日本組織培養学会

会員通信

第26号

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※※京都市東山区山科御陵 京都薬大

※ 第40回研究会要項決まる

別紙（色頁）の如くに研究会が開催されます。（宿泊に関しては別項を参照して下さい）

※ 幹事会および総会議事録

6月19日(木)，金沢大・薬・放射薬品化学教室において幹事会が開かれ，下記の事項について協議された。また，これらの協議事項は翌20日(金)の第39回研究会総会においていづれも可決承認された。

なお，幹事会出席者は野瀬，久米川，佐藤，堀川，黒木，難波，二階堂の新旧各幹事のほか，会計担当の山田氏，会員通信担当の梅田氏，ビブリオグラフィー担当の乾氏であった。

(1) 幹事長：幹事改選に伴い，1975年度の幹事長は東大・医科研・癌細胞の野瀬滑氏に決定。

(2) 新幹事：本年2月におこなわれた幹事選挙の結果，以下の4氏が当選され，新幹事となったが

（東部） 黒木登志夫氏，武田久雄氏

（西部） 難波 正義氏，二階堂修氏

このうち黒木氏が渡欧，武田氏が渡ソされるため，くりあげて 丸野内棣氏（三菱生命研），佐藤茂秋氏（国立がんセンター）にお世話を願うことになった。なお，本来なら次点の須田立雄氏（東京医歯大）がくりあげ当選になるところであるが，今回に限り特殊事情を認めて辞退を承認した。

(3) 会計報告： 担当の山田氏より別紙（会計通信第25号参照）にもとづき昭和49年度会計報告があり，承認された。

なお，種々問題はあるが会費は当面1000円すえおきにされた。

(4) ビブリオグラフィー：黒木氏の渡欧に伴い，ビブリオグラフィー担当乾氏の補佐役として，新たに佐藤茂秋氏にお願いすることになった。（本年度のビブリオグラフィーに関しては別紙参照）

(5) 会員通信：原稿の集りがわるく，会員各位からどしどし原稿を送ってほしい旨担当の梅田氏より要望があった。なお，今後は長期海外出張の会員にも会員通信を送ることになった。また，会員が住所変更した場合には，学会事務センターに直接連絡するよう要望があった。

(6) 会員名簿作製： 再三の催促にもかかわらず未だ約110名の未回答者があり，作製は難行している。未回答者には今後再度催促して今年中には何とか完成にこぎつけたい旨作製担当の佐藤氏，堀川より報告があった。

(7) 新入会員：新入会員，新入賛助会員，退会会員（別項参照）については，すべて承認された。

(8) 次回研究会：下記の要領で開催する。

世話人：　独協大学医学部　山田　番氏

会　期：　昭和50年10月24日・25日

場　所：　独協大学医学部　講堂

特にシンポジウム形式のものはやらず，上皮細胞および血液細胞の培養等を中心としたウワークショップを企画中（別紙参照）。

(9) 次々回研究会：　世話人：九州大学　医療短大　　高木良三郎氏

(10) その他：　3年後に日本で国際老年学会（International Congress of Gerontology）が開催されるが培養細胞関係では東大・医科研勝田氏がオーガナイズする。Dr. Hayflickを始めとする外人3名と日本側から2名の合計5名位をシンポジウムのスピーカーに考えている旨勝田氏より報告があった。（別項参照）

（文責　堀　川　正　克）

☆ 昭和50年6月　新入会員名簿

所属機関	同住所・電話	氏名	専門分野
東北大学医学部 第3解剖学教室	980 仙台市星陵町2－1 (0222)74−1111 内線　472	石川　博	解剖学
三楽オーシャン株式会社 中央研究所	251 藤沢市城南4-9-1 (0466) 34-4176	沖　俊一	微生物学
資生堂研究所 第12研究室	222 横浜市港北区新羽町1050 (045) 542-1331	宇塚　誠	皮膚科学 薬物学
昭和大学医学部 泌尿器科学教室	141 東京都品川区旗の台1-5-8 (03)784-1151	池内隆夫	泌尿器科学
食品薬品安全センター 秦野研究所	257 神奈川県秦野市落合500	渋谷　徹	突然変異
帝国臓器製薬株式会社 薬理研究部	210 川崎市高津区下作延1604 (044)833-5151	中山隆治	生化学
東京慈恵会医科大学 阿部内科	105 東京都港区西新橋3-25-8	尾林紀雄	内科学 （糖尿病）
東京大学医学部 老年病学教室	113 東京都文京区本郷7-3-1 (03)815-5411 内線　8344	岡野一年	内分泌学 老年病学

所属機関	同住所・電話	氏名	専門分野
東京大学薬学部 生理化学教室	113 東京都文京区本郷 7-3-1 (03) 812-2111 内線 2398 内線 3487	 川島光太郎 花園文雄	 生理化学 細胞生物学
東京大学医科学研究所 附属病院外科	108 東京都港区白金台 4-6-1	森廣厚	外科学 腫瘍免疫
ベーリンガー・インゲルハイム・日本薬理学研究所	666-01 兵庫県川西市 矢間守高田103 (0727) 93-8585	西川順子	組織化学
金沢大学薬学部 生物薬品化学教室	920 金沢市宝町13-1 (0762) 62-8151 内線 437	大場義樹	生化学
京都大学医学部 放射能基礎医学教室	606 京都市左京区吉田 近衛町 (075) 751−2111 内線 4410, 4415 内線 4410〜4412	 内海博司 伴貞幸	 放射線細胞生物学 放射線生物学
京都大学薬学部 衛生化学教室	606 京都市左京区吉田下 阿達町 (075) 751-2111 内線 4537	高木政貴	生化学
大阪市立大学 医学部 解剖学 教室	545 大阪市阿倍野区旭町 1-4-54 (06) 633-1221 内線 2015	大西礼子	細胞生物学
愛媛大学医学部 第1解剖学教室	791-02 愛媛県温泉郡 重信町 (089964) 3811	高島廣一郎	解剖学・超微 細胞化学
鹿児島大学医学部 病理学教室	890 鹿児島市宇宿町 1208-1 (0992) 56-2211 内線 2112 内線 2113	 井坂英彦 梅原澄子	 病理学(癌) 〃

☆ 昭和50年6月 新入賛助会員

賛助会員	同住所・電話	連絡者
株式会社 医学生物学研究所	458 名古屋市緑区鳴海町四本木 16-3 (052) 622-5261	諸岡一泰

賛助会員	同住所・電話	連絡者
アミコン・ファー・イースト・リミテッド	102 東京都千代田区飯田橋 3-1-3 惣建ビル (03) 264-2491	加藤好雄

☆ 住所変更

新潟大学医学部 病理学教室	951 新潟市旭町通一番地757 (0252) 23-6161	大星章一
北陸大学薬学部 生理化学教室	920-11 金沢市金川町ホ3番地	毛利哲郎
	161 東京都新宿区下落合3-11-15	川喜田愛郎
名古屋保健衛生大学医学部細菌学教室	470-11 豊明市沓掛町田楽ケ窪	小川透

☆ 昭和50年6月 退会者

附田盤子（予研・ウイルス部）

桜井淑子（予研・ウイルス部）

☆ 海外留学

黒木登志夫氏

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150, Cours Albert-Thomas,
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増地広氏

University of California,
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Laboratory of Nuclear Medicine and Radiation Biology
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U. S. A.

※ 第40回研究会の宿泊御案内

宿泊所	住所・電話番号	人数	内容	交通
公立学校共済組合 くろかみ荘 宇都宮宿泊所	宇都宮市桜4-1-19 TEL (0286) 22-1981	2，3人 1室3人	3,000-4,500円 2食付 朝 300円 夕 800～1,500円	国鉄宇都宮駅から作新学院行バス足利銀行本店前下車徒歩2分 東武宇都宮駅から作新学院行バス足利銀行本店前下車徒歩2分
地方職員共済組合 みくら山会館 宇都宮宿泊所	宇都宮市塙田町1-1 はなわだ -39 TEL (0286) 22-1093 1558	12人 1室2～3人	2850-3690円 2食付 朝 300円 夕 800～1,500円	国鉄宇都宮駅から作新学院又は戸祭行バス県庁前下車徒歩5分 東武宇都宮駅から徒歩10分
警察共済組合 しもつけ荘 警友会館	宇都宮市塙田町 1-1-39 TEL (0286) 23-3810	12人 1室3人	1,980円 2食付	上に同じ
東武ホテル	宇都宮市一条1163 TEL (0286) 36-3063	100人	シングル 2,930円 ツイン 5,420円 セミダブル 一人3,150円 二人5,420円 朝 450円	国鉄宇都宮駅よりバス，東武宇都宮駅前下車
宇都宮グランドホテル	宇都宮市西原町142 TEL (0286) 35-2111	70人	シングル 3,000円 ツイン 5000円 朝 和食 700円 洋食 600,800円 夕 800～6,000円	国鉄宇都宮駅より江曾島又は石橋行バス不動前下車徒歩3分 東武宇都宮駅より江曾島又は石橋行バス不動前下車
益石荘	宇都宮市大谷1228 TEL (0286) 52-0524	45人 1室3～5人	4,000-7,000円 2食付	国鉄宇都宮駅，東武宇都宮駅とも大谷ヘルスセンター行観音前下車 但し宿泊者15名以上の場合はマイクロバスで東武宇都宮駅まで送ります。
平屋荘	栃木県下都賀郡壬生町 大字北小林1022-85	15人 1室1～2人	2800円 2食付	東武宇都宮線おもちゃの町駅下車徒歩5分

注意事項

1. 会場は東武宇都宮線おもちゃの町駅（東武宇都宮駅より15分）下車徒歩15分
2. 東武宇都宮駅と国鉄宇都宮駅は徒歩30分位（バス10分）はなれていますので御注意下さい。

⁂ 国際老人病会議について

1978年夏第11回の会議が日本で開催されます。これは一般演説と各種シンポジウムから成っていますが，そのシンポジウムの一つに〃Aging of Cells in Culture〃というのが計画されています。演者として日本人2人，外国人3人，計5人が予想されます。つきましては，この日本人演者2人を公募いたしますので，御希望の方はあと1年余以内位の培養学会で成果を御発表下さい。採否はその成果によって判定いたします。

⁂ 講演会のお知らせ

日　時　8月25日　午前10時

場　所　東大医科研　本館　2階会議室

演　者　Prof. F. H. Kasten
Louisiana State Univ. Med. Center, Dept. Anatomy

演　題　"Differentiated and undifferentiated neuroblastoma cells in vitro"
"Mitosis and differentiation of mammalian cardiac cells in culture"

⁂ 第39回研究会を終えて

堀川正克　（金沢大・薬・放化）

日本組織培養学会第39回研究会は6月20日(金)・21日(土)の両日，金沢大学医学部十全講堂で開催された。Host University が学生実習のため例年より約1ケ月も遅れて研究会は開催された。そのため，今度は雨が心配されたが，幸い前日まで降っていた雨もからりとあがり，会期中は何とか晴天に恵まれた。研究会が終るやいなや翌日からまたドシャ降りの雨になったのも皮肉といえば皮肉であるが，世話人側からするとまったく〃ついていた〃の一語につきる。

さて，地理的条件もあって前回の第38回研究会には及ばないまでも，今回の研究会で登録された参加者は正会員87名，非会員102名，学生25名の計214名であったから，まあまあの研究会であったと言わざるをえまい。それにも増して39回目にして初めて北陸の地で研究会が開催されたということに意義を見いだしたい。

今回は特に黒木氏（東大・医科研），黒田氏（国立遺伝研）の力をおかりして〃哺乳類細胞における突然変異の機構解析〃と題するシンポジウムを企画した。しかも一日ぶっ続けのシンポジウムである。当初危惧した質問のマンネリ化もなく，終始活発な質疑応答のうちに幕切れとなったのは両氏の名司会に負うところ大であるが，くわえて各演者によって発表された研究内容の深さ，充実さ，更にその重要性によるものであったろう。哺乳類細胞を用いての突然変異の研究は細胞の癌化および老化といった重要問題と関連して，今後更にその系の確立，それらを使っての機構解析が精力的に進められるものと思う。今回のシンポジウムがそのための trigger ともなれ

ば幸いである。

また，シンポジウムに関連してか，一般講演の中にもこれに類する内容のものが多々報告されたが，これも今回の研究会の特徴であったろう。事実，シンポジウムの部に入れるか一般講演でやってもらうか，その取捨選択に悩んだのは舞台裏のいつわざる苦労談でもある。ともあれ，ハエ細胞の培養が出，アコヤ外套膜培養による真珠作(?)とまでは行かないまでも，一般講演にも興味ある演題がずらりと並んだのは事実である。これらの研究が今後とも更に一段と発展することを祈って止まない。

最後に，長時間シンポジウムの司会を努めて下さった黒木氏，黒田氏，会をもりたてて下さった各セッションの座長および演者，また遠路をいとわず御参会いただいた出席者諸氏にこの場をかりてお礼申しあげる。

☆ 海 外 情 報

(1) アメリカ合衆国では各種のworkshop や training course がはやっているが，最近医科研勝田先生の手許に，本年度の組織培養関係のeducation program が届いた。

日本の本学会会員諸氏には既に終ってしまったものもあり，出席は不可能に近いと思われるが，御参考迄に要点を御紹介する。

主催はW. Alton Jones Cell Science Center (Lake Placid , NY12946) で培養学会が協賛？の形をとっているようである。 対象は大学院生，大学院卒業者，或は充分な基礎知識を持つ技術者で，費用は培養学会員で$110～550・会員外で一割高となっている。

	DATE	PROGRAM TITLE
	March 3-4	Applications of Tissue Culture in Dental Science
	March 6-7	Histones as related to the Cell Cycle
	March 11-13	Preparation of Cultured Cells for Electron Microscopy
	April 9-10	Perfusion Techniques in Tissue Culture
	April 21-24	Plant Propagation in Vitro
	May 15-16	Cell Mediated Immunity
	May 19-20	Cell Culture Techniques in Inborn Errors of Metabolism
	May 24-25	Culture of Liver Cells
	May 29-30	Cell Separation
	June 9-10	Identification of Cells in Culture
	June 16-20	Cryobiology
o	June 23-24	Water Standards and Quality Control
	June 25-27	Biohazards in Tissue Culture
	July 7-18	Vertebrate Cell Culture
	July 21-25	Vertebrate Organ Culture
	July 28-Aug.1	Invertebrate Cell and Organ Culture
	August 4-15	Plant Cell and Tissue Culture
	August 18-22	The Nutritional Requirements of Vertebrate Cells in Vitro
	August 27-29	Culture of Muscle Cells

Sept. 1-5	Cell Hybridization and Chromosome Mapping
Sept. 3-9	Cellular Radiation Biology
○ Sept. 16-20	The Preparation of Media for Animal Cell Tissue and Organ Culture I. Formulation II. Sterilization
Sept. 22-24	Quantitative Optical Techniques in Cell Culture
Sept. 29-Oct. 3	Detection of Mycoplasma
Oct. 6-7	Mycoplasma and Acholeplasma in Biological and Medical Problems
Oct. 16-17	Cell Culture in Studies of Senescence
Oct. 23-25	Culture of Nervous Tissue
Oct. 27-31	Primary Vertebrate Cell Culture
Nov. 3-7	Clonal Culture
Nov. 10-14	The Bulk Culture of Heteroploid Diploid and Primary Cells
Dec. 1-5	Karyology of Cultured Cells

日本の学会で真似るとなるといろいろの意味で大変であるが，やはり会社が中に入り，費用も高くならざるを得ない現状がうかがえる。しかし興味のあるのは○印をつけた標題もあり，このような形でも知見交換，正しい培養法の標準化には役立つかも知れないと思われる。

(2) これも勝田先生の入手した資料ですが，Annual Meeting of the European Tissue Culture Society が5月5日から7日迄開かれた。Programme の演題を拾ってみると，以下のようです。

Ⅰ Oncogenic virus and tissue culture (8題)

Ⅱ Masscultures (3題)

Ⅲ Growth characteristics of various cells in vitro (7題)

Ⅳ Special aspects of tissue culture (6題)

Ⅴ Drug action on cells in vitro (7題)

Ⅵ 一般演題(17題)

彼地の研究の傾向がわかればと御参考迄にお知らせしました。

(文責 梅田)

編集後記

会員通信夏号をお送りします。 夏休み前に発行すると計画していながら，いつも遅れて申しわけありません。 次回研究会での発表の予定に間に合うことを願っています。 幹事会議事録にもありますが，会員名簿，ビブリオグラフィー等の本会の事業に皆様御協力をお願いします。私共からのお願いとしては所属変更，海外出張等の変更がありましたら，これからはすべて学会事務センターに通知していただくことになりましたので宜敷く。 尚本号には川崎医大難波氏の御協力でDr.Hayflick の講演原稿をのせました。紙面の関係で小さな活字になりましたが御容赦下さい。 次号原稿は11月15日締切りの予定です。 会員皆様の御投稿を望んでいます。 (U)

HUMAN AGING AT THE CELLULAR LEVEL

Professor Leonard Hayflick, Ph. D.
Stanford University School of Medicine

(Lectured at Kawasaki Medical College
on September 19, 1974)

I am sure that you will appreciate that the subject of aging or gerontology is a very large subject and impossible to discuss in one hour to say nothing of the fact that I must speak very slowly during the 1 hour. So, I will confine my remarks to one very small area of the cell biology of aging, with your understanding hopefully that I am covering just a very narrow area.

I think that one of the major problems in the field of gerontology unlike other areas of biology is the definition of the subject under study, strangely enough you might be surprised to learn that the gerontologists or biologists who study aging cannot agree even on the definition of the subject, that is, what is biological aging. I thought that I would try to give you the best definition that I know by simply showing you the two extremes of the problem, which appear on the first slide that I have. So, if I can have the first slide, I will show you, this is the definition of aging, that is, what biological changes take place in going from this condition to this condition.
This is the easiest definition that I am able to give you. But I am sure you will understand that this is a very complicated subject that results in these very dramatic changes.

One of the important points that I would like to mention to you is that the study of aging is not necessarily the study of the diseases that occur in humans and it is important to realize that there is a distinction between the diseases

that occur as individuals age and the biological changes that give rise to age changes. And I think that it is worth underlining this point by showing you some data that reveal very dramatically the question of improvement in hygienic conditions and curing of diseases and their impact on the human life span. It is important also to recognize that there is a very important distinction between the human life span, which we think is a fixed number of years, roughly 90 or 95 years of age, and life expectancy, which is the number of years that one can expect to live, based on statistical data as a function of his current age. So, I would like to show you this data that will underline this very important point.

Now, this is what gerontologists call a survival curve. This is a family of survival curves obtained from data in which a cohort of 100,000 people have been followed from birth until death and each year the number of individuals who have died is noted and then the tabular data is compiled and represented in the form of this family of curves. I will not discuss each curve but just the extremes. The bottom curve is generated from data taken from individuals in British India from 1921 to 1930. And as you can see in the first few years of life, there is a dramatic number of deaths in the post natal period. Then in later years this begins to flatten out, and there are some individuals in this group who survive up until roughly the 90th year of age. The other extreme curve is from New Zealand in this period of time.

Here you see very few deaths in the early years, then there is a plateau, and the number of individuals dying then begins to increase until some individuals remain at this point and live until the maximum period of life. Now the main point that this table of information indicates is that as the curing of diseases occurs and as better hygienic conditions occur, then there is, what gerontologist call, a rectangularisation of the survival curve, the survival curves become more and more rectangular until the most rectangular curve is produced in this fashion. This would be 90 years. The implication then, is that with the curing of diseases and the improvement of hygienic conditions, what will happen is that humans will live until the stroke of midnight of their 90th birthday and then they will drop dead. And this is the implication of work done simply addressing oneself to bettering hygienic and living conditions and curing diseases.

The main point is that very little work is done on the question of the biological causes of aging, the underlying principle biological causes of aging which are the physiological and biochemical decrements that result in the age changes that all of us are familiar with. There are very few people who work on this problem, and it is important if there is any merit to extending the life span. It is important to recognize that very little work is being done on this question. There is also a subsidiary question in respect to whether it is important and necessary to do work

in an area of biology that might result in the increase of human longevity. This is a very important moral question, and a very important ethical question that unfortunately I will not have time now to discuss with you. But if there is any merit to increasing the human life span, there is of course very little work being done in this particular area.

Now to give you further information on the implications of curing the major causes of death in my country and in your country, I would like to show you some projections of data that makes for some very revealing information. And that is to ask ourselves the question what would pappen to human longevity if we were able by some miracle, tomorrow morning, to cure the major causes of death, that is, heart disease and cancer. And the information that I want to show you is on this next slide in which it has been determined what these effects would be. This is the gain in expectation of life at birth and at age 65 due to the elimination of various causes of death. Here we have the major causes of death, major cardiovascular renal disease, heart disease, vascular diseases;if these diseases could be cured tomorrow, then people born tomorrow would have a total gain in life expectation of about 17 or 18 years. People who are age 65 tomorrow would have somewhat less, but not significantly less, expectation of additional life. The next major cause of death would be cancer. And if cancer were to be cured tomorrow morning, the impact would be, that people born tomorrow would only have an additional 2·3 years of life

expectation, people 65 tomorrow only 1·2 years of additional life expectation. And I would think that these numbers are much lower numbers than you would have guessed, based on the amount of effort and money and energy of so many people who are investing their lives in learning more about these important causes of death. That is not to say that the study of these diseases is not important. It is certainly important. But I think that the impact on longevity is not very great. I would suspect that you would have thought that curing cancer will result in increase of life expectancy, perhaps 20 or 30 or more years. But this simply is not the case.

Now with this information as background, what I would like to do now is to give you some information in an area of cell biology and I think we can have the lights now and the slide off. I would like to give you some information in an area of cell biology in which we and others have been working for a number of years, that has some important impact on the study of aging. For this purpose, it will be necessary for all of you to have a good background in tissue culture techniques, because what I intend to discuss is based on a knowledge of tissue culture. Since I have been told that there are certainly many experts on tissue culture here today, there are still some people here who are not expert on tissue culture. So I will spend only 5 minutes, giving you some basic background information which will be necessary for you to understand the rest of my presentation.

Many of you of course know that it is possible to take tissue from any animal and to grow the cells from this tissue in culture. This is done ordinarily by taking the tissue and exposing it to a chemical preparation or enzyme preparation called trypsin which results in the separation of the individual cells from the tissue that you then can collect in a centrifuge tube and this can be centrifuged and cells collected as a pellet. The trypsin of course is then discarded and the cells now can be put into a variety of glass bottles and cultured. Generally, the type of culture vessel that people uses is something of this shape. This would be a side view and this a top view. The cells will be introduced into culture vessel, in this manner, and in a period of a few hours cells would begin to stick the glass surface and then begin to grow. Now the medium that is used for the growth of cells in tissue culture is very complicated. I will not say any more about the media than to say that in general it consists of 13 aminoacids, 7 or 8 vitamins and a series of inorganic salts, the kind of salts that you would expect, potassium, calcium etc. This is the chemically defined portion of the growth media, but normal cells will not grow in a chemically defined media. So that the media must include 10% of serum and the serum used is generally serum from calves or horses. The development of media has not resulted in any chemical definition of all of the components necessary to grow normal cells in tissue culture. It is possible to grow a number of abnormal cell populations

in a chemically defined media. But much of what I want to discuss with you this afternoon has to do with the cultivation of normal cells. So we now have the cells growing in the culture vessel and in a matter of a few days or a week the cells are growing quite rapidly and they reach a point where they have now covered entire surface of the glass vessel. This condition is known as a confluent culture. It is also sometimes called monolayer culture and the most important event that takes place at this time is the fact that when normal cells cover the entire surface and touch all of their neighboring cells,mitotic activity essentially stops. Abnormal cells or cancer cells may continue to grow a little bit under the circumstances and this is a very important area of investigation now, to discover what the signals are that tell a cancer cell to contiune to divide under these circumstances of confluency and a normal cell does not continue to divide very much.

If you are interested in obtaining more cells, that is more than is contained in this vessel, then it is necessary to undertake a new or different technique, and that technique is known as subcultivation. We will remove the media from the culture vessel, the cells will remain behind, trypsin is then introduced to raise the cells from the surface, separate them from their neighbors, and then the cells can be distributed into two daughter bottles further subcultivation. So this is called a subcultivation. It also goes under the term "a split" and we refer to do this as "a 1 to 2 split

ratio", which means that we have increased surface area of the first bottle by a factor of 2. So that the number of daughter cultures is twice the number of the mother culture. Now it is possible to do subcultivations or 'splits' by making three bottles, or five, or ten or whatever number, from the first, but mathematics becomes a little difficult and since I want to keep simple, we will assume that the 'splits' are done by the 1 to 2 ratio, although theoretically everything is the same. So at the end of the first week we would have two cultures, at the end of 2nd week we will have four, and eight, sixteen, and so forth. So that the numbers of cultures would increase by powers of 2. Now this then is basically the technology that I wanted to introduce you to.

And now I would like to discuss some of theoretical implications of culture in cells in tissue culture by the method that I have just outlined. If one takes tissue from a variety of sources including humans and grows the cells from such tissue in culture, if you start with a scrap of tissue and you plot the number of cells that are produced from the tissue as a function of time, then you will generate data that will give you a curve looking something like this. This would be the primary culture, the first culture, and after a week you will then do a subcultivation so that you have two cultures, four, eight, sixteen so forth, for a number of weeks, until you reach a point when the cells stop dividing and begin to die. And we have divided this curve into three parts, Phase I, Phase II, which is the period of

active cell replication, Phase III which is the death of the cells in tissue culture. Now to give you some notion of the time parameter, if you culture tissue from a embryo, and the type of cell that generally grows most well in tissue culture, is called the fibroblast,if you culture human embryonic tissue in culture, the period of time that they can grow is something like 10 or 12 months. And the number of population doublings is equivalent to 50±10. Now this is the usual course of activity of the cultivation of normal cells in culture.

When you culture normal human cells, there can occur in these cultures very rarely, and until few years ago under circumstances that were particularly unknown, a new cell type which can arise from cultures of normal cells. The frequency that this occurs in human material is less than 0.01% of the time. For other animal species the frequency of the new cell arising is much greater. Mouse tissue, for example, may give rise to this new cell type within a frequency of 40% or perhaps even 50%.

Now I would like to point out the major distinctions between these two types of cells. The first type of cell that I have been discussing we will call "cell strain" and the type of cell that arises rarely in tissue culture we will call "a cell line". Now I want to emphasize that I will be defining these two terms in a very precise way but the literature, the scientific literature, is often not very clear on the distinction between these two terms and you

must know more about the author's work before you can understand whether he is working with this type of cell or with this type of cell. So we will now contrast the major properties between a cell strain and a cell line.

The first chracteristic that I have indicated already is the cell strain has finite capacity for replication or multiplication in tissue culture. A cell line, on the other hand, has an infinite capacity for replication. There are a number of these cell lines that are known, perhaps the most popular is the HeLa cells. This is the cell line that is derived from human cervical carcinoma in early 1950s by George Guy of John Hopkins University and this cell has been growing in laboratories throughout the world for these past 25 years. As I indicated that there are approximately 500 or 600 at least of these cell lines that have been described, another famous one is L cell derived from mouse tissue and so forth. The cell lines have developed in laboratories throughout world under spontaneous circumstances and at very low frequencies if you are working with human tissues.

The second property that distinguishes these two types of cells has to do with their karyology, or their chromosome configuration. If you look at the chromosomes of a cell strain derived from normal tissue then if for example the tissue came from your forearm or wrist, then you would expect that these cells would be diploid and for men of course the number of chromosomes would be 46. So depending on the tissue that one shows you would expect the strain to have the

karyotype of the tissue origin, and that is in fact what happens. The cell line, on the other hand, which is HeLa a good example, has a spectrum of chromosome numbers around some modal value with some cells having as few as 50 chromosomes, some having perhaps as many as 350, the modal value is usually in the 70's somewhere, and we refer to this condition as heteroploid.

The third property that distinguishes these two types of cells I will use as a general heading for a number of variables. That is to say, if you look at the following kinds of properties, staining characteristics, biochemical properties, or properties of the cells they are inoculated into a variety laboratory animals, you come to the conclusion that a cell strain has properties of normal cells and that a cell line has properties of abnormal cells, not only are these properties abnormal, but in many cases the properties are properties of cancer cells. So that this phenomenon, where a cell line has resulted, has emerged from a culture of normal cell is a very important phenomenon, and goes under the name of "transformation". This phenomena of transformation was recognized by cell culturists many years ago, but very little could be done about the problem, especially with human tissue because the frequency of transformation was so low that you could not construct experiments to answer important questions about this problem. Since the importance of that was generally recognized that when it occured, it represented the emergence of cancer cells in a population of normal cells in culture. This was

the situation for a number of years until the middle 1960s, when it was discovered that you could transform normal human cells each time if you added to the culture a virus called SV40. This virus has the unique capacity to transform normal human cells into cancer cells. As far as I know this is the only agency by which this can be done with the exception that Dr. Namba and I, when he was working in my laboratory have now evidence that this transformation can be accomplished by adding to the culture a chemical carcinogen,called 4NQO. But aside from these two situations, there is no other way that one can routinely transform normal human cells. There are a number of other viruses that can transform a variety of other cells from other animal species, and these viruses are such as polioma, and some of the adenoviruses etc., are all known as oncogenic or cancer causing viruses.

Now with this information, I would like now to concentrate on the question of the death of normal cells in tissue culture, and what we currently know about this question in respect to the most recent research on this area. But before I go any further, perhaps we can look at some of the slides that I have that will illustrate some of these points that I have been making. Some years ago, we were lucky enough to see one of these spontaneous transformations occurring in a culture of normal human amnion cells. These are human amnion cells here, after one month in culture, these amnion cells which are easily obtainable from hospitals and represent normal human cells, after about one month, we saw in these cultures the

emergence of a transformed population. Here you can see on the left the transformed cells which look very much different from the untransformed or cell strain type of cell here on the right. These cells then have all of the properties of the type of cell I indicated here, the cell line type of cell. Now if you look at the center of one of these islands of transformation and the transformation occurrs in small islands in the culture, you will see a tremendous amount of mitotic activity. When cells divide in tissue culture, they retract their processes, become spherical, go through the mitotic cycle and then become flattened out again. And here you see for example, a mitotic figure with chromosomes on the metaphase slate, here are also representations of cells in mitosis. So one of the features of one of these transformations is the tremendous increase in mitotic activity. Now this amnion was taken from my daughter at her birth, some 16 years ago, so this culture has been in contiunous cultivation in our laboratory and in a number of other laboratories for 16 years.

But I would like now to talk more precisely about normal human fibroblasts which have the greatest capacity for proliferation in tissue culture. This is a semiconfluent culture of normal human fibroblast showing one mitotic figure. After a few more days these cells will become more dense and the confluent condition will exist as you see here, here again as one mitotic figure. But this represents the confluent culture at which time, if you are interested in

obtaining additional cells, is necessary to subcultivate this culture and the subcultivations I described to you before. Compare this slide with the next slide which shows you the way these cells look during phase III. The cells now have completely lost their morphological characteristics. There is a considerable amount of debris in the culture. These then represent cells that are somewhere at this part of the curve. There is still some continuation of metabolic activity but in general over a period of 2 or 3 weeks the cells stop dividing, begin to look unhealthy and then begin to die and in a matter of 4 or 5 or 6 weeks most of the cells are dead. So this is the appearance of cells in culture in phase III.

One of the important points that I would like to remind you of appears in the next slide. Since it forms the basis for an experiment that I would to describe to you, and that is simply to remind you that it is possible to distinguish between male and female fibroblasts in tissue culture by the presence of the bar body in nuclei of female cells, and the absence of the bar body for the inactive exchromosome in a nuclei of male cells. I will say more about that in a few minutes. To those of you who might be interested, it is also possible to clone normal human cells. We can isolate tissue from your forearm, grow the cells out, and then isolate one cell and from that one cell produce progeny as you see here, this is progeny or daughter cells from a single cell, giving rise each of these to a colony or a clone of normal human

cells, which is an important question in respect to some genetic studies. Now if you take normal human cells as you have seen in previous slides and expose it to SV40, and transform them as I have indicated, this is what you see. This is WI-38 which is a normal human diploid cell population that is one of the cell populations that we do much of our work with. This cell of course has a doubling potential of about 50 population doublings and then the cells die. But if you expose the cells to SV40, they become altered or transformed. And this is their appearance, and of course the transformed cells have all these properties.

Now, I would like to give you some further information on the question of the relevance of these observations to aging. One of the most important questions that has been raised about the cultivation of cells with a finite life time in tissue culture is the question of whether the death of these cells is due to some error in laboratory technique; it is possible of course for cells to die in tissue as a result of faulty media or the presence of viruses or any number of extrinsic factors. There have been many experiments that have been done that have essentially excluded this possibility. But I would like to describe just one experiment but, I think, gives the most valuable information on this question. If you take female cells, female normal human cells at the 10th population doubling level (PDL), that is somewhere here, and mixed them with an equivalent number of male diploid cells at the 40th PDL and keeping unmixed control

cultures separate, after the mixture is made, if you wait 30 more subcultivations and look at the cells that remain, you will find that the only cells remaining are female cells. You will also find that the female cell control is still viable. The cells are still growing, because 10 + 30 = 40. You will find however that several weeks before, you looked at the mixture, the male cell component is dead, because it is now somewhere here. Now if the death of the male component is due to media, bad media or viruses or faulty technique, then it would be necessary to argue that this problem could distinguish between male and female cells, which of course we know is not possible. So this kind of experiment, have led us to believe that the death of normal cells in tissue culture is an intrinsic cell phenomenon. There is some kind of clock mechanism within cells which dictate the number of population doublings that they can undergo apparently in vitro and as I will show you later also in vivo.

When we first observed this phenomenon, it was thought that one other possible explanation could be that cells in vivo might be able to produce some essential metabilites that they are unable to produce in vitro. And it was argued that possibly some essential metabolite is being deluded out as the cells traverse this course and when they run out this metabolite, all of the cells will then die. And this hypothesis can be tested by looking at this question from a purely mathematical standpoint. If you assume that the molecule

that must be deluded is the smallest molecule which will be hydrogen, then you ask yourself the question how many hydrogen molecules must be present in this first cell, so that you can get one molecule in the cell at the 50th population doubling, that is at this poing. And if you do the mathematics, you come to the conclusion that each cell initially must weigh at least 3 times more than we know they weigh, and be composed entirely of hydrogen in order for simple a dilution to occur so that you end up with one molecule in each cell. So that we think this explanation is also not a valid explanation.

Now this left us several years ago with the difficult situation of being unable to explain why normal human and animal cells ultimately die in tissue culture. And we thought at first that the suggestion that this was aging at the level of the cell, was a rather interesting possibility but probably not true. And we use this as a method to devise subsequent experiments and I must say that in subsequent years as a result of work in our laboratory and many other laboratories, it appears that this hypothesis may be a valid hypothesis, and I would like to give you some of the recent work that has been done on this question, to show you the relevance of this observation to some of the variables that we know of in the field of aging.

One of technological breakthroughs that allowed us to do some interesting experiments in this field was the possibility of freezing cells or preserving cells for long periods of

time. As you can appreciate, when you culture normal human diploid cell, after 12 months, the cells are gone. It is possible, however, to take the surplus cultures after each population doubling, for example, you would take these two cultures and freeze them, and the next week you will take those two cultures and freeze them, and the third week these cultures and freeze those. So that at each population doubling level you would have frozen in your bank cell cultures from each population doubling to that final period. The important question to be asked is when you remove the cells from liquid N_2 which is where we ordinarily store such cells, what happens to the clock? Do the cells remember at what population doubling they were when you reconstitute them, and continue on, or do they recycle back to the origin, and then go through, or is there some other possibility that happens? And this hypothesis was tested, and I would like to show you some of the results on the next slide. If you reconstitute the cells from a variety of population doublings you generate what looks like a very complicated genealogy or family tree. Here is the culture, the original culture from 0 passage until the 50th passage here, and these vertical lines indicate ampules that have been reconstituted. This is number 5 and that was reconstituted and then grown in tissue culture until it reach Phase III at about 47. There are others you can see here. This one at 14, or 13 was reconstituted, and it died at the 50th population doubling. The important point is that all of the reconstituted

ampules gave rise to cultures that deteriorated or reached Phase III between 40 as you see here, and between 60. So the answer to the question is that the cells do remember at what population doubling they were when frozen and continue to replicate so that the total cumulative number of population doublings is 50, and this is an important point.

Now by increasing the number of cultures each week by multiples of 2, you can easily appreciate that number of populations, the number of cells will increase tremendously. The number of potential cells that can be produced from a culture that has population doubling potential 50, is approximately 20 million metric tons of cells. So WI-38 which was frozen many years ago has been used in laboratories throughout world, and we still have plenty of cells left, because the potential yield is this. This is some of the history of WI-38 which was frozen at the 8th population doubling level, most of it was frozen then, and each time an ampule was reconstituted we gave it a roman numeral as you see here. The number of population doublings that resulted from each thawed ampule is indicated here, average is about 50 and range again between 40 and 60. These are numbers of weeks each culture has been preserved and this is a very old slide, because we now have WI-38 frozen for 12 years, which is over 600 weeks. So WI-38 has been frozen longer than any other normal human cell and, after 12 years, the average is still about 50 and the range between 40 and

60. So the memory of the cells is very good indeed.

Now if what I have been discussing is related to aging, then 50 is the number that is important for human embryonic tissue, but we would like now to know the number of population doublings that normal human adult cells will undergo in tissue culture and this data is indicated on the next slide. Here you see a list of about 12 or 15 human diploid embryonic cultures that have undergone a number of population doublings averages is 50, ranging, between 40 and 60. And here are human diploid cell populations from human adults, these are the number of population doublings, the age of each donor and the number of population doublings possible in the case is 20 and range between 10 and 30. So there is a very dramatic difference between the number of population doublings that adult tissue and embryonic tissue will undergo in culture.

This work was done before we had experience with the details of the knowing how to count these doublings accurately, but a couple years ago Dr. J. Martin from University of Washington did a very interesting study on 100 human skin biopsies taken from individuals who were either embryonic or up to 90 years of age. And he determined for all one hundred the number of population doublings that the fibroblasts from each culture would undergo and I would like to show you this data. The number of cell doublings is indicated here, the age of the donor indicated here on abscissa, the open circles indicate the number of the population doublings that the cultures in each group underwent. And his statistical

analysis indicated that there is a decrement of 0.2 of population doubling per year of life. So the older the person, the fewer number of population doublings are able to be seen.

Now, if this is true for human material, the next question is whether this is a general phenomena that may have some bearing on the life span of other animals, and one of the most interesting questions is to determine the number of population doublings that can be undergone by other animal populations. And I would like to show you this slide. This is work done by four different laboratories with chicken embryo fibroblasts, the number of population doublings as you see, is indicated here, the average about 23.5. Now I should point out at this time that one of the most important experiments done in the tissue culture field that had a bearing on gerontological or aging questions was done with chicken embryo fibroblasts. Many of you will recall the work that was done many years ago, in the 1920s, by A. Carrel who claimed to be able to culture chick heart cells for something like 34, or 35 years. Carrel's work was very important, because if it is possible to culture normal animal cells for periods of time, greatly in excess of their maximum life span, then clearly aging is not a result of decrements in the function of individual cells but must be a result of actions taking place at higher levels in the single cell, that is tissue level or organ level. So that the experiment of Carrel is very important. Because it

contradicts what has been found recently. We now know the explanation for the cultures of Carrel that lived for 34 years. And the explanation is the following. The culture media that was used at that time was prepared from chick embryo extract (CEE) and that CEE was prepared and then centrifuged in such a way that many cells were still floating in supernatant fluid, so that when you put a pipette into the centrifuge tube, you took up centrifuge tube in the pipette new fibroblasts each time you fed the culture, that was supposed to be grown for 34 years. And this is generally regarded to be the explanation for this contradiction. No one has ever been able to confirm Carrel's work even to culture normal chick fibroblasts for 2 or 3 years.

The next question then is the number of population doublings that cells from other species will undergo, and here you see some of this data. For man as I have indicated the range is between 40 and 60. For embryonic fibroblasts the maximum life span about 110 years, let's say, chicken material between 15 and 35, the maximum life span about 30 years, the mouse between 14 and 28 population doublings and the maximum life span thought to be about 3.5 years, the Galapagos tortise, this work was published very recently by Goldstein, the range of population doublings between 90 and 125, the maximum life span is thought to be about 175 years. So there does appear to be some relationship between the age of the mean maximum life span which is reflected in a direct way in the number of population doublings, and the age of

the donor which is reflected in an inverse way, in respect of the age of the donor.

Now I would like to mention very briefly that it has been possible to repeat this kind of work in animals where the same situations seen in tissue culture has also resulted. If you take tissue from a laboratory animal, for example, the mouse, and this has been done for a variety of tissues from mice and also other animals and transplant this normal tissue, and this can be done, then you will find that the number of transplants that can be effected are finite, 4 or 5 or something of this sort, whereas the continuous transplantation of cancer cells of course can occur indefinitely. So that there is now in vivo evidence for this in vitro phenomena. I would like to say in ending here that I do not believe that animal's or individual's age, because their cells stop dividing. What has been discovered recently is that as the cells age in tissue culture roughly in this period, there occur a number of dramatic biochemical changes resulting in physiological decrements that I think are much more important in respect to our understanding of aging phenomena than the fact that the cells ultimately stop dividing. I don't have time to discuss all of these biochemical changes but I thought I would just show you a list of them which is incomplete list, to give you some idea of the amount of work that has been done in this area and without discussing each of these individually, but you can see some of parameters increase, other decrease, some do not change.

Finally I would like to summarize by giving you some information on three of the current theories of aging, since these current theories also are applicable for possible explanations of the Phase III phenomenon in tissue culture. There are many theories of aging, but I think the three most important ones are as follows; One is that there are specific genes for aging. That is in the developmental sequence in animals as developmental changes are programed by genetic information. Some people think that at the very end there are specific genes that shut down the metabolic machinary and force the cell to close down its functional reproductive capacities. Another possibility is that there is simply a loss of genetic information, that is accurate genetic information, that as developmental sequences occur, infomation containing molecules may in fact incur errors and as these errors accumulate, they may accumulate for a period of time, and then they reach a point where the whole cell stops dividing or stops functioning and these age changes occur. Of course there are methods for repairing errors in biological material but as we all will appreciate these repair mechanism are also, in themselves, not perfect. The final information that I would like to give you is the possibility that when the DNA molecule, the redandacy of message, may be directly related to the longevity of a particular species. If the same message was repeated many times in information containing molecules and if errors accumulated here, such that this information after a period of time became invalid of nonsense information, then the next sequence containing the same bit of information could take over, and this could occur again. So that the number of times that the redandant message appeared could be directly related to the longevity of the species, and many people feel that the evolution of the maximum life span of individual species, which is much more specific than the life span of individual members of a species, that this evolution occured following lines of this sort.

1975 年 Bibliography 原 稿 募 集

本年もBibliography 編集の季節になりました。下記の如く原稿を募集致しますので，1974年の年号のついた論文の英文抄録を漏れなくお送り下さい。

1. 〆　　切 ： 昭和50年11月15日
2. 宛　　先 ： 東京都中央区築地5－1－1
   （〒104）　国立がんセンター・
   生化学部・　佐 藤 茂 秋
3. 執 筆 要 綱 ： 昨年，一昨年に同じ。（写真印刷致しますので，抄録は電動タイプ（打抜きカーボン紙）を使用し，スペース内に必らず治めて下さい。）
4. 抄 録 用 紙 ： 本会員通信に各一部同封致しますが，余分に必要の方は 佐藤茂秋宛お知らせ下さい。

毎年のことながら，原稿の集まりが悪くて編集者一同発行に際し苦労致します。本年こそは，1回の原稿募集でBibliography が発行出来る様，皆様の御協力をお願い致します。

昭和50年7月

乾　記

日本組織培養学会第40回研究会

1. 会　　場：独協医科大学講堂
〒321-02 栃木県下都賀郡壬生町大字北小林880
2. 日　　程：10月24日(金) 12時30分より一般講演。夜，懇親会
10月25日(土) 9時より一般講演。Workshop
3. Workshop：培養技術を中心に次の3つの課題に分けて行います。奮って御応募下さい。
① 肝細胞培養
② リンパ球：T・B細胞分離培養
③ 骨髄細胞培養
4. 参加費：会員・学生500円，非会員800円
5. 懇親会費：1,000円
6. 講演申込み〆切：同封の講演申込み票（一枚）に記入のうえ，8月25日(月)までに送付して下さい。
7. 抄録原稿〆切：同封の抄録用原稿用紙（一枚）に黒インク楷書で記入のうえ，9月10日(水)までに必着のこと。（期限厳守）
8. 講演時間：講演，質疑応答を含めて一人30分を予定しています。
9. 宿　　舎：宿舎案内（別項）を参考にして各人で早目に申込んで下さい。
なお，抄録内容によっては一般講演又はWorkshopは変更されることがありますので御了承下さい。
10. 申込み先および連絡先：〒321-02 栃木県下都賀郡壬生町大字北小林880
独協医科大学第一病理学教室 山田　　番
TEL（02828）6-1111，内線 2180

キリトリセン

日本組織培養学会第40回研究会講演申込み票

〒321-02 栃木県下都賀郡壬生町大字北小林880 独協医科大学第一病理学教室 山田 番
一般講演・Workshop（御希望の方に○印をつけて下さい）

演　　題：

（ふりがな）
発表者氏名：（演者に○印をつけて下さい）

連絡先：

プロジェクター：（1台しか使用できません）

16㎜映写機：要　　不要　（いずれかに○印をつけて下さい）

その他の希望事項：

〆切日：8月25日(月)