ROUTINE AUTORADIOGRAPHIC ANALYSIS OF DNA EXCISION-REPAIR

Report of prenatal and postnatal diagnosis in eleven families

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SAVARY J.B., VASSEUR F., DEMINATTI M.M. – Routine autoradiographic analysis of DNA excision-repair. Report of prenatal and postnatal diagnosis in eleven families.

Ann Génét, 1991, **34,** n° 2, 76-81.

SUMMARY : DNA excision-repair of UV induced damages was investigated by unscheduled DNA synthesis and quantitative autoradiography. The method has been routinely used on lymphocytes for postnatal diagnosis of xeroderma pigmentosum and PIBIDS syndrome. Ten XP-families including 13 clinical XP patients and 9 XP-risk children, and one family with one clinical PIBIDS case and one PIBIDS-risk child were screened. Each of the 14 affected patients were biologically ascertained with a significant excision-repair defect. Among the 9 XP-risk children without clinical manifestations, the DNA excision-repair was defected in 4 cases considered as biological XP, and normal in 5 cases considered as biologically normal subjects. Likewise the PIBIDS-risk child exhibited a normal excision-repair. According to the age of the XP or PIBIDS-risk children, and the delay of appearance of clinical manifestations, the method should not present neither false positive nor false negative results and allows the infraclinical diagnosis. The protocol was extended for prenatal diagnosis on amniocytes and fetal cord blood. Excision-repair analysis on normal cultivated chorionic villi cells has been performed allowing a further first trimester prenatal diagnosis.

KEY-WORDS : DNA excision-repair. – Xeroderma pigmentosum. – PIBIDS. – Prenatal diagnosis. – Chorionic villi.

SAVARY J.B., VASSEUR F., DEMINATTI M.M. – Analyse autoradiographique en routine de l'excision réparation de l'ADN. Diagnostic prénatal et postnatal, à propos de onze familles. *(En Anglais).*
Ann Génét, 1991, **34,** n° 2, 76-81.

RÉSUMÉ : L'excision-réparation de lésions de l'ADN induites par les UV a été étudiée par la synthèse d'ADN non programmée et par autoradiographie quantitative. La méthode a été appliquée en routine sur lymphocytes au diagnostic postnatal du xeroderma pigmentosum et du syndrome de PIBIDS. Dix familles XP regroupant 13 patients cliniquement atteints et 9 enfants à risque, ainsi qu'une famille présentant un cas clinique de PIBIDS et un enfant à risque, ont été analysées. Les 14 sujets atteints ont été confirmés sur le plan biologique par des déficits significatifs de l'excision-réparation. Parmi les 9 enfants présentant un risque mais sans manifestations cliniques de XP, l'excision-réparation de l'ADN est déficitaire dans 4 cas qui sont considérés comme des sujets XP sur le plan biologique, et normale dans les 5 autres cas considérés comme normaux sur le plan biologique. De même, l'enfant à risque pour le PIBIDS a présenté une excision-réparation normale. Compte tenu de l'âge des sujets à risque, et du délai d'apparition des signes cliniques, la méthode ne semble présenter ni faux positifs ni faux négatifs et permet un diagnostic infraclinique. Le protocole a été appliqué au diagnostic prénatal sur amniocytes et sang fœtal prélevé au cordon. Une étude de l'excision-réparation a été effectuée sur une culture de cellules trophoblastiques normales laissant entrevoir la possibilité de diagnostic prénatal au cours du premier trimestre.

MOTS-CLÉS : Excision-réparation. – Xeroderma pigmentosum. – PIBIDS. – Diagnostic prénatal. – Villosités choriales.

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INTRODUCTION

Environmental carcinogen or mutagen agents may corrupt experimentally or spontaneously the genetic material. Enzymatic mechanisms normally repair structural damages and restore the original genetic information. In most cells of most tissues, at least three different repair systems can operate to save DNA from permanent damage (Cleaver, 1974 ; Cleaver, 1980) :

1) photoreactivation (direct reversal repair),

2) excision-repair (elimination of damage before replication),

3) post replication repair (replication on damaged DNA). These systems, especially excision-repair, are particularly important in the skin where they restore DNA damages caused by the ultraviolet irradiation. Induced DNA damages and *in vitro* repair studies have pointed out a pathology of excision-repair. Cleaver (1968) described the first case of excision-repair defect in skin fibroblasts from patients with xeroderma pigmentosum (XP), and this was afterward confirmed on epidermal cells (Epstein et al., 1970), and on lymphocytes (Burk et al., 1971). Since, the deficient DNA repair has been reported in other dermatological diseases : hydroa vacciniforme (Andrews et al., 1985), PIBIDS syndrome which is the abbreviation of photosensitivity - Ichtyosis - Brittle hair - Intellectual impairment - Decreased fertility - Short stature (Van Neste et al., 1985), trichothiodystrophy (Stefanini et al., 1987), basal cell carcinoma (Vasseur et al., 1989) and one case of Rothmund Thomson (Vasseur et al., 1990).

Two types of methods have been used for the quantitation of DNA excision-repair. All are based upon a repair incubation, following artificial *in vitro* DNA damages induced either by a 254 nm UV-C irradiation (Cleaver, 1968), or by chemical agents as 4-nitroquinoline-1-oxide (Walker, 1981), N-acetoxy-2 acetylaminofluorene (Ahmed and Setlow, 1979), aflatoxin B_1 (Leadon et al., 1981). The first pattern of methods which reveal repaired DNA lesions, used either the incorporation of labelled nucleotides in repair patches, i.e. : unscheduled DNA synthesis (Rasmussen and Painter, 1964 ; Cleaver, 1968), or host cell reactivation of UV irradiated viruses in human cells (Rabson et al., 1969 ; Lytle et al., 1982). The second pattern of methods reveal unrepaired DNA lesions, i.e. : biochemical procedures as alkaline sucrose sedimentation (Walker, 1981), classical chromatography for UV photoproducts detection as pyrimidine dimers (Günther and Prusoff, 1967) or high pressure liquid chromatography of carcinogen-DNA adducts (Leadon et al., 1981) ; enzymatic procedures as UV photoproducts detection with *micrococcus luteus* endonuclease (Fornace, 1982) ; immunological disclosure of DNA damages (Plascia and Braun, 1967 ; Munns and Liszewski, 1980) and biological procedures using *in vitro* induction of mutations (Glover et al., 1979).

In this paper we report adaptations of UV-induced unscheduled DNA synthesis (UDS) previously described by Cleaver (1968) or Burk et al. (1971). These protocols have been used routinely for postnatal detection of DNA excision-repair defects on lymphocytes from clinical affected patients and their unaffected collaterals considered as risk-patients. The method was then extended to prenatal diagnosis on amniotic fluid cells, fetal cord blood, and chorionic villi.

MATERIAL AND METHODS

In postnatal diagnosis purpose, DNA excision-repair has been investigated by UV induced UDS on lymphocytes, and compared with normal cells. In 10 XP-families, 13 patients with classical clinical XP and 9 XP-risk children were screened. In one PIBIDS family, one affected girl and her elder unaffected sister (PIBIDS-risk child) were analyzed too.

In prenatal diagnosis purpose, UDS has been performed in one XP-family and the PIBIDS-family. Amniocytes for the XP- (2 cases) and the PIBIDS-detection (1 case), fetal cord blood cells for the PIBIDS-detection (1 case), have been compared with either normal amniocytes or normal lymphocytes. DNA excision-repair was investigated on a primary culture of a normal chorionic villi sample too.

UDS on blood cells and fetal cord blood cells

UDS was performed without culture. Lymphocytes from heparinized blood samples were suspended into 0.5 ml aliquots of TC 199 medium containing 20 % fetal calf serum (FCS). *In vitro* DNA damages were induced by UV exposure under a 6 watts, 254 nm UV-C lamp (Bioblock). Cells were irradiated with various UV doses ranging from 0 up to 20 J/m2, as measured by a VLX 254 radiometer (Bioblock). DNA repair incubation with 5 μCi/ml 3H thymidine (specific activity 83 Ci/mmole, Amersham), was performed for a 3 hour period in 20 % FCS TC 199. Then an excess of unlabelled thymidine was added one hour before harvesting. Cells were washed 3 times with TC 199, fixed twice in ice cold methanol acetic acid (3:1) and spreaded on slides.

UDS on skin fibroblasts, amniocytes and chorionic villi cells

UDS required cultures and subcultures. Primary cultures were initiated in Chang medium (Hana Biologic INC). After one trypsinization, a subculture was obtained on slides set into 3 cm petri dishes containing 20 % FCS RPMI medium. In order to label S-phases, cells were incubated for a 1 hour period with 1 % FCS TC 199 containing 5 μCi/ml 3H thymidine (labelled TC), then UV irradiated from 0 up to 50 J/m2. DNA repair incubation was achieved in labelled TC with 10 mM hydroxyurea (Sigma) for a 3 hour period. One hour before harvesting, an excess of unlabelled thymidine was added. Slides were then washed 3 times, immersed 15 min in methanol acetic acid (3:1) and air-dried.

TABLE I. — **Excision-repair postnatal data following a 6J/m2 UV irradiation on lymphocytes from patients among 10 XP-families and one PIBIDS-family. Excision-repair values are expressed in relation with normal cells assumed as the 100 % reference level.**

Family	XP Diagnosis	XP risk	PIBIDS diagnosis	PIBIDS risk	Age (year)	% excision-repair (6J/m2 UV-irradiation)	Biological diagnosis
XP-I	case 1	case 2			5 1	49 17	XP XP*
XP-II	case 3 case 4	case 5 case 6 case 7 case 8 case 9			12 4 17 15 7 2 1	25 28 100 100 100 36 100	XP XP normal normal normal XP* normal
XP-III	case 10	case 11			4 3	48 56	XP XP*
XP-IV	case 12				6	27	XP
XP-V	case 13	case 14			4 10	20 100	XP normal
XP-VI	case 15				22	25	XP
XP-VII	case 16				20	25	XP
XP-VIII	case 17 case 18				13 12	50 36	XP XP
XP-IX	case 19 case 20				15 19	18 16	XP XP
XP-X	case 21	case 22			3 1	23 25	XP XP*
PIBIDS-XI			case 23	case 24	7 11	12 100	PIBIDS normal
Control						100	normal

XP is classical xeroderma pigmentosum. PIBIDS is Photosensitivity, Ichtyosis, Brittle hair, Intellectual impairment, Decreased fertility, Short stature. XP* is early biological XP diagnosis without clinical manifestations.

Autoradiographic analysis

Autoradiographic studies and quantitation of UDS were standardized whatever the investigated tissue may be. Preparations were washed first 3 times in 2 x SSC (1 x SSC is 0.15 M NaCl and 0.015 M sodium citrate) containing 10 mM sodium pyrophosphate pH 7, and second with distilled water. Slides were air-dried after dehydratation in an ethanol serie (70 %-90 %), and then dipped in Kodak NTB 2 (Eastman Kodak) or K5 (Ilford) liquid nuclear emulsion, stored 4 °C for a 3-30 day exposure. Autoradiographies were processed according to the supplier's protocol. Quantitative analysis of UDS was a previously described (Vasseur et al., 1990) : number of grains per cell was averaged from 50 random cells before irradiation (background value = I_1) and after UV irradiation (I_2). Specific excision-repair label (I_2-I_1) was compared with normal cells assumed as 100 % reference level.

RESULTS

In our procedures, induced UV damages were repaired in a 3H thymidine containing medium. Autoradiographic results reflected the amount of repaired patches. In normal repairing cells, one might expect as a rule, strong labelled cells after UV-induced UDS and a weaker label in repair defected cells.

Postnatal diagnosis purpose

Postnatal diagnosis of excision-repair was performed on lymphocytes (table I). The results are presented as percentage from normal cells processed in each experiment. In our 10 XP-families, DNA excision-repair was defected in the 13 clinical XP cases with excision-repair levels ranging from 16 % (case 20) to 50 % (case 17). Among the 9 XP-risk children,

TABLE II. – **Prenatal XP and PIBIDS data of excision-repair investigations. Excision-repair is expressed as a percentage from normal cells assumed to be the 100 % reference level.**

Family	Pregnant subject	Investigated tissue	% excision repair	Fetal diagnosis
XP-VI	case 15 pregnancy 1 pregnancy 2	 amniocytes amniocytes	 100 100	 unaffected unaffected
PIBIDS-XI	mother of case 23	amniocytes + fetal blood + skin fibroblasts (aborted fetus)	12.5 18.5 13.5	} = affected ascertained

TABLE III. – **Results of UDS on a primary culture of a chorionic villi sample after 2J/m2 (a), 5J/m2 (b), 10J/m2 (c) and 30J/m2 (d) UV irradiations.**

	Average number of grains per cell		
Procedures	before irradiation (background) (I_1)	after irradiation (I_2)	specific excision repair label ($I_2 - I_1$)
(a)	2.88	24.7	21.82
(b)	2.88	71.0	68.12
(c)	2.88	82.7	79.82
(d)	2.88	113.4	110.52

DNA excision-repair levels were 17 % (case 2), 36 % (case 8), 56 % (case 11) and 25 % (case 22). These 4 cases were considered as biological XP with significant excision-repair defect. The 5 remaining XP-risk cases (case 5-6-7-9-14) presented 100 % excision-repair levels. In the PIBIDS family, DNA excision-repair was defected in the affected child with a 12 % residual activity (case 23) and normal in the PIBIDS-risk child with a 100 % activity (case 24).

Prenatal diagnosis purpose

In XP-VI family, two prenatal diagnoses were performed on amniocytes from case 15 (table II). In both pregnancies, excision-repair levels were 100 % and fetuses were diagnosed as unaffected. In PIBIDS-XI family, amniocytes and fetal cord blood from mother of case 23 (table II) were investigated. Results were in agreement with an affected fetus with a 12.5 % excision-repair level on amniocytes and 18.5 % on fetal blood cells. Parents opted for termination of pregnancy. Excision-repair analysis on skin fibroblasts from the aborted fetus, ascertained the prenatal diagnosis with a 13.5 % residual repair activity.

Early prenatal excision-repair investigations have been achieved on one normal chorionic villi sample (table III). Before UV Irradiation, the background (I_1) was 2.88 grains per cell. With 2-5-10 and 30 J/m2 UV doses, average number of grains (I_2) were respectively 24.7-71-82.7 and 113.4 per cell. Specific excision-repair labels (I_2-I_1) were 21.82 - 68.12 - 79.82 and 110.52 grains per cell.

DISCUSSION

DNA damages induced by natural or artificial UV-light, are normally repaired by biological mechanisms (i.e. excision-repair). It has been previously described an excision-repair defect disease : xeroderma pigmentosum associating premature ageing of sun exposed areas, and a high rate of skin malignancy (Cleaver, 1968). Quantitation of DNA excision-repair has been performed on various tissues, and biological diagnosis of excision-repair defect is easily available. Among DNA damaging methods we opted for the UV-C irradiation instead of carcinogen or mutagen agents.

TABLE IV. – **Comparison of fibroblast and lymphocyte autoradiographic backgrounds with and without post-incubation in unlabelled thymidine containing medium.**

Experiment number	Cell type	Background levels (grains per cell)		Background decrease
		Without post-incubation in unlabelled thymidine	with post-incubation in unlabelled thymidine	
1	fibroblasts	20.5		
2	fibroblasts	19.9	$\bar{n} = 33.85$	
3	fibroblasts	52		
4	fibroblasts	43		89.7 %
5	fibroblasts	–	3.29	
6	fibroblasts	–	3.64 $\bar{n} = 3.46$	
7	fibroblasts	–	3.46	
8	lymphocytes	5.7		
9	lymphocytes	7.5	$\bar{n} = 7.67$	
10	lymphocytes	8.5		
11	lymphocytes	9		85.7 %
12	lymphocytes	–	1.12	
13	lymphocytes	–	1.09 $\bar{n} = 1.09$	
14	lymphocytes	–	1.07	

$\bar{n}$: average number.

Thus our protocol may be routinely and safety used without chemical poison and avoid the retentivity of the damaging effect of such compounds. Unscheduled DNA synthesis with 3H-thymidine incorporation in repair patches, and quantitative autoradiography do not require any specific equipment. They reflect the DNA excision-repair at the cell level better than global methods. Although excision-repair has been investigated on interphase-cells, scheduled DNA synthesis may interfere because S-phase cells incorporate thymidine too. This disadvantage does not occur with non stimulated adult lymphocytes. With dividing cells (monolayer cultures - fetal blood cells). The problem was outlined with a prelabelling before UV irradiation because replicating cells appeared heavily labelled and are discarded from our interpretation. During repair incubation, free 3H-thymidine in the cells (pool of unincorporated thymidine) produces the biological background. This may somewhat decrease the sensitivity of the method, especially in slight excision-repair defects. As shown in table IV, a post-incubation with unlabelled thymidine reduced backgrounds in fibroblasts (87.7 %) or lymphocytes (85.7 %). Indeed the unlabelled thymidine replaces the free 3H thymidine in the pool. Without post-incubation in unlabelled thymidine, backgrounds were significantly different between fibroblasts (33.85 grains per cell) and lymphocytes (7.67 grains per cell). This may be a consequence of a difference in cell permeability, and may explain our longer autoradiographic exposure on lymphocytes (30 days) as compared with cultivated cells (3 days).

In our post-natal investigations, biological results ascertained the 13 clinical XP diagnoses. Among the 9 XP-risk children, 4 cases were biological XP and 5 cases were biologically normal. These data seem to be neither false positive nor false negative results, according to the age of affected or unaffected patients (table I), and the delay of appearance of the disease in these families. The 4 biological XP children with significant excision-repair defect, may be considered as potentially clinical XP. Likewise, one might expect that the 5 XP-risk children with normal excision-repair, will remain clinically unaffected with classical XP. Similar data were obtained in the PIBIDS-XI family. So early infraclinical diagnosis of excision-repair defect is available. Nevertheless it must be kept in mind that as previously described (Vasseur et al., 1990), some XP-risk cases may be XP-carriers presenting on lymphocytes a 100 % excision-repair with low doses UV irradiation but a slight defect with high rates of UV.

The method appeared suitable for biological excision-repair diagnosis. Prenatal diagnosis of XP on amniocytes was pointed out by Regan and Setlow (1971), and first performed by Ramsay et al. (1974). Our results are in agreement with those previously reported (Halley et al., 1979 ; Barthélémy et al., 1983 ; Arase et al., 1985). Excision-repair on amniocytes may be either normally expressed discarding XP (XP-VI family, case 15), or defected allowing a positive prenatal diagnosis (PIBIDS-XI family, mother of case 23). As shown in table II, excision-repair has been investigated on fetal blood cells too. Results are in accordance with those of amniocytes, and have been ascertained on fetal skin fibroblasts after therapeutic abortion. According to the time of cordocentesis or amniocentesis, and the various delay of autoradiographic exposure (30 days for blood cells - 3 days for amniocytes), prenatal diagnosis of excision-repair is achieved earlier with amniocentesis, although requiring a primary culture. Further

improvement in prenatal diagnosis would be the excision-repair analysis on chorionic villi sample. Effectively, our results on cultivated chorionic villi cells show an expression of excision-repair (fig. 1). The specific excision-repair label enhances with increasing UV doses (table III) as in other various tissues studied. Thus, chorionic villi is quite suitable for a first trimester diagnosis of excision-repair defect.

NOTE ADDED IN PROOF

Case 8 from XP-II family was biologically XP without clinical features at 2 years old. At this time, 4 years old, this child presents the first clinical manifestations of XP.

ACKNOWLEDGEMENTS

Authors thank Pr Thomas (Service de Dermatologie B, Hôpital Huriez, CHR de Lille), Pr Bergoend (Service de Dermatologie A, Hôpital Huriez, CHR de Lille), Pr Bonnetblanc (Service de Dermatologie, CHR de Limoges), Pr Meynadier (Service de Dermatologie, CHR de Montpellier), Pr Bazex (Service de Dermatologie de Toulouse), Pr Lauret (Service de Dermatologie, CHR de Brest), Pr Bonerandi (Service de Dermatologie, CHR de Marseille), Pr Carli Basset (Service de Dermatologie, CHR de Tours), Pr Farriaux (Service de Pédiatrie, CHR de Lille), Pr Mallet (Service de Pédiatrie, CHR de Rouen), for allowing us to study their patients.

Authors wish to thank Pr Boekolt for correcting English manuscript.

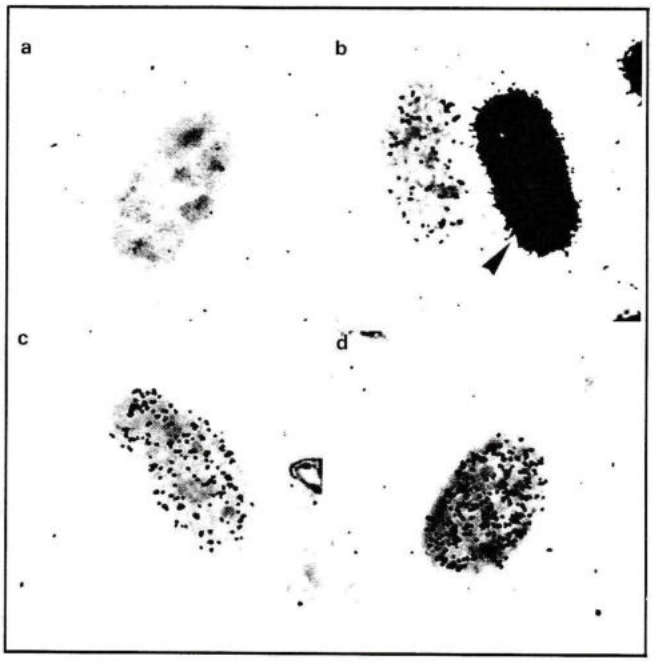

Fig. 1. – Autoradiographic analysis of excision-repair on normal cultivated chorionic villi cells. Unirradiated cells showing background (a), and following a 5J/m2 (b), 10J/m2 (c), 30J/m2 (d) UV-irradiation. Heavily labelled cell is S-phase (arrow). Note increasing label with increasing UV doses.

REFERENCES

1. AHMED E.F., SETLOW R.B. – DNA repair in xeroderma pigmentosum cells treated with combinations of ultraviolet radiation and N-ace toxy-2-acetylaminofluorene. *Cancer Res,* 1979, *39,* 471-479.
2. ANDREWS A.D., HALASZ C.L.G., POH-FITZPATRICK M.B. – Abnormally low UV-induced unscheduled DNA synthesis in cells from a patient with hydroa vacciniforme. *Photodermatology,* 1985, *2,* 315-318.
3. ARASE S., BOHNERT E., FISCHER E., JUNG E.G. – Prenatal exclusion of xeroderma pigmentosum (XP-D) by amniotic cell analysis. *Photodermatology,* 1985, *2,* 181-183.
4. BARTHELEMY H., CLAUDY A.L., LAURAS B., FREYCON F. – Prenatal diagnosis of Xeroderma pigmentosum. *Arch Fr Pediatr,* 1983, *40,* 198.
5. BURK P.G., LUTZNER M.A., CLARKE D.D., ROBBINS J.H. – Ultraviolet-stimulated thymidine incorporation in xeroderma pigmentosum lymphocytes. *Lab Clin Med,* 1971, *77,* 759-767.
6. LEAVER J.E. – Defective repair of DNA in xeroderma pigmentosum. *Nature,* 1968, *218,* 652-656.
7. CLEAVER J.E. – Repair processes for photochemical damage in mamalian cells. *In :* Lett J.T., Adler H., Zelle M. *Advances in Radiation Biology, 4,* p. 1, New York, Academic, 1974.
8. CLEAVER J.E. – DNA damage, repair systems and human hypersensitive diseases. *J Environ Pathol Toxicol,* 1980, *3,* 53-68.
9. EPSTEIN J.H., FUKUYAMA K., REED W.B. – Defect in DNA synthesis in skin of patients with xeroderma pigmentosum demonstrated *in vivo. Science,* 1970, *168,* 1477-1478.
10. FORNACE A.J. Jr – Measurement of *Micrococcus luteus* endonuclease sensitive lesions by alkaline elution. *Mutat Res,* 1982, *94,* 263-276.
11. GLOVER T.W., CHANG C.C., TROSKO J.E., LI S.S. – Ultraviolet light induction of diphteria toxin-resistant mutants of normal and xeroderma pigmentosum human fibroblasts. *Proc Natl Acad Sci,* 1979, *76,* 3982-3986.
12. GÜNTHER H.L., PRUSOFF W.H. – Protective effect of 6-azathymidine and 6-azauracil against the lethal effect of ultraviolet irradiation. II Isolation of thymine photoproducts from the nucleic acids of *Streptococcus faecalis. Biochim Biophys Acta,* 1967, *142,* 304-313.
13. HALLEY D.J., KEIJZER W., JASPERS N.G., NIERMEIJER M.F., KLEIJER W.J., BOUE J., BOUE A., BOOSTMA D. – Prenatal diagnosis of xeroderma pigmentosum (group C) using assays of unscheduled DNA synthesis and postreplication repair. *Clin Genet,* 1979, *16,* 137-146.
14. LEADON S.A., TYRRELL R.M., CERUTTI P.A. – Excision-repair of aflatoxin B1-DNA adducts in human fibroblasts. *Cancer Res,* 1981, *41,* 5125-5129.
15. LYTLE C.D., NIKAIDO D., HITCHINS V.M., JACOBSON E.D. – Host cell reactivation by excision-repair is error free in human cells. *Mutat Res,* 1982, *94,* 405-412.
16. MUNNS T., LISZEWSKI K.T. – Antibodies specific for modified nucleosides : an immunochemical approach for the isolation and the characterization of nucleic acids. *Prog Nucl Acid Res Mol Biol,* 1980, *24,* 109-165.
17. PLESCIA O.J., BRAUN W. – Nucleic acids as antigens. *Advanc Immunol,* 1967, *6,* 231-252.
18. RABSON A.S., TYRRELL S.A., LEGALLAIS F.Y. – Growth of ultraviolet damaged herpes virus in xeroderma pigmentosum cells. *Proc Sox Exp Biol Med,* 1969, *132,* 802-806.
19. RAMSAY C.A., COLTART T.M., BLUNT S., PAWSEY S.A., GIANELLI F. – Prenatal diagnosis of xeroderma pigmentosum. Report of the first successful case. *Lancet,* 1974, *ii,* 1109-1112.
20. RASMUSSEN R.E., PAINTER R.B. – Evidence for repair of ultraviolet damaged deoxyribonucleic acid in cultured mammalian cells. *Nature,* 1964, *203,* 1360-1362.
21. REGAN J.D., SETLOW R.B. – Xeroderma pigmentosum : a rapid sensitive method for prenatal diagnosis. *Science,* 1971, *174,* 147-150.
22. STEFANINI M., LAGOMARSINI P., GIORGI R., NUZZO F. – Complementation studies in cells from patients affected by trichothiodystrophy with normal or enhanced UV photosensitivity. *Mutat Res,* 1987, *191,* 117-119.
23. VAN NESTE D., CAULIER B., THOMAS P., VASSEUR F. – PIBIDS : Tay's syndrome and xeroderma pigmentosum (Letter). *J Am Acad Dermatol,* 1985, *12,* 372-373.
24. VASSEUR F., FONTAINE F., CAULIER B., SAVARY J.B., THOMAS P., DEMINATTI M.M. – DNA excision-repair analysis on leukocytes. Early diagnosis of xeroderma pigmentosum (XP) and prenatal diagnosis. *J Inv Dermatol,* 1989, *92,* 147.
25. VASSEUR F., THOMAS P., CAULIER B., SAVARY J.B., DEMINATTI M.M. – Apport de l'étude de l'excision-réparation dans les génophotodermatoses. *Nouve Dermatol,* 1990, *9,* 424-430.
26. WALKER I.G. – Alkaline sucrose sedimentation analysis as an indicator of repair capability of xeroderma pigmentosum fibroblasts for 4-nitroquinoline-1-oxide damage. *Carcinogenesis,* 1981, *2,* 691-695.