

A Note on Serum p,p'DDE and p,p'DDT Residues in Persons Taking Sustained Oral Doses of Vitamin B₆

by

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Pyridoxine, or vitamin B₆, acts as a cofactor in numerous types of metabolic reactions. These include such biochemical transformations as decarboxylation, deamination, transamination, desulfhydration, etc. (1,2). Although pyridoxine appears to exert most of its functional diversity in the intermediary metabolism of amino acids, it is also believed to be involved to some extent in lipid metabolism (2). Since this essential vitamin does participate in such varied mechanisms and since it is present in liver tissue, we have investigated the possibility, however remote, that pyridoxine is somehow involved in the hepatic microsomal enzyme pathways (3,4) responsible for the breakdown of stored organochlorines such as dichlorodiphenyl-trichloroethane (DDT). If this were indeed the case, it would seem that an increased dietary intake of pyridoxine would be accompanied by noticeable changes in the DDT-derived body burden. Although it appears that serum organochlorine levels may not prove in all cases to be a reliable index of the more extensive body burden of adipose tissue (5), serum residues are the most practical parameter to use at this time, and significant changes in pesticide metabolism should theoretically be observable in serum.

Materials and Methods

Twenty-four adult members of our chronic exposure study group were selected as participants. All but two of these volunteers had been routinely monitored by the Idaho Community Study on Pesticides Program for at least three years. Consequently, their serum pesticide levels, past medical histories, and degree of prior occupational exposure had been well documented throughout this period. To minimize variability, only males were chosen. Ages ranged from 33 to 68 and all members of the group were in good health. None were receiving any medication known to be capable of enhancing hepatic microsomal enzyme induction. Nine of the subjects were "control" persons who had never been occupationally exposed to pesticides. The remaining 15 were "exposed" individuals, all of whom had histories of rather extreme occupational exposure to pesticides for at least three years.

In November, 1971, a 10 ml whole blood sample was obtained from each person and the serum was analyzed for organochlorine content. At this time, vitamin B₆ therapy was initiated at oral doses of 50 mg/day, and maintained for a period of twelve

weeks. A total of four subsequent serum pesticide analyses were then made at three week intervals while the B₆ regimen was in force. Serum pesticide levels prior to and during the pyridoxine therapy were then compared with one another, as well as with serum levels that had been monitored at various intervals during the two previous years.

The serums were separated by centrifugation and pesticides were extracted with hexane by means of a semi-automated modification of the procedure of Dale, *et al.* (6). Two ml of serum were placed in a round bottom tube with 6 ml of nanograde hexane. This mixture was then agitated gently for two hours at 55 rpm on a rotary mixer. Five ml of the hexane layer was then removed by pipette and evaporated by steam to a volume of 1 ml on a two ball Snyder column. A five microliter portion of this was then injected into a Micro-Tek 220 gas chromatograph equipped with dual columns and tritium foil electron capture detectors. Column 1 was 4 per cent SE-30, 6 per cent QF-1 on chromosorb W, H.P., 80-100 mesh. Column 2 was 1.5 per cent OV-17, 1.95 per cent QF-1 on chromosorb W, H.P., 80-100 mesh. Nitrogen flow in the SE-30, QF-1 column was 90 ml/minute, while that in the OV-17, QF-1 column was 70 ml/minute. Approximate elution times for p,p'DDT were: SE-30, QF-1, 20 minutes; and OV-17, QF-1, 19 minutes. Both columns were maintained at a temperature of 200° C, while the detector temperature was 205° C and the injection chamber at 220° C. Quantitation was based on relative peak heights, and qualitative retention time of aldrin (Octalene). Per cent recovery was based on the prior addition of an aldrin spike as an internal standard.

Results and Discussion

Results of the various pesticide analyses are shown in Table 1. From these data, it is apparent that the pyridoxine therapy did not bring about any significant changes in serum pesticides in either the control or exposed participants. Serum titres of both p,p'DDE and p,p'DDT were not appreciably altered by the vitamin. In all of the 24 persons analyzed in Table 1, the 50 mg daily pyridoxine regimen was rigorously enforced. With the exception of one member of the original study group, the twelve week dosage period proceeded without incident. However, one "exposed" volunteer (whose data has been excluded), asked to be terminated from the study after six weeks of participation. This 35 year-old agricultural worker complained that the vitamin "seemed to make him feel impotent."

As a corollary to this investigation, 30 (16 male, 14 female) institutionalized, mentally retarded patients at the Idaho State School and Hospital, Nampa, Idaho, were given 50 mg oral doses of Vitamin B₆ daily for a period of four weeks. Baseline serum pesticide levels for these patients, none of whom were taking drugs, had previously been well established in a prior study (7).

TABLE 1
Serum p,p'DDE and p,p'DDT Levels in Persons Taking Vitamin B₆

Group	Subject	Age	p,p'DDE				p,p'DDT			
			1970 (1)	1971 (2)	Pre B ₆ (3)	parts per billion Post B ₆ (4)	1970	1971	Pre B ₆	Post B ₆
Control	1	43	32	23	32	27	13	5	2	4
	2	40	49	25	50	50	5	4	1	8
	3	54	41	54	44	42	12	20	18	16
	4	37	26	30	28	26	3	4	3	2
	5	44	--	38	38	39	--	9	8	7
	6	57	47	55	61	61	6	11	10	10
	7	65	78	98	98	82	7	12	10	10
	8	51	50	69	73	59	18	40	35	28
	9	50	41	50	47	53	4	16	4	6
Exposed	\bar{x}	49.0	45.5	49.1	52.3	48.8	8.5	13.4	10.1	10.1
	S.D.	8.9	15.6	24.0	22.1	17.7	5.3	11.4	10.7	7.8
	10	50	90	83	117	111	16	13	8	16
	11	33	--	59	46	42	--	11	6	8
	12	68	53	51	90	75	9	15	23	16
	13	58	49	58	36	55	9	12	6	7
	14	51	34	45	25	24	24	22	15	14

TABLE 1 (continued)

Group	Subject	Age	p,p'DDE				parts per billion				p,p'DDT			
			1970 (1)	1971 (2)	Pre B ₆ (3)	Post B ₆ (4)	1970	1971	Pre B ₆	Post B ₆	1970	1971	Pre B ₆	Post B ₆
Exposed	15	34	42	52	62	57	5	5	5	6	5	5	5	6
	16	54	34	57	57	56	4	6	4	5	4	6	12	5
	17	54	57	52	67	72	8	10	12	28	12	10	12	6
	18	46	31	75	29	27	7	8	5	6	7	8	5	6
	19	57	44	52	46	51	9	12	12	12	9	12	12	12
	20	43	54	83	61	58	10	13	7	10	10	13	7	10
	21	46	62	73	62	63	9	10	7	9	9	10	7	9
	22	52	73	29	62	76	8	20	6	10	8	20	6	10
	23	54	125	64	123	142	27	30	42	41	27	30	42	41
	\bar{x}	50.0	57.5	59.5	63.1	64.9	11.2	13.4	11.3	13.4	11.2	13.4	11.3	13.4
	S.D.	9.3	26.2	15.0	29.4	31.0	7.0	6.7	10.2	9.9	7.0	6.7	10.2	9.9

(1) Average of four quarterly analyses made during 1970.

(2) One analysis made during the peak spray season, July-August, 1971.

(3) One analysis made in November, 1971, one day prior to B₆ therapy.(4) Average of four analyses made at three week intervals while B₆ regimen was in force.

As with the more extensively examined exposed and control individuals analyzed in Table 1, pyridoxine therapy in the retarded group did not appear to produce any obvious changes in serum p,p'DDE and p,p'DDT levels.

Blood (or serum) is obviously the most convenient tissue to obtain and analyze for pesticide content, and epidemiological monitoring in this fashion is now rather commonplace. However, the fact remains that the circulatory system is essentially a transport medium, rather than a storage site. Thus, the accuracy obtained by predicting changes in body burden on this basis of serum levels alone is difficult to establish. Despite this compartmental effect, it does seem reasonable that increased microsomal depletion of organochlorine stores would be readily noticeable in serum, either by decreased levels of the pesticides themselves or by increased levels of their metabolites. Since this was not found to occur, we tentatively conclude that the catabolism of p,p'DDE and p,p'DDT in man is apparently not influenced by pyridoxine.

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