

## EFFECTS OF BIO-NORMALIZER ON SERUM COMPONENTS AND IMMUNOLOGICAL FUNCTIONS IN HUMANS

SANTIAGO, L.A.<sup>1,2)</sup>, UNO, K.<sup>3)</sup> KISHIDA, T.<sup>3)</sup>, MIYAGAWA, F.<sup>4)</sup>, OSATO, J.A.<sup>2,5)</sup>, AND MORI, A.<sup>1)</sup> (1: Department of Neuroscience, Institute of Molecular & Cellular Medicine, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700; 2: Osato Research Institute, Gifu 500; 3: Institut Pasteur de Kyoto; 4: FALCO Biosystems, Kyoto; and 5: The United Graduate School of Agricultural Science, Gifu University, Gifu 500)

We studied the effect of the one-month administration of Bio-normalizer on the immunological, hematological, biochemical, and antioxidant functions of the blood and serum of 14 healthy and unhealthy subjects, and observed an increased mean rate of interferon- $\gamma$  production in both groups while a downtrend then uptrend or vice-versa were noted in the production of interferon- $\alpha$ , 2-5A synthetase activity, and phytohaemagglutinins or concanavalin A- stimulated proliferative activity. The exceptionally high levels of GOT and GPT and the lipid components in three unhealthy subjects were significantly decreased after 14 days of treatment. The thiobarbituric acid-reactive substances increased while the superoxide dismutase activity did not change. The ability of Bio-normalizer to increase interferon- $\gamma$  producing capacity provides greater resistance for lymphocytes or helper T cells to combat infection and diseases.

### Introduction

Bio-normalizer, a health food product of the fermentation of herbal plants such as papaya was found to be a potent hydroxyl radical scavenger (9). In *in vivo* studies, Bio-normalizer was shown to provide antioxidant protection in the animal models of epilepsy (9,10), brain ischemia-reperfusion injury (8), and aging (11). It was also demonstrated to inhibit the chromosome-breaking effects of carcinogens in the bone marrow of mice (1), enhance the natural killer (NK) cells activity, and reduce the toxohormone L-induced lipolysis in rats (7). In humans, Bio-normalizer normalizes the decrease in blood glucose and superoxide dismutase (SOD) activity after alcohol consumption (6). To probe further into its purported therapeutic actions, we studied its effects on the hematological, biochemical, serological and antioxidant activities of the human serum components, as well as the immunological functions.

### Materials and Methods

Six grams of Bio-normalizer (Sun-O International Inc., Gifu) were orally taken by 14 subjects (healthy: 11; with liver malfunctions: 3). 25 ml of peripheral blood was drawn in heparinized tubes at 0, 2, and 4 weeks of Bio-normalizer treatment (April 13, April 27 and May 11, 1993). Measurement of interferon (IFN)- $\alpha$  and - $\gamma$ , 2-5 A synthetase were done using the whole blood method (2); the natural killer (NK) cells were analyzed by the cytotoxicity assay using Cr-labelled K562 cells whereas; the phytohaemagglutinins (PHA) and concanavalin A (con A) - stimulated proliferative activity were

determined by standard methods. The hematological test consists of the routine analysis for WBC, RBC, HGB, hematocrit, platelet count and HbA1c. Routine biochemical and serological tests for BS, total protein, albumin A/G, ZTT, LDH, GOT, g-GTP, AL-P, total bilirubin, TG total cholesterol, BUN, uric acid, creatinine, amylase, HBs antigen, HBs antibody, LP (X1, X2), blood sugar, lactic acid, and pyruvic acid were done. The antioxidant functions of Bio-normalizer were measured in serum TBARS and SOD activity using fluorometry and electron spin resonance spectrometry/spin trapping technique. The Student's t-test was used to evaluate the statistical difference.

### Results and Discussion

Table 1 summarizes the effects of Bio-normalizer on the human immune response. IFN- $\alpha$  is usually produced within a few hours of virus infection in humans followed by IFN- $\gamma$ , virus-specific killer cells, and neutralized antibodies produced by B cells. It is considered that IFN- $\alpha$  and - $\gamma$  reflect to non-specific and specific immunological functions, respectively. It was found that the normal value of IFN- $\alpha$  which ranges between 4,000 to 13,000 units/ml falls within the range of 80% in healthy subjects. IFN- $\alpha$  and - $\gamma$  production declined in patients with cancer (3), diabetes, liver diseases and chronic renal failure (5), and rheumatoid arthritis (4) and decreased rapidly depending on the severity or stage of the disease. The IFN- $\alpha$  production slightly decreased after the second week then went up to the original level on the fourth week of Bio-normalizer administration. The normal range of IFN- $\gamma$  production is from 15-45 units. IFN- $\gamma$  production increased with time of Bio-normalizer

Table 1. Effects of Bio-normalizer administration on the immunological response in humans.

Parameters	Unhealthy			Healthy		
	0	2 (weeks)	4	0	2 (weeks)	4
IFN - $\alpha$ production (IU/ml)	10607 $\pm$ 3271	5325 $\pm$ 1601	7367 $\pm$ 2982	8534 $\pm$ 3192	3986 <sup>a</sup> $\pm$ 1850	6652 <sup>b</sup> $\pm$ 3108
INF - $\gamma$ production (IU/ml)	21 $\pm$ 6	42 $\pm$ 27	79 $\pm$ 29	26 $\pm$ 22	38 $\pm$ 38	51 $\pm$ 36
2-5 A synthetase activity (pmol/dl)	2337 $\pm$ 297	3030 $\pm$ 1529	1457 <sup>c</sup> $\pm$ 155	1626 $\pm$ 462	2630 $\pm$ 1693	2561 $\pm$ 1963
NK activity (%)	52.3 $\pm$ 9.7	59.6 $\pm$ 18.0	39.6 $\pm$ 11.0	45.8 $\pm$ 10.9	48.0 $\pm$ 9.0	43.3 $\pm$ 12.6
PHA response (cpm)	37211 $\pm$ 15252	35702 $\pm$ 5596	34475 $\pm$ 12596	41935 $\pm$ 9629	38140 $\pm$ 13653	38728 $\pm$ 11144
Con A response (cpm)	33890 $\pm$ 3481	34475 $\pm$ 6128	33890 $\pm$ 4337	33983 $\pm$ 7754	35086 $\pm$ 9218	33061 $\pm$ 8708

<sup>a</sup>p < 0.005 vs. 0 week; <sup>b</sup>p < 0.05 vs. 2 weeks; <sup>c</sup>p < 0.025 vs. 0 week

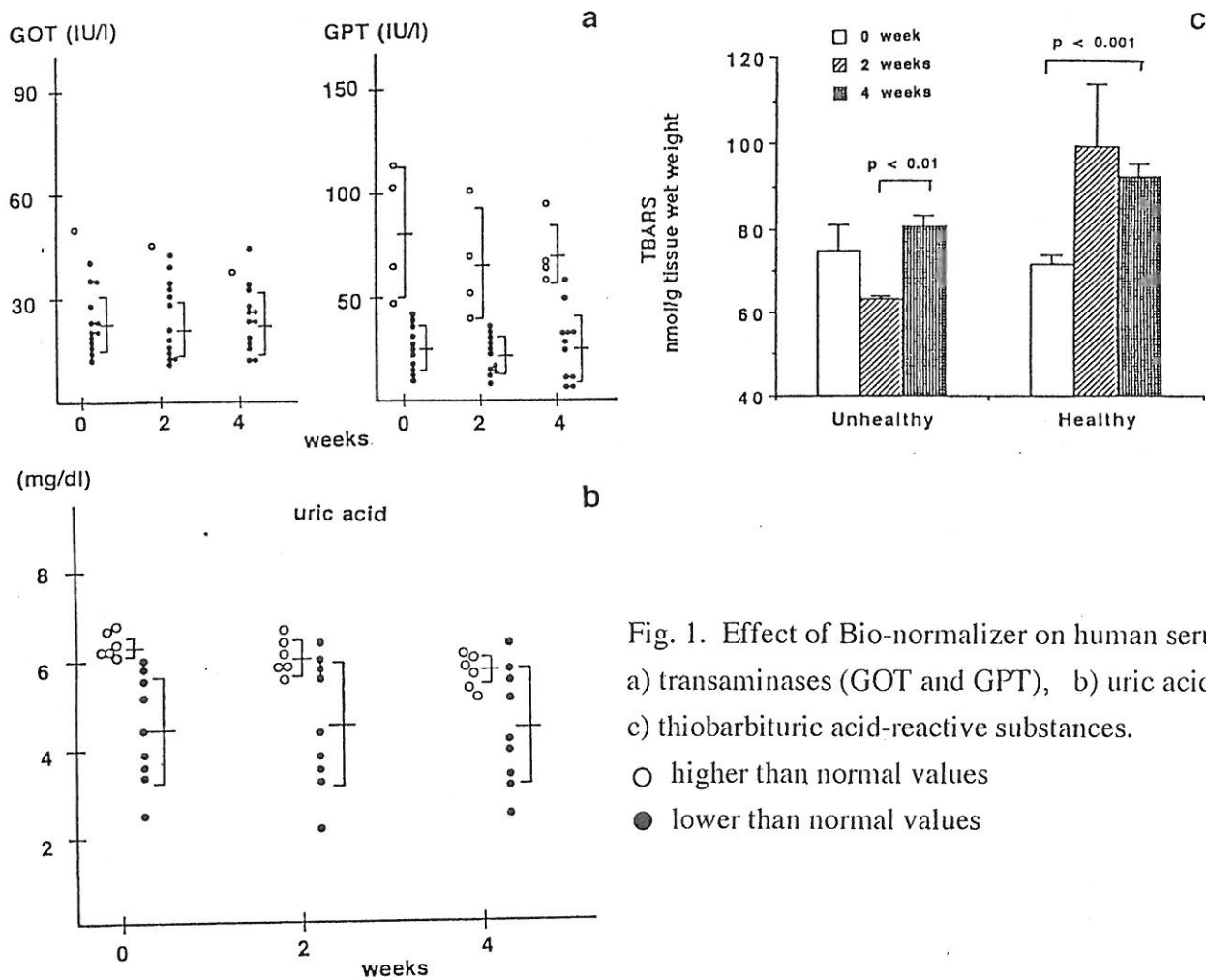


Fig. 1. Effect of Bio-normalizer on human serum a) transaminases (GOT and GPT), b) uric acid, c) thiobarbituric acid-reactive substances.

○ higher than normal values  
● lower than normal values

treatment in healthy and unhealthy subjects. This suggests that Bio-normalizer may have some effect on lymphocytes specifically on the helper T cells since no changes were observed in the NK cells activity (an important defense mechanism during the early stage of cancer) and in PHA response. However, the CD4 and CD8 values need to be further studied. While the 2-5 synthetase reflects the extent of IFN production in the body, we could not offer a better explanation why there was no change in its activity except that perhaps Bio-normalizer has indirect inducing effect on IFN- $\gamma$  productivity, and may prime up the macrophages, lymphocytes or polymorphonuclear lymphocytes to activate the immune defense mechanism of the body.

As noted in Fig. 1a, the pre-GOT level was 45 IU/l or more in one case, but its level declined at two weeks and four weeks after treatment. One out of 14 cases indicating pre-levels of 40 IU/l or less showed activated GOT level at four weeks after taking Bio-normalizer, although it was at 45 IU/l or less. In four cases, the pre-GPT levels were over 45 IU/l. Two cases showed more than 100 IU/l but declined to less than 100 IU/l four weeks later with Bio-normalizer intake. In 11 cases, the pre-treatment GPT levels were less than 45 IU/l of which two cases showed increasing enzymatic activity although the levels were less than 60 IU/l.

Uric acid concentration was more than 6.0 mg/dl in six out of 15 cases before taking Bio-normalizer and with one exception, all the six cases showed uric acid concentration declining to less than 6.0 mg/dl after taking Bio-normalizer. The other nine cases showed uric acid concentration of less than 6.0 mg/dl before taking Bio-normalizer, but the levels increased up to 6.3 mg/dl after taking Bio-normalizer (Fig. 1b). These results suggest of Bio-normalizer's role in maintaining the uric acid level.

Regarding serum lipids variation, the phospholipids and HDL cholesterol are illustrated in Fig.1c. The average PL concentration was  $303 \pm 48$  mg/dl prior to Bio-normalizer intake and  $255 \pm 32$  mg/dl after four weeks of taking Bio-normalizer. It should be noted that the phospholipids concentration declined significantly ( $p < 0.05$ ) with Bio-normalizer intake. As for HDL cholesterol, there was no significant change between pre- and post- Bio-normalizer intake (data not shown). While the serum SOD activity did not change (data not shown), TBARS increased with continuous intake of Bio-normalizer in both groups of subjects (Fig.1c), though the increase in serum TBARS is not well understood.

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