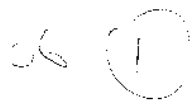


# Biolab Medical Unit

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Biolab reference: MAWL/SROS/D06

Patient: MR JOHN GRAHAME HOYTE

Date: 26-04-2006

DOB: 17-10-1955

Your reference:

Sex: MALE

Doctor: DR S MACKENZIE ROS Sample Date: 26-04-2006

SUB-DEPT OF CLINICAL

SAMPLE:	TEST:	RESULT:	UNITS:	REFERENCE RANGE:
LEUCOCYTE	MAGNESIUM	46	ng/1000	42 - 76
LEUCOCYTE	ZINC	6.4	ng/mil.cell	5.4 - 8.2
FUNCTIONAL	SODase INHIBITION	35	%	>40

Stephen Davies MA BM BCh FACN

John McLaren-Howard D.Sc. FACN

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Biolab reference: MAWSC/S268/DO6 Patient: Mr John Graham Hoyte

Date: 26-04-2006

Doctor: Dr S Mackenzie-Ross

## PESTICIDES AND RELATED SUBSTANCES IN FAT CELLS

Please note: these tests are performed for medical purposes only. We cannot enter into legal disputes. (In the table, PLOA is the provisional Limit of Acceptability based on reference data from 57 controls. We do not have this data for all of the substances that may be found but we add extra comments where we can).

The following pesticide-related substances were detected in this FAT CELL sample:

(Note the units, mg/kg, relate to 1000X the level used for serum levels)

NAME (some trade names are listed over page)	mg/kg	Note over page	PLOA and control range (n = 57) (*may be hazardous at ANY level ND = not detected).
Aldrin	ND	1	< 0.01 (0 - 0.013)
Dieldrin	TRACE	1	< 0.04 (0 - 0.05)
Lindane	0.24	1	< 0.05 (0 - 0.05)* ←
DDT	0.06	1	< 0.18 (0.02 - 0.21)
DDE	0.14	1	< 0.21 (0.03 - 0.23)
DDD	0.07	1	< 0.04 (0 - 0.04)
HCB	0.41	1	< 0.55 (0.03 - 0.57)
MIREX	0.03	1	< 0.05 (0 - 0.05)
TOXAPHENE	TRACE	1	< 0.07 (0 - 0.08)
PENTACHLOROPHENOL	0.44	2	< 0.18 (0 - 0.21) → ←
TRICHLOROPHENOL(S)	0.10	2	< 0.08 (0 - 0.07)
p-DICHLOROBENZENE	0.21	2	< 0.42 (0.01 - 0.52)
o-DICHLOROBENZENE	0.03	2	< 0.05 (0 - 0.06)
DICHLOROPROP (2,4-D)	ND		< 0.20 (0 - 0.21)*
PCBs (total)	0.14	1	< 0.30 (0.02 - 0.41)
PBBs(BDE) FLAME RETARDANTS	0.37	*...*	< 0.05 (0.01 - 0.08) ←
ORGANOPHOSPHATES (TOTAL)	0.19	3	< 0.10 (0 - 0.19)*
CARBARYL (SEVIN)	ND	4	0.15 (0 - 0.16)
a-NAPHTHOL (FROM CARBARYL)	ND	4	< 0.03 (0 - 0.04)*
CARBAMATES (TOTAL)	TRACE	4	< 0.28 (0 - 0.30)
PROBABLY THE CARBARYL PHOSPHATE		3	ND

See over page for a list of the major pesticides that have been looked for using gas-liquid chromatography. The list is not exhaustive. Some other pesticides may not be detected but it may be impossible for us to identify some chemicals that are found. If this occurs it will be noted above.

Nanogen<sup>TM</sup> MX1 and MX3 pesticide quality control materials are run routinely. Additional individual pesticide standards are prepared in our own laboratory.

Further comments (if appropriate):

Please see additional report on blood-cell-membranes. Test added without charge to pursue the above.\*

Dr John McLaren Howard DSc FACN

Dr Stephen Davies MA BM BCH FACN

Pestrep.doc

\* ALSO, TRANSLUCATION STUDIES FOR FOC.

JMA

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Biolab reference: MAWC/1205/006

Patient: Mr Iden Graham Hoyte

Date: 26-04-2006

Doctor: Dr S Mackenzie Kerr.

## VOLATILE ORGANIC COMPOUNDS IN FAT CELLS

(tests performed for medical purposes only. We cannot enter into legal disputes.)

	Result mg/kg	Population average (provisional findings)
Benzene .....	0.7 *	0.4
Toluene .....	0.10	0.15
Ethylbenzene .....	<0.1	<0.1
Xylene(s) .....	0.24	0.2
Styrene .....	<0.1	<0.1
Trimethylbenzene(s) ....	<0.1	0.1
Dichloromethane .....	<0.1	<0.1
Chloroform .....	0.15	0.12
1,1,1-Trichloroethane ...	0.09	0.05
Trichloroethylene .....	<0.1	<0.1
Tetrachloroethylene .....	<0.1	0.15
Dichlorobenzene(s) .....	0.25	0.5
Bromodichloromethane	<0.1	<0.1
Carbon Tetrachloride ...	TRACE	0.1

### Aliphatic compounds

N-Pentane .....	0.7 *	<0.4
2,2-Dimethylbutane .....	<0.1	<0.1
Cyclopentane .....	<0.1	<0.1
2-Methylpentane .....	0.9 *	0.3
3-Methylpentane .....	0.9	1.1
N-Hexane .....	1.9 *	1.6
N-Heptane .....	1.4 *	<0.2

### Additional findings/comments:

\* Increase in petroleum-related VOCs.

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Biolab reference: MAWL/S205/D 06 Patient: Mr John Grahams HOYTE  
 Date: 26-04-2008 Doctor: Dr S Mackenzie-Kerr

## DNA ADDUCTS (genomic DNA from leucocytes)

See over page for background to the test and technical information

### Findings (organic chemical adducts):

We check for adducts to the following groups of chemicals and some individual compounds. These are circled if found and detailed below. *Aromatic amines (general) Nitrosamines Halogenated phenols & benzenes Vinyl halides Acrylamide Aldehydes Chloro-ethylenes Aflatoxins & mycotoxins Malondialdehyde Lindane Toxaphene other organochlorine pesticide Tetrachlorvinphos other organophosphate pesticide others:-*

### Findings (metal adducts):

We check for adducts to the following metals. These are circled if found and detailed below: *Lead Cadmium Mercury Aluminium Antimony Arsenic Strontium Tin Nickel Copper Manganese Chromium Cobalt Iron Zinc - normally associated with DNA others:-*

### Detailed findings:

Total DNA extracted from leucocytes = 54 ug (Reference 30 - 60\*)

ADDUCT OR CHEMICAL GROUP	QUANTITY ng/ml*	Associated GENE(s) (if identified)	RELATED TO (if known)
1) MALONDIALDEHYDE	17	Spread	LIPID-PEROXIDATION-RELATED-ADDUCT
2) ANTIMONY	24	ON CHROMOSOME 21 POSSIBLY BLOCKING Zn/Cu-SOD gene	TOXIC METAL
3)			
4)			
<u>Zinc</u> Normally DNA-associated	16 Ref: 21 - 74*	<u>Zinc-DNA association</u> <u>Low**</u> Normal High (circle the finding)	**[poor zinc association: DNA is vulnerable to toxic insult (especially by toxic metals) and mutations]

\* quantitative results are related back to and expressed as ug or ng in the leucocytes in 1ml whole blood.

Further comments on a separate sheet Yes/No

dna-adducts-quant-report.doc (John McLaren Howard)



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Reference: MAWS/5205/DO6 Patient: John Graham HOYTE  
 Date: 26-04-2006 Doctor: Dr S Mackenzie-Ross

## ATP (adenosine triphosphate), studies on neutrophils

ATP is hydrolysed to ADP and phosphate as the major energy source in muscle and other tissues. It is regenerated by oxidative phosphorylation of ADP in the mitochondria. When aerobic metabolism provides insufficient energy, extra ATP is generated during the anaerobic breakdown of glucose to lactic acid. ATP reactions require magnesium. ADP to ATP conversion can be blocked by environmental contaminants as can the translocator [TL] in the mitochondrial membrane. [TL] efficiency is also sensitive to pH and other metabolic factor changes. [TL] defects may demand excessive ADP to AMP conversion (not re-converted to ADP or through to ATP). Defects in Mg-ATP, ADP - ATP conversion and enzyme or [TL] blocking can all result in chronic fatigue - a factor in any disease where biochemical energy availability is reduced.

### ATP whole cells:

With excess Mg added (Standard method of measuring ATP)	1.26 nmol/10 <sup>6</sup> cells	1.6 - 2.9
Endogenous Mg only (Measured ATP result is lowered during intracellular magnesium deficiency)	0.77 nmol/10 <sup>6</sup> cells	0.9 - 2.7
Ratio ATP/ATP <sup>Mg</sup>	0.61 .....	> 0.65
<b>Results (circle):</b>	ATP normal	ATP low
	Mg normal	Mg low

### ADP to ATP conversion efficiency (whole cells):

ATP <sup>Mg</sup> (from above)	1.26 nmol/10 <sup>6</sup> cells (1*)	1.6 - 2.9
ATP <sup>Mg</sup> (inhibitor present)	1.02 nmol/10 <sup>6</sup> cells (2*)	< 0.3
ATP <sup>Mg</sup> (inhibitor removed)	1.07 nmol/10 <sup>6</sup> cells (3*)	> 1.4
ADP to ATP efficiency [(3*-2*)/(1*-2*)] x 100 = 20-8 %		> 60
<b>Results (circle):</b> Inhibitor site:	normal	partially blocked
		SUBSTANTIALLY blocked

Conversion (ADP to ATP): normal poor very poor

### ADP-ATP TRANSLOCATOR [TL] (mitochondria, not whole cells):

	ATP (pmol/10 <sup>6</sup> cells)	Ref. range	change %	Results (circle):
Start	205	290 - 700		
[TL] 'out'	239	410 - 950	16.5 > 35%** (increase)	function: normal VERY poor
[TL] 'in'	76	140 - 330	61.9 > 55%*** (decrease)	function: normal poor

in vitro \*\* reflects mitochondrial membrane supply of ATP to the cytoplasm  
 in vitro \*\*\* reflects normal use of ATP

Any additional comments and fluorescence microscopy results (if requested) will be found over the page)

- \*<sup>1</sup> Very low whole-cell ATP. Effect amplified by poor Mg Availability.
- \*<sup>2</sup> 80% block of active site leading to very poor ADP → ATP reversion.
- \*<sup>3</sup> low mitochondrial ATP. Very poor provision of ATP by mitochondria.

JMO

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1066

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Biolab Reference: *MAWL/Saerth/06*

Patient: *John Graham HOYTE*

Date: *25-04-2006*

Doctor: *Dr S MacKenzie-Ross*

## Cell-Free DNA in blood plasma

### Patient's result:

		<u>Reference range</u>
Cell-Free DNA	<i>21-2</i> ug DNA per litre of plasma	up to 9.5

Comments: *Highly significant increase in cell degradation.*

**Method summary\*** Blood plasma is incubated with EDTA, a detergent and a proteinase prior to salting out of the proteins. The DNA is then precipitated with ethyl alcohol and re-dissolved in Tris-acetate-EDTA buffer. The DNA is measured in an ultramicro cuvette using a Pharmacia GeneQuant™ analyser.

\*Schmidt B, Weickmann S, Witt C, Fleischhacker M. Improved Method for Isolating Cell-Free DNA  
*Clin Chem* 2005; 51(8); 1561-2

### Notes that may be helpful:

**Background.** Most of the cell-free DNA present in blood plasma is associated with the degradation of cells. Very low levels are present in healthy people. Increases are associated with serious illnesses such as malignancy, stroke, autoimmune diseases, severe infections and chronic fatigue syndrome.

**Cell-Free DNA in chronic fatigue syndrome (CFS).** In studies performed at Biolab, 88% of 87 CFS patients had increased levels in their blood plasma. 93% of the 75 patients where the duration of the disease was between four months and five years and nine of the 12 patients whose illness had persisted for more than five years and up to 14 years had positive findings.

The levels of Cell-Free DNA equate well with the severity of CFS and they reliably follow clinical improvement and, in some patients who have been monitored regularly, increases have effectively *predicted* clinical relapse. CFS is an addition to the list of diseases where Cell-Free DNA has prognostic as well as diagnostic implications.

Copies of a referenced internal document entitled **Cell-Free DNA** are available from Biolab.



Dr John McLaren Howard DSc FACN

DNA-cell-free-report.doc [JMH]

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Biolab Reference: MAW/SAS/DOG

Patient: Mr John Graham HOYTE

Date: 26-04-2006

Doctor: Dr S Mackenzie - ROIT

## Mitochondrial membrane - Translocator protein [TL]

[TL] scavenges ADP from cytoplasm and returns ATP from re-conversion with 'new' ATP from oxidative phosphorylation [TL] can be blocked by xenobiotics and/or partial detoxification products. The site is very pH sensitive and can be affected by local or general acidosis including the organic acid accumulation from over-dependence on anaerobic metabolism.

The efficiency of [TL] is also compromised by increased intracellular calcium or reduced intracellular magnesium.

This test sequence examines the white blood cell mitochondria using phase-contrast and dark-field microscopy and explores the mitochondrial membrane and the [TL] site using a series of specific and group-specific fluorescence probes.

A technical summary can be found over page and a reference list is available on request.

<u>Phase-contrast &amp; dark field microscopy</u>	<u>Poor</u>	<u>Normal</u>	<u>High</u>	
Numbers of mitochondria		✓		
Mitochondrial clumping		✓		
Mitochondrial membrane structure	SOME DISTORTION OF OUTER MEMBRANES			
Mitochondrial-DNA fluorescence binding			✓	

	< 6.8	6.8-7.4	> 7.4	<u>Result</u>
<u>The pH at the outer mitochondrial membrane</u>	✓			6.7 *1
	<u>Normal</u> < 200	<u>High-norm</u> 200-300	<u>High</u> > 300	<u>Result</u>
<u>Ca<sup>2+</sup> at the outer mitochondrial membrane</u>		✓		270 -umol/l

<u>Mitochondrial membrane binding of:</u>	<u>Low</u>	<u>Normal</u>	<u>High</u>	
Protein(s)		✓		
Lipids		✓		Diolain Y (N)
Esterases		✓		

<u>Other substances bound:</u>	<u>ND</u>	<u>Trace</u>	<u>High</u>	
Glutathione conjugates		✓		
Organic sulphate conjugates	✓			
Peptide complexes	✓			
Lactic acid & keto-acids			✓	(Lact) Keto *1
Chlorinated pesticides		✓		
PCBs (poly chlorinated byphenyls)	✓			
PBBs (poly brominated byphenyls)			✓	
Dichlorobenzene	✓			
Organophosphates (including OP pesticides)		✓		
Toxic metal(s)	✓			Metal = *2
DNA/RNA (probably viral)	✓			
Others:				

<u>Essential elements associated with mt-membranes:</u>	<u>Low</u>	<u>Normal</u>	<u>High</u>	
Potassium (K)		✓		
Magnesium (Mg)	✓			
Zinc (Zn)	✓			

\*1 Effect from early switch to anaerobic metabolism (tendency to lactic acidosis)

\*2 DEFINITE TRACE OF OP COMPOUNDS ON MITOCHONDRIAL MEMBRANES

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JMAG

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Biolab Reference: MAWY/S205/DOG

Patient: Mr John Graham Hoyte

Date: 25-04-2006

Doctor: Dr S Mackenzie - Ross

## PESTICIDES in BLOOD CELL MEMBRANES

(INCLUDES SOME OTHER LIPIDS EXTRACTED FROM THE BLOOD SAMPLE)

(PLOA = provisional limit of acceptability, based on UK data)

Results are given as mg per kg fat).

CHEMICAL:	RESULT	CONTROLS RANGE (n = 19)
ALDRIN	ND	0 - 0.01
DIELDRIN	0.02	0 - 0.04
LINDANE	0.29	0 - 0.05
DDT	0.05	0.02 - 0.20
DDE	0.17	0.03 - 0.27
DDD	0.04	0 - 0.04
HCB	0.50	0.03 - 0.62
MIREX	0.02	0 - 0.04
TOXAPHENE	0.02	0 - 0.05
PENTACHLOROPHENOL	0.27	0 - 0.16
TRICHLOROPHENOL(S)	TRACE	0 - 0.05
DICHLOROBENZENE(S)	0.14	0.01 - 0.35
DICHLOROPROP (2,4-D)	ND	0 - 0.16
PCBs (TOTAL)	0.09	0.02 - 0.44
PBBS [BDE] (TOTAL)	0.52	0.01 - 0.08
<i>(Flame retardants/thermal modifiers in plastics)</i>		
CARBARYL (SEVIN)	NSD	0 - 0.12
ALPHA-NAPHTHOL (from carbaryl)	NA	0 - 0.04
CARBAMATES (TOTAL)	≈ 0.05	0 - 0.22
ORGANOPHOSPHATES (TOTAL)	≈ 0.3	0 - 0.19
→ Almost certainly Tri CASYL PHOSPHATE but there may be a trace of MEVINPHOS (PHOSDRAN)		

(Numbers in brackets refer to notes over the page)

Note that this is a research procedure performed for medical purposes only. It may prove useful when toxic chemical exposure is thought to be a factor in changes in cell membrane integrity.