28). ES cells were also used for neuronal transplants (29), including production in the transplanted brain of dopaminergic neurons (30). This may be useful for Parkinson's disease and in diseased rats the use of dopamine cells from cloned bovine fetuses (42-50 days) improved motor performance (31). Recently murine ES cells differentiating only as aggregated embryoid bodies were transplanted in the spinal cord of rats 9 days after a traumatic contusion and shown to promote some recovery, the cells contributing both glial cells and neurons (32). Reconstitution of the hematopoietic system of irradiated mice by ES cells differentiated 4 days was also attempted (33). In all these ES cell transplantations, immune rejection was alleviated by cyclosporin treatments.

It is envisaged that human stem cells could one day be similarly used for transplantation. Thomson et al. (34) have applied the methods developed for animal stem cells to derive a number of human ES cells from human blastocysts. Individuals undergoing IVF for clinical purposes donated, with informed consent, frozen and fresh cleavage-stage embryos that they no longer intended to use for reproduction. These embryos were cultured to blastocysts, which is routinely done as part of IVF (because blastocysts have a better implantation rate). The inner cell mass, isolated by opening the embryos with antiserum lysis, were cultured on irradiated mouse embryonic fibroblasts as a feeder layer for the ES cells. The pluripotent capacity of these human ES cells was demonstrated by injection to immunodeficient mice, to form teratomas in which various differentiated cell types could be demonstrated. Other groups (35) have similarly reported blastocyst-derived human ES cells, but an alternative source of pluripotent stem cells may be germ-line cells (EG) isolated from gonadal ridges of human fetuses aborted at 1-2 months (36). These EG cells form, like ES cells, embryoid bodies in which various types of differentiated tissue cells can be demonstrated to develop.

For human transplantation, it would be preferable to prevent rejection of the grafts by using immunologically compatible cells. This could be achieved (37) by generating large panels of different ES cells and using for each patient in need of transplant those ES cells that match his own haplotype. Another way could be to engineer ES cells without histocompatibility antigens, but this may cause tumorigenicity. The concept of therapeutic cloning proposes another approach which would create ES cells genetically identical to the patient genotype by producing

embryos through nuclear transfer from one of the patient's cells into an enucleated oocyte. These cloned embryos would serve as source of ES cells for producing transplants. This approach would also prevent need of donation of embryos produced for IVF, but has its own ethical problems (see below).

Experimental evidence indicates that reprogramming a cell from an adult organism (as a patient in need of transplant) and restoring its pluripotent ES-like potential could be possible. Thus, bovine fetal fibroblasts (made from 55 day fetuses and marked by a transgene) were fused with enucleated oocytes and developed to blastocysts (7.5 day stage) from which ES cell lines were established (38). The pluripotent character of the nuclear-transfer derived ES cells was demonstrated by formation of ectoderm, mesoderm and endoderm upon injection into other blastocysts, some of which were subsequently grown to chimeric calves. With bovine embryos, the efficiency of the nuclear transfer for ES cell production could be 37% (24), but it is realized that this may be much harder in primates. Up to now only transfer of totipotent blastomeres was reported to have produced live rhesus monkeys (18). For therapeutic cloning to be practical in man, adult cells should be used for nuclear transfer. Lanza et al. (24) mentioned that in 1996 they fused human lymphocytes and oral mucosal epithelial cells with enucleated bovine oocytes from which 1/56 developed into near blastocysts from which ES-like cells started to grow on feeder layer fibroblasts. A recent study (39) shows that bovine oocyte cytoplasts may allow nuclei from skin fibroblasts of different species, including monkeys, to develop to near-blastocyst embryos. Interspecies embryos raise ethical problems, but allowing limited studies on their use for ES cell derivation will contribute to understand and resolve the difficulties in developing therapeutic cloning for transplants in humans.

Ethical Issues in Use of Human Stem Cells for Transplantation

The shortage of organs and tissues for transplantation is a major problem in medicine. It is not clear yet how far the ES cell for transplant approach can be pushed, in particular whether reconstruction of tri-dimensional organs could be feasible. But even cells and tissue transplants would be of importance as already illustrated with dopaminergic neurons (for Parkinson's disease), myelinating glial cells (for demyelinating diseases including Multiple Sclerosis), hepatocytes that could replace liver organ transplants (see also Table 1 in ref. 24). A source of immunocompatible hematopietic stem cells as replacement for

the very common bone marrow or cord blood cell transplantation would have a profound medical impact. Hence, research on ES cell for transplants is ethically justified as it will save lives. There are different possible methods to obtain human ES cells, and each has its own ethical quandaries.

Deriving ES lines from human blastocysts raises the very difficult ethical dilemma of using human embryos for this end. Some see this issue no less problematic than the ethics of selective abortion, and often its politics (40). One has nevertheless to realize that the clinical practice of IVF creates multiple embryos that are not all used for implantation, and there are many surplus or spare embryos in fresh or frozen state. All embryos must be produced for procreative purposes but for some this purpose will no longer be fulfilled because pregnancy has already been established and the parents no longer have parental plan for the spare preimplantation embryos. Some accept that 'the moral standing of frozen embryos derives more from its role in the context of human reproduction than in its physical properties' so that the informed 'choice of donating spare embryos for important medical research that cannot be done by other means is ethically superior to either destroying them or keeping them perpetually cryopreserved' (40). But, if one does not accept that premise, there may be other possibilities. Many studies aiming at improving IVF efficacy show that many embryos do not implant in the mother's uterine endometrium and that it is possible to recognize these defective embryos in a week-long *in vitro* culture during which blastocysts form. A phenomenon called fragmentation occurs in these embryos, due to death of some of their cells, but it is possible that the remaining living cells could be placed on feeder layers for derivation of ES cells. Such embryos being anyhow rejected by the IVF physician, they are not even potentially human beings, minimizing the ethical problem. Another way would be if ES cells could be derived from biopsies of embryos, taking a few cells in analogy to what is done for preimplantation diagnosis or now proposed as a monitoring method for embryo development potential (41). Some argue that preimplantation embryos are not 'individuals' until the primitive streak forms, since prior to that time twinning can occur (23). Clearly ethical positions of use of human embryos will vary according to cultural and religious perception of the status of preimplantation embryos.

Therapeutic cloning as a means to obtaining human ES cells requires human oocytes, which may be monitored as are at present gamete donations (assuming the rate of success is good enough not to require unreasonable numbers of oocytes). As implantation of embryos

produced by nuclear transfer for reproduction is prohibited, it may be argued that reconstructed embryos following nuclear transfer are not potentially human beings. If there is no consensus on this view (40), genetic change could be made in the donor nucleus to prevent any potential of fetal development. Technical difficulties in reprogramming adult human somatic cells in human enucleated oocyte may justify using bovine or other animal oocyte cytoplasts for making the embryos from which human ES cells will be derived. These hybrid embryos would not have the status of human embryos, but some would argue that the presence of animal mitochondrial DNA creates intolerable chimeras. If proven safe, the public may be educated to understand that the animal cytoplast just serves as a momentary vehicle but that the derived ES cells would be really no different from many human-animal hybrid cells routinely used in somatic cell genetics. Another advantage is that stocks of animal oocyte cytoplasts may be prepared in advance and kept frozen.

Deriving ES cells from germ-line cells obtained from aborted fetuses (36) raises the danger that fetuses may be aborted for this purpose. To avoid transplant rejection, many ES cells lines would have to be derived from fetuses representing a large panel of histocompatibilities. It may then be as logical to use fetal cells for generating transplantable tissues. Engraftable human neural stem cells have been obtained from 3.5 months fetuses and transplanted in the mouse brain (42). But immunocompatibility will have to be insured, whereas it is built-in for therapeutic cloning.

Future research may elicit somatic nuclei reprogramming not in oocytes but in ES cell cytoplasm (37). Few would support the view that ES cells are similar to embryos. ES cells are pluripotent but not totipotent as are blastomeres from the early embryo up to the morula stage, which can develop in an independent embryo. Mouse ES cells can contribute to most tissues when injected into blastocysts, but are unlikely to be able to form the embryo envelopes and placental trophocytes, and hence are not totipotent embryos (37). However, embryos produced by nuclear transfer of ES cells to enucleated oocytes can be used for reproductive cloning of mice (7). This advance has another consequence: it will not be easy at all to keep a strict boundary between therapeutic and reproductive cloning. Making human ES cells for therapeutic cloning may indeed be the easiest way to overcome the low efficiency of reproductive cloning. Once an ES cell line will be made from a human adult, it will provide unlimited supply of cells for further nuclear transfers.

The use of ES cells for transplants is also not without risks. There may be somatic mutations during the cultures, and these will not be selected against, as in reproductive cloning by fetal death. The ES cells have tumorigenic potential as they can form teratoma tumors, which contain most type of tissues including hair, teeth, bone or kidney and intestine tissues. In the reported animal transplantation experiments, tumors were not often observed possibly because the ES cells receive from the organs signals that insure their full differentiation, or because the time lapse was short enough. Suicide genes could be introduced as a fail-safe device to kill the cells if tumors develop. Another way would be to be able to cause differentiation *in vitro* in defined cell types by appropriate growth factors and cytokines. These could also be purified from undifferentiated ES cells by using selectable genes driven by differentiation-specific promoters (27, 43).

A conclusion, appropriate to this update on cloning research, can be borrowed from Solter and Gearhart (37): 'It is our view that these and other benefits of nuclear transfer and cloning far outweigh the possible harm, but they can only be achieved through determined experimental effort. It will ultimately be up to society to decide which way to go, but one must hope that the decision will be an informed one and not based on irrational fear, ignorance, and prejudices'.

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Chapitre / Chapter 3

ETHICAL CONSIDERATIONS RELATED TO XENOTRANSPLANTATION

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Given the great shortage of human organs that can be used for transplantation, the field has turned to the possible use of pig organs for transplantation to humans: xenotransplantation. Xenotransplantation would, if it could be developed to the point where pig organs survive and function in humans and the therapies needed to achieve survival are reasonable, save many tens, perhaps even hundreds of thousands of lives per year. Most investigators in the field believe that we are not yet at the point where organ xenotransplantation can be performed clinically; there are still formidable obstacles that must be overcome, that currently lead to rejection of pig organs in non-human primates, and that would likely do the same in humans. However, there should be hope that these barriers can be overcome with the development of additional therapeutic approaches, be they genetic engineering of the donor organ (expressing new genes to block rejection in the organ that is transplanted) or the development of new immunosuppressive agents.

While xenotransplantation would offer this significant and important advance in medical therapy, it also raises a number of ethical issues. These issues include mixing of species, genetic engineering of the donor pigs, the problems of informed consent in xenotransplantation and several others. We shall not detail these issues here. Rather, it is our purpose to focus on our own current activities in ethics, governance and society as it relates to xenotransplantation. We shall focus on only one issue in this paper: namely, that of the infectious risk attendant to xenotransplantation.

The Ethical Issues

Pig organs carry the genetic information in their DNA for retroviruses, the type of virus that causes malignancy and that caused the AIDS epidemic. There are other infectious organisms carried by pigs. It is possible that one of the pig viruses (let us use the virus as the example of the general infectious problem), which causes no disease in pigs, could infect the cells of the human recipient and spread to the general population. Everyone agrees that such a risk exists, although there is disagreement about whether the likelihood of such an event occurring can be accurately stated. Some argue that we cannot know what the probability is that a recipient human would be infected with a pig virus and then transmit that virus to the general population; others argue that such a risk would be small, or even infinitesimal. No matter the magnitude of the risk, if a pig virus were to spread beyond the patient to the general population, the potential negative effect of the event would be enormous.

The highly unusual nature of this risk is that it affects individuals other than the patient. The introduction of 'informed consent' in medicine has been used to allow the patient to understand the nature of the benefits a given treatment would have as well as to comprehend the risks that she/he would be undertaking if they undergo the treatment in question. In the case of xenotransplantation, it would not only be the patient who would be at risk, but individuals in the population who are potentially not even aware that they are being put at risk.

A Moratorium to Allow Public Involvement in Decision-Making

Based on this unusual situation of risk, we suggested that there be a moratorium on clinical xenotransplantation that poses such a risk until certain steps have been taken. We suggested that if the public is to be put at risk, every effort should be made both to inform the public about the risk and to develop a methodology that would allow input from the public. It is important to define the use of the term 'public' here. In terms of informing the public, every effort should be made to inform the largest number of individuals in society as possible, in a manner that allows the members of the public to understand the issues. The 'public' could be quite different in terms of the individuals from whom one would obtain feedback. The 'public' might be a significant proportion of the population (as in national referenda as they are held in Switzerland or relatively small groups that have input from larger segments of the population as in the discussions of such issues as takes place in Denmark).

We suggested that for the United States of America one might organize a national committee to consider the issues. Such a national committee should be comprised of individuals who are open-minded, sensible and broadly-concerned citizens from many walks of life, thus representing a range of philosophical backgrounds and disciplines. Ethicists must be actively involved; physicians and scientists familiar with technical aspects of the problem should also be included. As part of their own educational process, the committee would invite experts in relevant disciplines to answer questions and give advice. These should include, but not be limited to, those involved in the science of xenotransplantation, epidemiology, ethical aspects of the problem, animal welfare and rights, the medical profession, commercial efforts in transplantation, as well as the law and economics. Potential transplant recipients should be consulted. It will be important for the committee to have input from experts from the US Food and Drug Administration and the Centers for Disease Control and Prevention.

Education of the members of the national committee would be only a preliminary to their participating in decision-making about the future of xenotransplantation. The fundamental aim is to develop a consensus about the risks posed by present clinical trials, whether these efforts in xenotransplantation should be abandoned or expanded and, if the latter, under what conditions. The members of the national committee could use other methods of public consultation, such as Town Hall meetings held in various locations in the country, to broaden their input. These efforts in the United States would have to be coordinated internationally with similar ones internationally. Whatever safeguards are needed to avoid infectious spread to the population would have to be adhered to in all countries concerned.

There are some historical precedents to deal with concerns about risks to the public of infection from novel procedures, such as agents produced by genetic engineering. The Asilomar proposals set standards dealing with recombinant DNA research. The fact that worst-case scenarios that underlay that caution did not materialize is no reason to suspend caution in this case.

No matter what the nature of the 'public' that will have input, it is critical that that public is well informed about the issues. Clearly all bodies having an interest in the issues, no matter how extreme their views, should be heard by the national committee. However, individuals who have a conflict of interest with regard to xenotransplantation should not make or try to influence the final decision of the national committee. It would be the difficult

task for the national committee to balance the rights of the individual versus the rights of the public. They would have to weigh the immediate needs of the patient against the speculative risk to the population.

Our suggestion for a moratorium was based on the rights of the population to render their views, not unlike the informed consent we offer to the patient, if we were going to do xenotransplantation, thereby putting them at risk. Further, we felt that the population should have a right to be heard before they were put at risk, again not unlike informed consent for the individual patient. Our suggestion for the moratorium was challenged.

Some of the major objections to the moratorium were the following. First, that we had previously put the population at risk without a moratorium and the type of discussion with the public that we were now proposing for xenotransplantation. The specific instance brought up was that of the use of antibiotics which would give rise to resistant organisms thus putting others at risk of infections that we might not be able to treat. We argued that if a discussion of the type we are now proposing for xenotransplantation were held several decades ago about antibiotics and their use, the results of that discussion might well have curtailed the excessive use of antibiotics as went on for many years and still does. This would likely have led to a slower arising of resistant organisms.

A second argument against the moratorium was that the only way to find out if there is an infectious risk is to do the experiment: namely, to transplant organs to humans. This would presumably be done in a measured manner with careful monitoring of the patients. We argued that the decision whether to undergo the risk of a carefully monitored trial was still something that required input from the public. It is correct that the only way to ascertain the magnitude of the risk is to transplant to humans. However, the issue here is whether the public is willing to accept the risk of xenotransplantation. No matter how carefully we monitor the patient, we cannot eliminate the risk. The question then is: who has the right to decide whether to undergo that risk. Our answer was that only those being put at risk, i.e. the patient and the public, have that right.

A third argument concerned the plight of the patient who will die if she/he does not receive a transplant. A moratorium would deny xenotransplantation to a certain number of such patients. This is a horrible conundrum in which to place anyone having to make a decision. The need of the individual patient is always so commanding. However, it must be the task of the national committee (or other body representing the public) to balance that immediate pressing need with the rights of the public. With xenotransplantation, there is another factor to consider. We are not yet

ready to do organ xenotransplantation. We are thus in the fortunate position of being able to consider the ethical aspects related to organ xenotransplantation while trying to perfect the techniques needed to have successful xenotransplantation to humans. For the moment, thus, the ethical discussion is not delaying the application of organ xenotransplantation.

The Current Status of Regulation of Xenotransplantation

Several countries have regulated xenotransplantation. In the United States of America, the Food and Drug Administration (FDA), in collaboration with other agencies including the Center of Disease Control and Prevention (CDC), has the power to make decisions whether to move forward with xenotransplantation. In fact, the FDA has permitted a limited number of trials of transplanting pig cells into the brains of patients with degenerative diseases of the brain. Some investigators have also used *ex vivo* perfusion of pig livers with blood of patients whose livers have failed. (This procedure involves having the patient's blood flow through a pig liver that is kept outside the patient's body, with the purpose of having the pig liver function to replace the function of the liver of that patient. The procedure can be used either to keep patients alive long enough that they can then get a human liver transplant, or in some cases to keep the patient alive until that patient's own liver starts to function again.) No permission has been given to date to transplant pig organs to humans, however that has not been ruled out. No moratorium exists in the United States of America.

In contrast, the Council of Europe has adopted a moratorium for xenotransplantation. Several countries in Europe have a *de facto* moratorium, with some having established committees that will decide whether organ xenotransplantation will be performed. In most cases, these committees include ethicists and a consideration of the ethical issues, something that has not been a significant focus in the United States. Other countries in Europe have regulations that do not necessarily bar xenotransplantation until the public has been consulted.

An International Effort Aimed at Discussion of the Ethical and Social Issues Attendant to Xenotransplantation

The possibility of an infectious epidemic arising because of organ xenotransplantation is one that concerns all countries. International borders would clearly not limit viral spread. Other aspects of issues related to xenotransplantation are also of concern not only in the richer countries in which xenotransplantation would presumably first be used, but also to developing countries.

A group of individuals interested in this problem area have taken the initiative to develop an international effort to take up these issues of ethics and governance. This initiative plans to address the concerns about xenotransplantation. In addition, however, the initiative uses xenotransplantation as a model of the type of technology that will require ethical as well as technical review in the future as new technologies raise ethical issues.

An international meeting was held at Meech Lake, Canada with the purpose of discussing various questions related to the ethical aspects of xenotransplantation. Approximately 40 individuals representing around 10 countries from both the industrialized and the developing world were present. The ethical, scientific, economic and public consultation areas were discussed. A consensus developed from that meeting that listed three priorities for a group that should carry on from there: to develop an international ethic for xenotransplantation, to involve both the developing and developed world, and to use public consultation.

As a result, a 'study group' guided by the co-chairs, Dr Farkonda Hassan of Egypt and Dr Strachan Donnelley of the Hastings Center in the United States, has formed. That group is promoting the writing of 'white papers' in areas in which the current information is either incomplete and/or is the subject of controversy. These white papers will form the basis of a number of initiatives. To provide a greater degree of detail in the areas of the white papers and in other areas, a website is being established that will have information in a variety of areas of interest and that will link to other web sites dealing with xenotransplantation. In addition to the white papers, there will be 'informational' papers about issues such as the state of regulation in different countries and methods of public consultation that have been used and evaluated in other spheres.

The activities that are underway and being planned include the following. National committee-like[1] efforts are being established in several countries including some in the developing world. The study group will coordinate the efforts of and results from the activities of various national committees. Group discussions in the United States (and perhaps in other parts of the world) are being initiated in various fora. These will hopefully include, but not be limited, to colleges and high

1. We anticipate the national committees will vary in their membership and in their method of procedure in the different countries from the model we suggested for the United States of America.

schools. As a part of this effort, a questionnaire is being prepared that will probe various issues. Connections to and involvement with bodies that have interest in such matters is being established.

Having the Public Influence Governance

The goal of these efforts is to establish a methodology that will allow public consultation on technologies that require ethical discussion in addition to a technical review and to translate the 'decisions' reached by public consultation to the sphere of governance. This last step represents a major challenge in some areas of world, but is an essential if this whole process is to have meaning.

At the present time, public consultation is championed by many. However, how to consult the public in a helpful and meaningful manner is still a much-discussed issue. Even if we could agree on methods of public consultation, there is still the critically important issue of how the opinion of the public can be translated into public policy. For the United States of America, at least, this involves potential confrontation between those that lobby the government to achieve their goals and those who are affected by the actions in question.

It is critical that issues such as xenotransplantation are put into perspective. While there is general agreement that there is the infectious risk, it is important to keep in mind that this is yet another incremental risk that we would undertake in our lives. In that regard it is no different from the building of nuclear power plants, a topic that has been influenced extensively by the public.

It is also important that we do not hold back potentially life-saving procedures for longer than is absolutely necessary. We must develop a system that allows for the simultaneous evaluation of the technical aspects of any invention and the ethical ones. The ethical ones would hopefully be undertaken even as the new procedure is being considered for use, again so as not to delay its application if it is regarded as a valuable one when considering all factors.

Chapitre / Chapter 4

STATE-OF-THE-ART ON EMBRYONIC STEM CELL RESEARCH*

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Introduction

Scientific progress in various fields of biology and medicine have opened a very wide perspective for the knowledge of living organisms, including man. Questions related to the structure and function in biology are addressed from the point of view of the different levels of organization. Molecules, cells, organisms are the basic levels and it is essential to understand the relationship and hierarchy that exist among them.

This scientific progress, that happened very fast, also prompted the development of technologies that enable not only to address scientific questions but to manipulate biological materials, be it molecules, cells or organisms. We usually speak about genetic manipulation to refer to the process that leads to very specific permanent changes, deliberately generated, in a particular gene of a cell. Such changes will be transmitted to the progeny of the particular cell since it affects the genetic material, that is to say what the progeny cells inherit from their mothers.

Genetic technologies for this type of manipulation were introduced about 25 years ago and raised many expectations for the advancement of knowledge. After such a relatively short period of time those expectations have been more than fulfilled in many respects, giving rise at the same time to the emergence of new technologies, new possibilities and new questions.

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* I am deeply indebted to Professor Carlos Alonso (*Centro de Biología Molecular Severo Ochoa*-CSIC) for his invaluable help in the preparation of this presentation by providing most of the text and material.

We are now in the situation in which the full complement of genes of a given species (the genome) can be analyzed at the molecular as well as the functional level. The contribution of each single gene to the characteristics of the corresponding organism can be addressed. If we consider that the human genome consists of about 100,000 genes, the complexity of the questions that can be addressed from the point of view of genomics and related approaches becomes very apparent.

But, it must be emphasized that the technologies involved in genetic manipulation not only represent an adequate strategy to understand life but to manipulate it.

The isolation of genes, referred to as 'gene cloning', prompted the possibility of introducing permanent changes in the cells by reintroducing the manipulated gene in the corresponding cell. Similarly, by cell cloning and manipulation one could affect the characteristics of the pluricellular organisms.

I understand that this presentation should deal mostly with this aspect by analyzing the state-of-the-art in research with particular types of cells that have the potential to give rise to organisms. Obviously, we have to place this research in the perspective of the Universal Declaration on the Human Genome and Human Rights that UNESCO had the courage to adopt. The advance of knowledge and technology in this particular field happens continuously and we must interpret the new findings from the point of view of the Declaration.

Development

Pluricellular organisms result from the development of one single cell, the zygote, that undergoes a whole process of divisions originating a whole progeny of cells carrying the same genes. The new organism develops by both multiplication and differentiation of the cells. The visible differentiation of cells is only the last of a progressive sequence of states in each of which the cell becomes committed to narrower range of types into which it can develop.

The degree of differentiation and commitment of specific cells is addressed in experimental embryology either by explanting them to be cultured in neutral medium or by transplanting them to other sites in the embryo. These methods can be used to study the mechanisms that lead to growth and differentiation of the cells thus developing a new organism.

Completion of development gives rise to an adult. With regard to the division and differentiation capacities of the cells, the adult mammal consists essentially of three classes of tissue. First, there are those that, like nerve and muscle tissue, are incapable of further cell division. Second, there are those that, like tissue of the liver or kidney, do not normally grow but may do so following removal of part of the tissue or after hormonal stimulation. Third, there are the renewal tissues, in which differentiated cells are constantly produced from an undifferentiated stem cell population.

The blood is a typical renewal tissue consisting of different cells that arise from a single type of stem cell, which is found in bone marrow together with larger numbers of mature cells and cells in intermediate stages of maturation. These are real pluripotent stem cells (a stem cell from which many different types develop) as it has been shown by many experimental approaches. Other stem cells, such as those that line the crypts of the small intestine also constitute proliferation units that can differentiate, thus fitting in the category of stem cells.

The question of the fate of the genetic material in each differentiated cell of a given organism and how it controls the whole process of multiplication and differentiation have been addressed by cloning techniques, a strategy that was attempted years ago in amphibians and which has recently produced significant results in mammals.

Cloning of Mammals: The New Breakthrough

Cloning means the production of a precise genetic copy of a molecule (including DNA), cell, tissue, plant, animal or human. In the present context it refers to the process of producing individuals genetically identical to some other living or dead individual. Cloning by nuclear transfer involves the complete removal of genetic material (chromosomes) from a matured oocyte or an egg to produce an enucleated cell (cytoplast). It is replaced by a nucleus containing a full complement of chromosomes from a suitable donor cell (the karyoplast), which is introduced into the recipient cytoplast by direct micro-injection (in amphibians) or by fusion of the donor and recipient cells (in mammals).

Both nucleus and cytoplasm are transferred into the recipient cells, but subsequent development of the embryo is thought to be controlled by the interaction of the recipient cytoplasm and the nuclear genes of the donor chromosomes. If this produces a normal animal, the result demonstrates that the donor nucleus is totipotent (i.e. after nuclear transfer to an enucleated egg the zygote so formed subsequently develops and produces all the fully differentiated cellular tissue of the normal animal).

It was commonly accepted prior to the Dolly experiment that a donor nucleus originating from an adult mammalian cell would already have been irreversibly programmed. However, the experiment showed in fact that the quiescent nucleus of the donor cell was totipotent.

The mammary gland cell used in the Dolly experiment came from a mixed heterogeneous culture. As the authors indicate, it is not clear whether there was something exceptional about the particular nuclear donor cell which was important, whether it was a mammary cell, a stem cell or some other. The authors of Dolly think that the novelty of their cloning procedure lies in enforcing the quiescence of the donor cell before transfer to the recipient egg in order to facilitate the reprogramming of gene expression in the reconstructed egg to its totipotent state.

The scientific discovery initiated by the birth of Dolly has been confirmed. Cloning has become of interest and the Dolly experiment raised important questions, especially what would be the best nucleus for cloning, either from embryo, foetal or adult cells.

Since Dolly was derived from the adult nucleus of a 6-year old sheep, it remains to be seen:

1. if she has a shorter life span than normal, and
2. whether she or her progeny have an increased susceptibility to pathology or an increased rate of abnormalities arising from accumulated somatic mutations.

The principal motivation behind non-human mammalian cloning is to form new transgenic animals of medical importance. This can be done by genetic modification of the cell line from which the donor nucleus comes. The production of therapeutic pharmaceutical proteins remains a major and promising objective. The long lead time required for the development of these techniques should not be underestimated. However, nuclear substitution may one day serve as a therapeutic measure to combat abnormalities of mitochondria.

For cloning purposes, a crucial step forward would be the identification of well characterised embryonic stem (ES) cell lines either to provide a supply of nuclei for transfer or to be used for development after proper signalling. The ES cell lines, whilst maintaining their original totipotent state, can be genetically manipulated, subjected to somatic cell selection procedures and maintained by cryopreservation.

The analysis of multiplication and differentiation capacities of various types of cells, from embryos, foetal tissues or adults is the basis for many approaches that address the question of totipotency or pluripotency of stem cells.

Development and Stem cells

Stem Cells in the Connective Tissue

During development the mesoderm segregates into paraxial, intermediate and lateral plate mesoderm. The local renewable stem cell population for any given mesodermal tissue has been viewed as pre-committed to a particular tissue lineage, forming the intermediary phenotypes within that lineage. However, evidence for the existence of another subset of mesenchymal stem cells, namely lineage-uncommitted pluripotent mesenchymal cells, has come from various sources.

The ratio of progenitor stem cell clones to pluripotent stem cell clones may be up to 30:70. However, with a 95% loss in the number of clones during preservation and culture it is unrealistic to assign absolute values or even ratios to the actual numbers of progenitor and pluripotent stem cells per gram of tissue. Clonal analysis did, however, reveal the presence of both progenitor mesenchymal stem cells and pluripotent mesenchyinal stem cells within these tissues.

Interestingly, adult bone marrow also contains mesenchymal stem cells.

It is most likely that the mesenchymal progenitor and/or pluripotent stem cells with the potential to differentiate into multiple phenotypes reside within the connective tissue compartments of many tissues and organs. It has been proposed that:

1) pluripotent and progenitor mesenchymal stem cells are present throughout the lifetime of the individual;
2) pluripotent and progenitor mesenchymal stem cells are involved in the continued development of tissues, tissue replacement and repair and in certain disease states;
3) pluripotent and progenitor mesenchymal stem cells can be regulated by various endogenous active factors; and
4) pluripotent and progenitor mesenchymal stem cells are present in multiple species across the phylogenetic tree.

Progenitor and pluripotent mesenchyinal stem cells, with differentiation capabilities similar to those of embryonic cells, have been found in adult mice, neonatal, adolescent and adult rats and adult rabbit.

Recently, pluripotent stem cells have been cultured from human foetal tissue and have shown the ability to give rise to a variety of differentiated cell types found in embryonic germ layers.

Nervous System Stem Cells

The nervous system does have the ability to regenerate throughout life, the corresponding brain marrow cells being located in the subependymal zone (SEZ) and the other paraventricular areas such as the ependyma. The cells can be induced to proliferate as it happens with the hematopoietic stem cells.

Pluripotency and Proliferative Capacity of Neural Stem Cells

Before neuropoiesis was even known to occur in the adult central nervous system (CNS) it was noted that there are numerous analogies with the hematopoietic system and suggested the application of similar nomenclature. Stem cells are able to differentiate into all of the different types of cells in a given tissue, while maintaining a pool of themselves. In general, one speaks of stem cells when being certain of having found the ancestor of all cells with proliferative potential in a given organ. Self maintenance, asymmetric versus symmetric divisions, existence of mitotically quiescent forms, pluripotency and other putative characteristics have been extensively studied.

It has been shown that neural stem cells, which were isolated from the embryonic or adult striatum, can produce a broad range of hematopoietic progeny. There is no question that stem-cell biologists, especially neural stem-cell biologists, would have generally predicted that adult-brain-derived stem cells must have a rather restricted fate potential, that is, at best, they give rise to neurons and glia of the CNS. It is accepted now that pluripotency of stem/progenitor cells in both brain and bone might best be evaluated according to their degree of developmental or differentiation potential. Continuously expanded populations of stem cells might be endowed with the appropriate machinery required to express an otherwise silent genomic potentiality in response to an appropriate pattern of stimulation.

The isolation of neural stem cells has been described from foetal brain, adult brain and day 3-5 blastocyste derived ES cells. It is possible that ES cells derived from other sources (for example, primordial germ cells) could also give rise to neural cells.

Embryonic stem cells that are derived from early embryonic totipotent cells are capable of extensive proliferation without differentiation *in vitro*, when removed from the conditions that discourage differentiation. Thus, they may differentiate into multicellular embryoid bodies. These cell lines also contain high levels of telomerase, a ribonucleoprotein that maintains telomere length and has been implicated in replicative life-span or immortality.

Of course, human ES-cell lines offer the hope for cell-transplant therapies and new gene-drug discovery approach. Culture methods for the isolation and characterization of pluripotent precursors from neural crest, and the fetal and embryonic nervous system are well established, and interesting analogies exist between hematopoietic and neuropoietic research fields regarding technique and terminology. The *in vitro* generation of NSs and *de novo*-generated neurons from the adult mouse has presented the first completely convincing description of pluripotent stem/progenitor cells in the mature CNS.

It is conceivable that ES or hematopoietic cells also could be primed to differentiate into different neural cell populations, and to serve as optimal tools for gene delivery and therapy, because ideal grafts would provide exogenous therapeutic gene products as well as becoming integral components of the deficient host structure.

Transplants of neuropoietic cells into the embryonic rodent brain have revealed a rather dramatic incorporation of these cells into appropriate arrangements. Transplants of embryonic dividing precursor cells, in a rodent model of Parkinson's disease, have also led to some dopaminergic-neuron replacement and partial amelioration of abnormal behaviour.

The ability to propagate pluripotent mouse embryonic stem cells and then induce differentiation has enabled the production *in vitro* of tissues for functional studies, drug screens and experimental transplantation. A report has discussed the possibility of using neurones generated from mouse ES cells for transplantation. In the interim, a series of research papers and a number of stimulating reviews have described some of the molecular mechanisms that underlie the proliferation and differentiation of rodent neural stem cells derived from the developing or adult CNS, which

has provided the experimental groundwork for this new field. It would now appear that the areas of embryonic and neural stem cell biology will become increasingly more interwoven over time.

Pluripotent Embryo-Derived Stem Cells in Mice

Eperimental investigation of mammalian embryonic development is complicated by the inaccessibility of the embryo *in utero*. This has resulted in efforts to isolate and propagate in culture the founder stem cell of the foetus. In mice, pluripotent stem-cell lines can now be established that produce derivatives of all three primary germ layers: endoderm, mesoderm and (neuro)ectoderm. These ES cells are derived without the use of any immortalizing or transforming agents and are distinguished by a number of specific features. The most remarkable property of ES cells is their ability to reintegrate into the developing embryo. They can colonize all lineages to produce chimaeric animals that contain a mixture of ES-cell-derived and host embryo-derived progeny in all tissues, including the germ line. The viability and fertility of ES-cell chimeras demonstrates the intrinsically normal character of ES cells. They also establish the capacity of ES cells to give rise to functionally-mature adult cell types.

Transplantation of ES Cells

The differentiation of ES cells *in vitro* yields primarily embryonic or foetal cell types. In the context of cellular transplantation, the developmental plasticity and proliferative properties of lineage-restricted but still immature cells could be an advantage. Naive precursor cells might respond to inductive, trophic and migratory clues from the host micro-environment more appropriately than fully differentiated cells do. They could also show greater flexibility for integration into existing cytoarchitecture. However, adult tissue is probably unlikely to retain all the relevant instructive signals, and some degree of phenotypic commitment might be required prior to grafting.

Transplantation of Cardiac and Haematopietic Cells

Cardiac and haematopoietic derivatives have been transferred into adult mouse heart and circulation, respectively. In both cases, long-lived grafts can be obtained with evidence of functional maturation of the donor cells. But the complete reconstitution of the mouse haematopoietic system entirely from *in vitro* differentiated ES cells has not been achieved to date.

Transplantation of Neural Cells

The ability of ES-cell-derived neural cells to integrate into the developing brain has been investigated by *in utero* injection into the telencephalic vesicle of late gestation rat foetuses. Both neuronal and glial derivatives of injected cells were found to be widely distributed in various brain regions suggesting the appropriate specification of neuronal subtypes. Also differentiated ES cells have been administered intracerebrally into adult mice and immunosuppressed rats. Extensive projections from the graft sites into white and grey matter were found, with evidence of both serotonergic and dopaminergic neurones plus glia. Thus, it appears that ES-cell derived neural cells will be able to survive, migrate and differentiate in the intact brain.

However, a significant problem in using ES-cells progeny for transplantation has also been highlighted by these studies. The difficulty stems from our current inability to direct ES-cell differentiation efficiently. Existing differentiation protocols produce an assortment of cells from different lineages and of differing maturity. Consequently, the transplanted cell populations tend to be highly heterogeneous and the resulting grafts tend to be populated by multiple cell types. This can impede access of the donor neural cells to host tissue and might antagonize or suppress host-inductive signals. Yet, more serious is the potential for any undifferentiated ES cell present to continue proliferating and produce teratoma or even malignant teratocarcinomas.

A possible solution to the difficulties encountered when transplanting ES cell progeny would be to preselect the population for transfer, in order to eliminate irrelevant cells. This could be achieved by various means of tagging the cells and purification by sorting.

Can Human ES Cells Be Grown in Culture?

Until recently, ES cells that were capable of multi-lineage differentiation had only been reported in specific strains of mice. However, there are reports that indicate the tantalizing possibility that ES-like cells might be isolated from humans from surplus embryos donated by individuals undergoing infertility treatment or foetal tissues. The embryos were allowed to develop to the blastocyste stage and were then treated in essentially the same manner as for derivation of mouse ES cells.

These reports suggest that our experience with mouse ES cells might be transferred directly to humans. However, caution is still required at this early stage of our understanding in this new area. It is not certain

that human cells derived by either the blastocyste or germ-cell route will show equivalent properties to mouse ES cells. Clonal analysis has not yet been performed, which is a pre-requisite for a formal conclusion of pluripotent stem-cell identity. It might be more appropriate to describe these cells as human pluripotent stem cells (HPCs) rather than human ES cells.

The ability to expand the ES cell population is crucial, as many millions of cells will be needed for neural transplants. The proliferative capacity of these cultures is not clear, but it appears that they are rather more difficult to expand than mouse ES cells. It is also uncertain as to whether these human cells use the same intracellular signalling pathways as mouse ES cells in order to sustain the self-renewal cycle.

The ability of the human cells to produce foetal cell types *in vitro* also has to be confirmed. Surprisingly, only extra-embryonic cell types were detected when differentiation of the blastocyste-derived cells was induced *in vitro*. This could simply be because appropriate permissive or inductive conditions have not been identified.

Neural Precursor Cells Can Be Isolated from Human Foetal Tissue

The human blastocyste-derived cells generate neuroepithelial structures in teratomas. If this can be reproduced *in vitro*, it should be possible to isolate neural precursors which in turn might circumvent the need for foetal CNS tissue. However, it is not yet clear:

- how rapidly the human pluripotent cells can be expanded in culture,
- how stable they will be over repeated passages, or
- how pure the neural cells derived from them will be.

During development, stem cells in different organs become increasingly restricted with regard to their phenotypic potential.

The neuroepithelium generates all of the CNS and is thought to contain a population of founder neural stem cells. A more liberal approach has been taken with regard to defining neural stem cells when compared to ES cells, in part, because this field is very new and many aspects remain to be fully characterized. However, neural stem cells should at least be multipotent and capable of extended self-renewal.

In a very recent report, cloned single neural stem cells that were derived from the adult mouse brain have been shown to differentiate into blood cells following transplantation into irradiated mice. This exciting finding suggests that neural stem cells might have a wider potential for differentiation than was previously assumed, but has yet to be confirmed by other groups.

The extrapolation of these rodent studies on neural stem cells to the human remains in its early stages. Cells isolated from the 5-12 week-old human foetal CNS have been grown attached to a substrate in the presence of FGF2 and have been found to generate neurones, astrocytes and oligodendrocytes. Similar EGF responsive cells could be isolated from the mesencephalon of older foetuses (>13 weeks), but not younger ones.

Other groups have had problems in inducing the division of human neural precursors from young tissues (6-8 weeks) with EGF alone, and needed to use both 5% horse serum in combination with insulin-like Growth Factor 1 to expand the cells as neurospheres. This lack of EGF responsiveness might be due to the late development of EGF receptors. Furthermore, it is clear that mouse striatal precursors can be 'primed' with FGF2, after which they will then respond to EGF, suggesting that FGF2-responsive cells give rise to the EGF responsive cells.

As all of these studies have used population rather than clonal analyses, it is not possible to assess the multipotentiality of individual cells. However, in most studies, neurones, astrocytes and oligodendrocytes (in very small numbers) were found within the cultures following the withdrawal of the mitogen and the exposure to a suitable substratum. Thus, the favoured terminology for cells within these types of cultures is human neural precursor cells (HNPCs).

HNPCs Can Be Cloned and Integrate into the Developing and Adult CNS

Various authors have attempted to address whether HNPCs were in fact multipotent by taking individual FGF2-responsive cells, which were derived from the embryonic CNS, and generating a clonal line. In one example, such a line could be expanded *in vitro* and showed apparent multipotency on the basis of results obtained using immunocytochemical markers.

Parallel clones were generated from similar human cells immortalized by Myc. All clones appeared to be similar in culture while only some could engraft into the developing brain. This is the first evidence that a single human cell is capable of expansion *in vitro* and subsequently capable of producing neurones, astrocytes and oligodendrocytes, thus fulfilling the main criteria that define a neural stem cell.

However, the percentage of non-immortalized clones that gave rise to all three phenotypes is not known and no information was given regarding the length of time *in vitro* that the clones had been expanded prior to differentiation or grafting, or how this related to the numbers of neurones and glia generated. This is an important issue, as it is possible that the original stem cell will undergo both symmetrical and asymmetrical divisions *in vitro*, leading to a heterogeneous pool of both committed progenitors and new stem cells.

It was also shown that following grafting into the postnatal day 0 (P_0) mouse brain, human precursor cells expanded for short periods of time in culture could integrate into both developing forebrain and cerebellar structures, even replacing granule cells lost in the neurone-deficient cerebellum of the Meander tail mutant. Remarkable integration was also shown to occur when similar HNPCs, expanded for short periods of time and generated from bulk culture, were transplanted into the developing embryo. Human cells mixed almost seamlessly with the rodent ones forming a chimaeric brain. In this study, human oligodendrocytes were also found in dose proximity to rodent axons and might have been able to myelinate under these transplant conditions. Neurones were also found, although their specific neurochemical phenotypes were not reported. These studies show that HNPCs can differentiate in response to local developmental cues in the embryonic or neonatal rodent, suggesting a conservation of such signals across species and an inherent cellular plasticity.

But what happens when these cells are transplanted into the adult rat brain, a situation perhaps more analogous to that of clinical transplantation? HNPCs derived from the human embryonic cortex and expanded in culture for between two and four weeks (passaged every week) with EGF and FGF2 can survive transplantation into the striatum of adult rats with dopaminergic-neurone lesions, and develop into neurones and astrocytes. Some of these human neurones show extensive axonal branching into the host tissue. However, there was extensive migration away from the graft site and into the host brain. Many of these migrating cells were astrocytes. A small percentage of neurones expressed the gene for tyrosine hydroxylase and were able to reverse behavioural deficits associated with the lesion.

Although these results are encouraging, it will be crucial to obtain larger numbers of dopaminergic neurones before clinical transplantation programmes for Parkinson's disease could be considered using precursor cells.

Human Pluripotent and Neural Precursor Cells: Implications for Cell and Gene Therapy

It is interesting to note that neurogenesis continues in defined regions of adult human CNS, particularly the hippocampus, and that certain cells continue to divide into adulthood. However, the multipotency of these cells is not known at present. They could represent a population of resident stem cells, or a specialized neuronal progenitor that continues to divide throughout life. Thus, future therapeutic strategies might also be aimed at generating HNPC cultures from adult tissues (ideally from the same patient that requires the therapy), or stimulating these cells to divide *in situ*, migrate and repair local neuronal damage.

After decades of genetic and cellular research using model organisms, there is now the opportunity to study human neural tissues directly. This is important for a number of reasons. First, there are significant genetic differences between rodents and humans reflected by the fact that many antibodies, probes and drugs are species-specific. Furthermore, it is not clear that human neural precursor cells are very different from their rodent counterparts and need to be assessed independently: it is not possible to extrapolate from mouse to man directly. Thus, results with human cells will be more directly relevant to medical applications. Second, within the next few years the human genome project will provide a complete list of human genes. Using human tissues, the regulation and cellular function of such genes can be asked directly.

This is particularly relevant when combined with homologous recombination and ES-cell technology where gene alterations can be made in specific locations and all resulting progeny from these stem cells (including neurones) will subsequently carry this modification. In this way, specific genes could be deleted, modified or added in order to assess their function, mimic the pathology of common inherited disorders, or express therapeutic proteins, such as neurotrophic factors. Finally, ES and neural precursor cells could potentially be used in new fields of cell and gene therapy, which will require large amounts of human tissue.

Genetic manipulation could also provide a means of customizing stem cells for each patient in order to minimize concerns of immunological rejection. For example, genome-engineering technologies could be used to mobilize major histocompatibility loci. Minor loci would always remain with such an approach, however, and might present a problem.

The ultimate solution, therefore, would be to recreate stem cells from the patient's own tissue. In principle this should be achievable via nuclear transfer into an enucleated oocyte followed by development in culture to the blastocyste stage. Stem cells could then be isolated, expanded and selected prior to differentiation and autologous transplantation.

The challenge of translating our still rudimentary understanding of mouse embryology, neural stem-cell biology and nuclear reprogramming into a system for human cell therapy remains considerable. However, the main avenues of investigation are becoming clear and the prize in prospect is great. A recent publication has shown that HNPCs that have been expanded 10 million-fold migrate and differentiate appropriately when transplanted into neurogenic regions of the adult rat brain. These data further support the use of such cells in both experimental and clinical transplantation programmes.

Pharmaceutical or surgical interventions into neurodegenerative diseases have, in general, met with limited long-term success and are often compounded by serious side-effects. Whilst such avenues should continue to be explored and refined, a third approach, cell therapy, now warrants serious consideration. Current clinical trials suggest that the transplantation of dopaminergic neurones derived from primary human foetal tissues can have beneficial effects in patients with Parkinson's disease. In one case, post-mortem data have shown good survival of dopaminergic neurones within a transplant, proving that these cells can survive and mature within the adult human brain. Similar trials that involve cell therapy are being undertaken for Huntington's disease and are being considered for myelin-related disorders.

However, human foetal tissues are extremely difficult to obtain in sufficient quantities for transplants. Generation of neural tissue *in vitro* would, therefore, be a key milestone towards developing cellular and gene therapies for disorders of the CNS. Until recently, ES cells could only be generated from mice. However, two reports have now appeared that provide the first evidence to show the derivation of pluripotent stem cells from human embryos. These cells retain the potential to differentiate into various tissue lineages after growth in culture, which offers the prospect of developing novel cell-transplantation therapies. Meanwhile, in the

neuroscience arena human neural precursor cells were recently cloned and expanded for extended periods of time *in vitro* and transplanted successfully into foetal rodent brain. These findings on human tissues extend stem-cell research from being principally academic to being potentially medical.

In addition, the applications for generating human neurones *in vitro* should not be overlooked. In this context, *ex vivo* maturation of stem cells into terminally differentiated phenotypes with high fidelity to adult cells *in vivo* will be crucial for many purposes. These include their use as research tools for the genetic dissection of neuronal developmental and cell biology, and in the creation of new assay for pharmaceutical discovery, such as high-throughput screens for neuroprotective compounds.

Reflections

These data suggest a number of questions and avenues for future research.

Why does a tissue that, until recently, was characterized by its post-developmental mitotic quiescence, contain cells with notable proliferative potential that could possibly replenish lost components?

Could this newly appreciated proliferative cell population also contribute to or be the basis of tumorigenesis? Bone-marrow transplant malignancies occur in less than 1%. Given that most haematopoietic neoplasms develop clonally, and many might even originate from a haematopoietic stem/progenitor cells, cancer can potentially develop from a single infused cell that undergoes transformation. Although this is still a controversial issue in the haematopoietic transplant community, the possibility of undifferentiated neural stem/progenitors having tumorigenic potential is a concern for both neuro-oncology and future brain-cell-transplantation therapies.

In addition to the high mitotic potential exhibited by neural stem/progenitor cells, these cells share certain molecular features with neoplastic cells. It is a well-described phenomenon that ES-cell transplants to an adult host can develop into teratomas and teratocarcinomas. Recent observations of impressive pluripotency of even adult brain stem/progenitor cells, including their differentiation into different types of blood cell, as well as reports of haematopoietic cells invading the adult brain and differentiating into both microglial and astroglial lineages, suggest that we do not understand the potential tumorigenic roles of stem/progenitor cells in any tissue. A glycoepitope,

CD34, found on surfaces of hematopoietic cells, is also known to be transiently synthesized by neural stem cells during early neurulation and at least one type of brain tumor, gangliogliomas, which are thought to originate from developmental failures of brain maturation also express CD34.

While the bone marrow guarantees a life-long supply of blood cells, the adult brain does not seem to possess the capacity to repair significant damage on its own. Is it possible that, to a limited and perhaps easily exhaustible extent, lost brain cells are continuously replaced, and that this has simply been overlooked so far? While neural ancestors are predominantly found in areas characterized by dense accumulations of developmentally regulated molecules, including ECM molecules, the SFCs are found in the adult brain in regions such as the SEZ brain marrow that, during development, represent the last areas to contribute to a massive developmental neurogenesis: the regions of the M or SEZ associated with the anterior and posterior neuropores of the neural tube.

It is possible that the persistent, relatively quiescent cell populations in these areas simply represent a sort of leftover supply of 'bricks' as seen near any construction site when the building of a structure has been completed. A change in the composition of growth factors at the end of postnatal development might allow stem/progenitor cells to remain within marrow and perhaps other regions until something (for example, a genetic message or an environmental message or insult) happens that leads to their proliferation, differentiation or death. Given that adult SFCs and their progeny (the NSs) might be a heterogeneous population, it seems reasonable to assume that different SFCs were born at different stages of embryonic development, and this would, considering their preserved level of potency, impact significantly upon their potency and their potential use in cell-replacement therapies. It is, in any case, surprising that these cells do not undergo apoptosis, which is so prevalent during the building of the CNS. An appropriate timing of their birth and their protection by an appropriate environment are reasonable factors that could enable these cells, which might be simply too young to die, to persist for an entire life-span in the CNS.

At present, one cannot exclude the possibility that neural ancestors are located in well-defined areas of the adult CNS in a predetermined and organized manner, as is the case for all other regenerative tissues and organisms. This very provocative model would suggest that the adult brain is able to replace lost components, at least to a minor extent. A potential decline in the number of stem/progenitor cells recovered from aged brain dissociations, and the well accepted idea that the human brain decreases

in size with advancing age, could support this idea. One could even assume that the size of a well-organized stem/progenitor cell pool declines because of reparative processes, and it is possible that the number of available stem/progenitors per area is simply insufficient to replace lost brain material following an unforeseen, catastrophic event, as seen in the case of extensive neuronal loss of a single cell population in Parkinson's disease. Whether by coincidence or plan, the adult brain seems unable to respond to massive damage with impressive reparative sequalae, however, brain-marrow research might also reverse this dogma.

Ethical and Regulatory Issues

The regulations governing research with human embryonic and foetal material differ between countries. In the United Kingdom, research on human embryos is regulated by the Human Fertilisation and Embryology Act (1990), which prohibits the propagation of an intact embryo beyond 14 days. Pluripotent stem-cell cultures are established by desegregating embryos between 6 and 8 days. Research projects on these cells can only be conducted under a research licence issued by the Human Fertilisation and Embryology Authority. Neural stem-cell cultures are established from post mortem foetal material. There are no statutory restrictions on research with post-mortem foetal material, provided ethical approval is obtained from the relevant authority and tissues are collected under guidelines set out in the Polkinghome report and Department of Health guidelines.

Conclusions

The characteristics of embryonic stem cells can be summarized as follows:

- non-transformed,
- indefinite proliferative potential,
- stable diploid karyotype,
- clonality,
- formation of multi-differentiated tumours (teratomas) on ectopic transplantation,
- multi-lineage differentiation *in vitro*,
- permissive for genetic manipulation,
- incorporation in chimeras (not practical for human tissues),
- germ-line transmission in chimeras (not practical for human tissues).

The applications of *in vitro* generation of human neural tissue are:

- identification of inductive factors and mediators in human neurogenesis,
- determination of gene function in neuronal and glial differentiation,
- modelling neurodegeneration,
- pharmaceutical discovery,
- cell therapy,
- *ex vivo* gene therapy.

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