SYSTEM STUDY OF ALTERATIONS IN VIT A STATUS AMONG RURAL SOUTH VIETNAMESE EXPOSED TO AGENT ORANGE

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Beginning with the papers of Innami (1976) and Thunberg (1979) numerous experimental studies have been examining species-specific dose-dependent effects of TCDD-like compounds upon the vit A status involved in pathogenesis of the intoxication. In humans such effects are poorly investigated. We present initial results on differential diagnosing nutritionally and chemically-induced alterations in vit A status among rural South Vietnamese exposed to Agent Orange (AO).

The apparantely healthy villagers of the nationality "kinh" with similar socio-economic, nutritional and toxicological status (N=106, m, 31-50, malaria and HBsAg free, moderate smoking, low alcohol consumption, low use of certain pesticides: 0-4 contacts/y, Mean=.98, no correlations with the studied characteristics of homeostasis, not supplemented with physiologically active drugs and micronutrients for at least 2 m and 1 y pre-examination) were recruited from the villages Binh My and Chanh My. They were divided into 3 risk groups regarding potential exposure to AO as described by Roumak et al in this issue: I group - no contacts with AO, II - no direct contacts, a 15-25 y history of residence in the polluted environment, III - remote direct contacts: MET1,2 .23, a 15-25 y history of residence /1/.

Plasma a-tocopherol "E" (lipid adjusted), all trans retinol before "RO" and after oral administrations of test: 1 mg, 5 h, "R5" - "RDR-test"=R5-RO/R5x100% and pharmacologic: 30 mg, 1 m pre-examination, "RA" doses of retinyl acetate in oil, b-carotene "bCar", and total carotinoids "Car" were quantitated in fasting blood by isocratic HPLC /2/. Integral indices of remote direct exposure to AO and of health status, various physiological, biochemical and cytological characteristics were taken from the integrated data base /1/. Standard programs of the "R Base System Y" and "Statgraphics v.3.0" were employed to select patients with appropriate medico-biological characteristics and for statistical evaluations. Special program has been designed to calculate Z-values: Z=Xobs-Xst/SDst

and percentages of risk groups with excessive or inadequate values of the study parameter as compared to the normally distributed its reference values (Z-value based probability approach) /3/. The following procedure of "medico-biological screening" was carried out to select healthy reference group regarding the vit A status and to establish the "normal" local ranges for its characteristics within this group (a system approach):

Step 1. Selection of the reference group using epidemiological and clinical laboratory data related to the vit A status-maintaining and interferring factors:

medical (history of morbidity: integral indices of general health status and of pathologic conditions for different systems of the organism, frequency of parasitic and infectious diseases; current health status - by medical examinations),
physiological (weight-for-height index "W/H"; dark adaptation "DAdp" - with the ADMU-42 adaptometer, USSR),
cytological (ultrastructural changes in epidermis),
endocrinological (serum thyroxin, insulin) data available,
some characteristics of:
protein-calorie malnutrition (plasma protein, urea, creatinin, carotinoids as the discriminants),
inflammatory diseases (ESR1, leucocytes, Igs A, M, G),
hematopoesis and iron metabolism (Hb, RBC, Hb/RBC, zinc-protoporphyrin/heme, plasma iron),
(pro)vitamins status (E, E/Lip, Car, bCar, bCar/Car, urinary excretion of riboflavin),
liver injury (serum enzymes, bilirubin, porphyrinuria, cholestasis: coproporphyrin isomers I/III ratio, low RDR-test in vit A hypovitaminotics),
cyt P-450 system (basal and induced benz-a-pyrene hydroxylase in lymphocytes, urinary excretion of total and 4-OH- antipyrene metabolites),
renal functions (urinogramma; clearance of plasma porphyrins and creatinine; abnormally elevated RO), and
classification criteria (the "normal" ranges for some medico-biological parameters properly established for the Vietnamese /4/, ranges from 5-10th to 90-95th percentiles for other variables excluding characteristics of vit A status).

Step 2. Establishment of the "normal" local ranges for the studied characteristics of vit A status within the selected reference group using broader ranges of generally accepted criteria of normality for the vit A status in humans: $.10 < RO < .90$, $CV < 25\%$, RDR-test $< 20\%$, $RA\text{-}RO/RA \times 100\% < 15\%$, no correlations between RO and vit A-dependent characteristics of the homeostasis.

The following characteristics of the "normal" vit A status "A+" were established in the selected reference group by this method: RO=.561+/-.082 mcg/ml (MEANnor+/-SDnor, close-to-normal distribution - by "Normal Probability Distribution Plot"), RDR-test $< 17\%$, $RA\text{-}RO/RA < 10\%$ ($p < .05$), no correlations between RO, indices of health status, W/H, DAdp, changes in epidermis, Hb,

and plasma iron ("Spearman Rank Correlations") in this group (N=36). These criteria enabled us to demonstrate high stochastic prevalence of inadequate RO values in the entire populational sample (up to 55% probationeers) and to identify 2 types of mild hypovitaminosis A: the "usual" - .22 < RO < .65 mcg/ml, RDR-test > 18%, RA-RO/RA > 15%, "A-" status and the "hyposensitive" - RO < MEANnor-1.86SDnor, RDR-test < 15%, RA-RO/RA < 15%, "hA-" status with the stochastic shifts in some physiological parameters which can be associated with prolonged mild vit A insufficiency

Parameter, the "normal" ranges	Percentages of vit A-status group with excessive(+) or inadequate(-) values, rounded* A+ (N=54)	A- (N=30)	hA- (N=18)
W/H .298+/-.017**	13.7%+	.57%-	5.56%-
DAdp 32+/-13 sec	9.3%+	17.65%+	38.89%+

* Four persons with RO > MEANnor+2SDnor were excluded. ** Mean+/-SD /4/.

Some medico-biological characteristics of patients with different types of vit A status were compared:

Parameter°/ correlation with RO	Vit A status A+		A-		hA-	
RO,mcg/ml	.561	.082	.392	.141**	.348	.054**
E,mcg/ml	6.74	1.88/no	5.42	1.74/++	6.27	2.62/no
E/Lip,mcg/mg	.869	.245/no	.763	.322/+	.841	.321/no
Car,mcg/ml	.320	.169/no	.325	.164/no	.292	.161/no
bCar,mcg/ml	.219	.152/+	.122	.089**/+	.174	.121/no
bCar/Car,%	68.6	24.7/no	38.5	22.4**/++	59.4	24.2/no
IGHS[x]	.156	.117/no	.223	.166*/no	.212	.127/no
IGES[x]	.096	.113/no	.170	.148*/no	.156	.191/no
IRS[x]	.184	.199/no	.293	.241*/no	.240	.197/no
ESR1,mm/h	50.9	26.9/-	58.7	37.6/-	64.2	38.0/no
Leucocytes/mcl	6167	1895/no	5458	1174/no	5600	1706/no
Serum:						
AlcPhase,U/L	142.1	55.3/no	166.0	49.7/no	146.3	51.2/no
AST, U/L	29.2	18.8/no	18.2	6.2*/no	22.6	9.8/no
ALT, U/L	23.0	13.5/no	13.5	8.9*/no	18.2	11.0/no
Bilirubin,mcmol/L	6.87	1.50/no	7.15	1.58/no	7.19	1.60/no
Urinary:						
Porphyrins,pmol/ml	172	46/no	131	41/no	206	56/no
APMet,mcg/24h[xx]	8.66	2.10/no	6.66	1.61/no	13.26	3.89**/no
AP4OHMet,mcg/24h[xx]	.49	.14/no	.29	.08/no	.77	.06**/no

° Mean, SD. [x] Integral indices of general health status (IGHS) and of pathologic conditions for gastroenteral (IGES),and renal (IRS) systems of the organism /1/. [xx] Urinary excretion of total "APMet" and 4-OH- "AP4OHMet" antipyrene metabolites /1/. *, ** Significantly different from corresponding values in A+

group with $p < .05$ and $p < .01$ ("Two Sample Analysis"). +/- and ++/-- Significant positive/negative correlations with RO within each vit A status group with $p < .05$ and $p < .01$ ("Spearman Rank Correlations").

Incidence of A- and hA- status within each of 3 risk groups was determined as follows:

Risk group:	I (N=42)		II (N=29)		III (N=31)	
Vit A status:	A-	hA-	A-	hA-	A-	hA-
Persons	12/42	15/42	10/29	2/29	8/31	1/31
%, rounded	28.6	35.7	34.5	6.9	25.9	3.2

From these results it can be suggested that: I-the "normal" ranges for the studied characteristics of vit A status are similar in the adult vietnamese and europeoids, 2-bCar and E-enriched plants are the main source of vit A in this subpopulation and its inadequate intake resulted in wide spreading of mild nutritional (A-) hypovitaminosis A associated with the tendency to increased morbidity and with subclinical alterations in the homeostasis, 3-high incidence of the hA- status, elevated rates of porphyrins and antipyrene metabolites excretion /1/ are associated with history of remote direct exposure to AO and are not associated with the signs of liver injury (serum enzymes, bilirubin; cholestasis and plasma lipids - data not shown). Taking into consideration the minimized influence of age- and sex-related medico-biological, socio-demographic, nutritional, and toxicological confounding factors due to the selection procedure, it can be suggested that hA- status may serve as one more specific indicator of remote direct exposure to AO. Basing on the recent knowledges about dioxin-retinoids interaction in regulation of the genome expression in target cells, we may presume the existence of long-lasting hormone-like effects of AO-derived TCDD upon vit A-sensitive vit A-regulating functions of the liver in this subpopulation inadequately provided with the vit A. More research is needed to validate this phenomenon and to clarify its ethiology using liver biopsies and isotope dilution techniques.

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2 Stukalov Y, Orlov E. Determination of retinol, a-tocopherol, b-carotene, and total carotinoids by an HPLC method. Quest Med Chem (USSR) 1988;5:121-4.
3 Guthrie H. Interpretation of data on dietary intake. Nutr Rev 1989;47:33-8.
4 Vietnam Ministry of Health Care. Biological characteristics of the Vietnamese. Hanoi: 1971.