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Effect of a Rice-rich Diet on the Therapeutic Efficacy of Cyclophosphamide with Special Reference to the Enhancement of Transplantation Immunity

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The present study investigated the problem of whether the therapeutic efficacy of cyclophosphamide in the *in vivo* Ehrlich ascites tumor system can be improved by adjuvant use of hydrocortisone or of dietary hydrocortisone mobilizers. In the chemotherapy experiment, female ICR mice each received an inoculum of 1×10^6 cells of Ehrlich ascites tumor ip followed by 2 ip injections of cyclophosphamide 36 and 37 hr later (2.4 mg/mouse for the 1st injection, and 1.0 mg/mouse for the 2nd injection). The effects of both cyclophosphamide and adjuvant treatments were assessed in terms of either survival rate or cure rate in the 1-month experiment. The results obtained were as follows. 1) Prolonged use of hydrocortisone as an adjuvant can improve the survival of cyclophosphamide-treated mice. 2) Adjuvant use of a rice-rich diet for maintenance increased the rates of both survival and cure in the cyclophosphamide-treated mice. 3) The same maintenance of mice on a rice-rich diet increased transplantation immunity on the one hand, and induced a set of steroidal changes including hydrocortisone excess on the other hand. 4) Evidence is presented to indicate that the beneficial influence of a rice-rich diet on the drug effect is related to an increase of transplantation immunity in the host, and that there could be a causal relationship between hormonal and immunological changes in rice-saturated mice.

Key words: Cyclophosphamide — Transplantation immunity — Corticosteroid — Ehrlich tumor — Nutritional intervention

The contribution of glucocorticosteroid to cancer chemotherapy is unique in the sense that the hormone, besides its cytostatic effect on neoplasia, often reverses the destructive effect of other anti-cancer agents on the bone marrow function, and also produces a general improvement in the performance status of a cancer patient. The term "chemo-endocrine therapy" itself indicates that the combined use of a hormone with anti-cancer agents is expected to create a beneficial change which can not be achieved by a combination of solely cytotoxic compounds. The problem is that a hormone may at times exert a negative influence on both the physical activity of a cancer patient and the therapeutic efficacy of companion anti-cancer agents. We previously demonstrated that the therapeutic efficacy of cyclophosphamide on Ehrlich ascites tumor was decreased by the concomitant use of glucocorticosteroid, which was found to accelerate the inactivation process of its partner drug.[1)]

The present study was initiated to answer 2 questions: 1) Can the use of glucocorticosteroid have a positive influence on the therapeutic efficacy of cyclophosphamide? 2) If so, what is the mechanism that underlies the modulation of drug effect?

The results obtained indicated that the antitumor effect of cyclophosphamide was improved by either adjuvant treatment with hydrocortisone or the use of a rice-rich diet (physiological glucocorticoid mobilizer) for the maintenance of tumor-bearing mice, and that the beneficial influence of the diet could be accounted for by an enhancement of transplantation immunity that was detectable in mice fed a rice-rich diet. Evidence is also presented to suggest that the observed increase of host defense capability could have been mediated by the concerted effects of endogenous steroids whose activities at a target tissue were subject to change under various dietary conditions.[2)] Possible linkage between endocrinology and immunology is discussed in the light of recent progress in the study of autoimmune diseases including systemic lupus erythematosus.

Materials and Methods

Swiss/ICR female mice from the Atsugi Branch Farm of Charles River Co., Ltd., Atsugi, were used throughout the study. A standard pellet diet from Oriental Yeast Co., Ltd., Chiba, was given to mice in those experiments for which no nutritional specification was given for the maintenance of animals. The problem of nutritional intervention in cancer chemotherapy was studied by use of 5 experimental diets as follows: 1) powdered standard diet (MF), the nutritional contents of which were described in our earlier paper[3]; 2) fat-rich diet (MFF), a mixture of 85% MF (w/w) and 15% corn oil (w/w); 3) fat- and salt-rich diet (MFFS), a mixture of 75% MF, 15% corn oil and 10% NaCl (w/w); 4) rice-rich diet (R), a mixture of 90% rice flour (w/w) and 10% MF; 5) rice- and salt-rich diet, a mixture of 80% rice flour, 10% NaCl and 10% MF. Ehrlich ascites clone 1, a fast-killing tumor of the hypotetraploid family,[4] was developed in mice by introducing an inoculum of 1×10^6 cells per mouse, ip, in all chemotherapy experiments. Every mouse in the treated group received 1st (2.4 mg/mouse) and 2nd (1.0 mg/mouse) ip injections of cyclophosphamide at 36 and 37 hr after the above tumor inoculation. In a nutritional study with the 5 powdered diets, dietary conditioning for each group was started on the day of tumor inoculation (0th inoculation day) and terminated on the 30th inoculation day, the upper ration limit for each diet being set at 90 g/day/10 mice. All deaths before day 30 were recorded together with the dissection findings. The 30-day survivors were sacrificed and checked for tumor take by dissection. The adjuvant treatment with hydrocortisone, 1 mg/mouse, sc, on every 2nd day, starting on the 5th inoculation day, was continued with all living mice until the end of the experiment. The transplantability of Ehrlich ascites tumor in mice under two dietary conditions was assessed by the Reed-Muench method in terms of 50% tumor take dose, as described in our earlier paper.[5] The hormonal environment of a tumor-free mouse under various dietary conditions was investigated by estimating both the excretions of 15 urinary steroids in urine and the levels of 5 steroids and cholesterol in plasma. The names, abbreviations and physiological classification of urinary and plasma steroids will be given in the legends of Figs. 4 and 5. The details of urinary steroid analysis in mouse urine were described in another paper from our laboratory.[2] The urine collection, mostly covering a period of one month or longer, was conducted with each mouse in a metabolic cage.[2] Because of the above duration of urine collection, the excretion of an ovarian steroid represents an average over the whole reproductive cycle and is free from cycle-dependent variation. Likewise, the cycle-dependent fluctuation of an ovarian sterioid in plasma was minimized by preparing a pooled sample for a subgroup of 4 to 5 mice and performing steroid assay with each plasma pool. This maneuver also enabled us to prepare a quantity of plasma stock that is large enough to allow 5 consecutive steroid radioimmunoassays for each subgroup of mice. The mean and standard deviation for each steroid and for each group were calculated with the data of 5 to 6 subgroups so that the size of one group ranged from 20 to 30 mice in most experiments. Before the start of the steroid radioimmunoassay procedure, each plasma specimen underwent 2 ethanol extractions, as described by Murphy.[6] The 2 extracts were combined and evaporated to dryness in a water bath. The residue was then washed twice with ethyl acetate, and the 2 washings were combined and again evaportated to dryness in a water bath. The final residue was dissolved in Tris-buffer (pH 8.0) and processed according to the radioimmunoassay manual for each steroid.

The mathematical manipulations of hormonal data as well as the reasoning involved are detailed in our earlier paper.[7] The intergroup difference with a parametric data set was assessed in terms of the t value in Student's test. The statistical significance with a non-parametric data set was tested by either the chi-square test or the Wilcoxon rank t test.[8]

In view of the complexity of steroid interactions in the *in vivo* system, an overall assessment of the hormonal environment was attempted by investigating the weight change of some steroid-responsive organs. For example, an increase in kidney weight is taken as an indication of increased androgen activity,[9] whereas both an increase of forestomach weight and a decrease of glandular stomach weight could be related to a complex of glucocorticoid stimulation and androgen depression.[2,3] An excess of glucocortiocoid may increase liver weight through an acceleration of hepatic glycogen deposition.[10] Major chemicals used in this study were as follows: hydrocortisone acetate, Merck Sharp and Dohme, West Point, PA; cyclophosphamide, Asta-Werke AG, Brackwede, Germany. The radioimmunoassay kits for 5 plasma steroids were purchased from Bio-Yeda, Kiryat Weizmann, Rehovot, Israel. The ^{3}H-labeled steroid tracers with a specific activity of 50–100 Ci/mmol were all supplied by NEN Research Products, Boston, MA. Plasma Cholesterol was estimated by use of the Iatrolipo TC kit from Iatron Co. Ltd., Tokyo.

Results

Relation between the Therapeutic Efficacy of Cyclophosphamide and the Adjuvant Use of Hydrocortisone in Female Mice with Ehrlich Ascites Tumor The effect of cyclophosphamide, when measured in terms of survival time or cure rate, varied remarkably depending on how much time lag is inserted between the introduction of cyclophosphamide and the start of hydrocortisone treatment. Several preliminary experiments indicated that adjuvant use of the hormone started on the 3rd inoculation day or earlier could exert a negative influence on the drug efficacy of cyclophosphamide (data not shown here). The situation was reversed when the hormone treatment was started on the 5th inoculation day: in Fig. 1, the survival rate for the (Ex + F) group was significantly higher than that for the (Ex) group from the 17th till the 23rd inoculation day, though the hormone treatment produced no improvement in the rate of permanent cure. With a similar treatment design, the adjuvant use of estrodiol, tamoxifen, testosterone and progesterone did not improve the therapeutic efficacy of cyclophosphamide at all. Thus, there appeared to be some specificity to the ameliorating effect of hydrocortisone on the survival time of mice.

Effect of Nutritional Intervention on the Therapeutic Efficacy of Cyclophosphamide The possibility that the therapeutic efficacy of cyclophosphamide may vary under specific dietary conditions known to induce a change in the hormonal environment of the host was tested by the use of diets that were rich in carbohydrate, fat and salt, all of which are

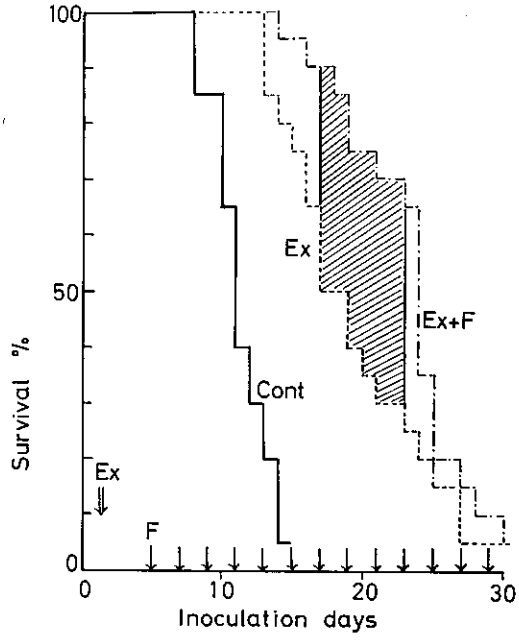

Fig. 1. The effect of cyclophosphamide (Ex) and hydrocortisone (F) treatments on the survival of female ICR mice bearing Ehrlich ascites clone 1 tumor. Each of the 3 groups consisted of 20 mice. Mice in the control group received no Ex injection. They were all maintained on a standard pellet diet during the 1-month experiment. The hormone treatment was started on the 5th inoculation day and terminated on the 29th inoculation day. Intergroup differences of survival marked by shading are statistically significant, as assessed by the chi-square test.

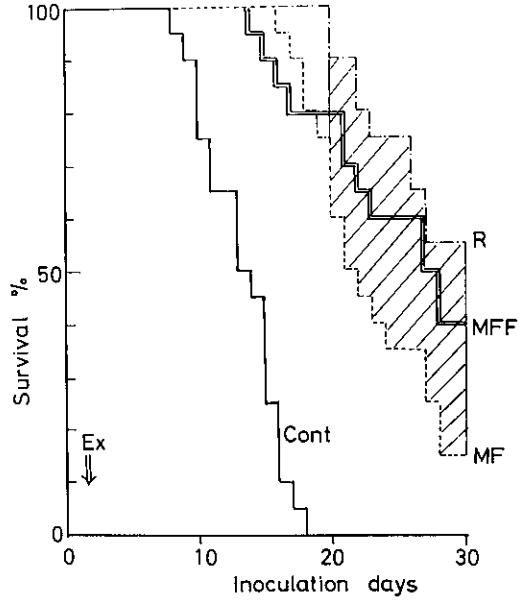

Fig. 2. The effects of cyclophosphamide (Ex) and nutritional intervention on the survival of female ICR mice bearing Ehrlich ascites clone 1 tumor. Each group consisted of 20 mice. Abbreviations used are: MF=standard diet; MFF=fat-rich diet; R=rice-rich diet. The dietary conditioning for each group was continued from the 0th to the 30th inoculation days. Intergroup differences of survival marked by shading are statistically significant by the chi-square test.

Table I. Effect of Nutritional Intervention on the Therapeutic Efficacy of Cyclophosphamide in Female Mice Bearing Ehrlich Ascites Clone 1 Tumor[a]

Group No.	Experimental diet[b]	Total No. of mice	Status of mice on 30th inoculation day		
			dead with tumor	alive with tumor	alive without tumor
1	MF	40	12	12	16
2	MFF	40	7	11	22
3	MFFS	40	12	11	17
4	R	40	6	8	26[c]
5	RS	40	10	9	21

a) A tumor inoculum of 1×10^6 cells was given ip on the 0th inoculation day to each mouse.
b) The dietary conditioning covers the period from the 0th to 30th inoculation day. Five diets were used as follows: MF, standard diet; MFF, fat-rich diet; MFFS, fat- and salt-rich diet; R, rice-rich diet; RS, rice- and salt-rich diet. Refer to the text for details.
c) The number of cured survivors for group 4 was significantly larger than that for group 1 by the chi-square test.

Table II. Modulating Influence of a Tumor Promotor, Diacylglycerol (DG), on the Therapeutic Efficacy of Cyclophosphamide (Ex) in Female Mice with Ehrlich Ascites Clone 1 Tumor

Group No.	Ex	Diet	DG[a]	Total No. of mice	Status of mice on 30th inoculation day			
					dead with tumor	dead without tumor	alive with tumor	alive without tumor
1	−	MF	−	10	8	0	2	0
1′	−	MF	+	10	8	0	2	0
2	+	MF	−	25	14	0	4	7
2′	+	MF	+	25	11	3	0	11
3	+	R	−	25	3	0	8	14[b]
3′	+	R	+	25	9	1	7	8

a) The DG treatment, 25 μg/day/mouse sc, was started on the 1st inoculation day and was terminated on the 29th inoculation day (a total of 29 injections).
b) The numbers of both cured survivors (cure rate) and all survivors (survival rate) for group 3 are significantly larger than those for group 2 by the chi-square test.

supposed to increase the activity of endogenous corticosteroid.[2, 3, 11] As shown in Fig. 2, the use of a rice-rich diet significantly improved the survival rate of cyclophosphamide-treated mice. The overall assessment of diet effects is summarized in Table I, which shows that a rice-rich diet, when compared with a standard diet, significantly improved the cure rate (the rate of tumor-free survivors). Other intergroup differences were statistically insignificant. The hormonal nature of the rice diet effect was tested by introducing the 2nd hormone modulator, diacylglycerol, into the test system. This compound is known to act at the gene level as an amplifier of hydrocortisone action.[12] Table II shows that a rice-rich diet increases the cure rate in the cyclophosphamide-treated mice, and that the beneficial effect of the diet is nullified by superposing diacylglycerol on it. The compound alone exerted no detectable influence on the course of tumor development. These findings suggest that the observed diet effect could be of hormonal nature. It is likely that more than one steroid hormone is involved in the improvement of the therapeutic efficacy of cyclophosphamide in rice-fed mice.

Relation between the Immunological and Hormonal Aspects of Rice Diet Effect in the Host versus Tumor Interaction The next experiment investigated the problem of whether the adjuvant effect of a rice-rich diet

is related to an increase of transplantantion immunity in the host. Practically, the relation between the size of tumor inoculum and the response of the host to the allograft tumor was compared between the standard- and rice-diet groups. As shown in Fig. 3, the use of a rice-rich diet retarded (or suppressed) the progress of tumor development at 2 tumor inoculum doses. Table III summarizes the final assessment of tumor transplantability in the 2 diet groups.

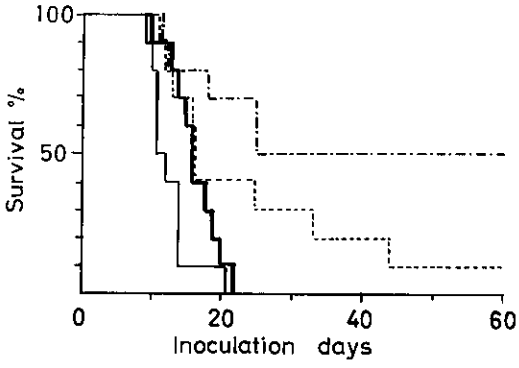

Fig. 3. Survival of female ICR mice as a function of the tumor inoculum size and of the dietary conditioning. MF and R denote a standard diet and a rice-rich diet, respectively; —— MF, 1×10^6 cells/mouse; —— R, 1×10^6 cells/mouse; ----MF, 1×10^5 cells/mouse; - - - - - R, 1×10^5 cells/mouse. The intergroup difference of survival at a low inoculum dose (1×10^5 cells/mouse) was found to be statistically significant by the Wilcoxon rank t test ($0.01<P<0.05$). Refer to the text for details.

The 50% tumor take dose (an index of transplantation immunity) of a female ICR mouse increased by over 1 order in the rice-diet group as compared with the standard diet group. It was indicated that a rice-rich diet did increase transplantation immunity to improve the therapeutic efficacy of cyclophosphamide. The physical characteristics of the 2-month survivors from the same experiment are presented in Table IV. Mice in the rice diet group are significantly larger than those in the standard diet group in both body weight and liver weight, and are significantly smaller in the weights of the kidney and glandular stomach. Probably, an excess of glucocorticoid in a rice-fed mouse stimulated accumulation of both abdominal fat (body weight increase) and hepatic glycogen (liver weight increase), and an androgen deficiency in the same mouse induced regression in the kidney (kidney weight reduction) and glandular stomach (gastric atrophy). These findings are in good agreement with our former reports on diet-hormone relationship.[2, 3] The effects of a

Table III. The Influence of Dietary Conditioning on the Transplantability of Ehrlich Ascites Clone 1 Tumor in Female ICR Mice

Diet[a]	Tumor cell dose[b]	Fate of tumor: take	Fate of tumor: rejection	Take at this and smaller dose	Rejection at this and greater dose	% take[c]
MF	1×10^1	3	7	3	28	9.7
〃	1×10^2	3	7	6	21	22.2
〃	1×10^3	4	6	10	14	41.7
〃	1×10^4	3	7	13	8	61.9
〃	1×10^5	9	1	22	1	95.7
〃	1×10^6	10	0	32	0	100.0
R	1×10^1	0	10	0	41	0
〃	1×10^2	0	10	0	31	0
〃	1×10^3	2	8	2	21	8.7
〃	1×10^4	2	8	4	13	23.5
〃	1×10^5	5	5	9	5	64.3
〃	1×10^6	10	0	19	0	100.0

a) Dietary conditioning covers the period from the 0th to 60th inoculation days.
b) The tumors were inoculated intraperitoneally, and the mice were observed for 2 months.
c) TTD_{50} (50% tumor-take dose), when plotted on a logarithmic scale, was estimated to be $10^{3.41}$ for the MF group and $10^{4.64}$ for the R group.

Table IV. The Influence of Dietary Conditioning on the Physical Constitution of Female Swiss Mice

Parameter	MF diet group mean±SD (n mice)	Rice diet group mean±SD (n mice)	$t^{a)}$
Body weight (g)	39.4±6.5 (48)	42.3±5.8 (51)	2.341
Liver weight (mg)	1847±182 (48)	2202±157 (51)	10.421
Kidney weight (mg)	545±44 (48)	441±21 (51)	−15.149
Spleen weight (mg)	157±20 (48)	162±23 (51)	1.151
Forestomach weight (mg)	99±14 (48)	97±12 (51)	−0.765
Glandular stomach weight (mg)	199±42 (48)	168±29 (51)	−4.295

a) A statistically significant difference ($P<0.05$) is indicated with an underline.

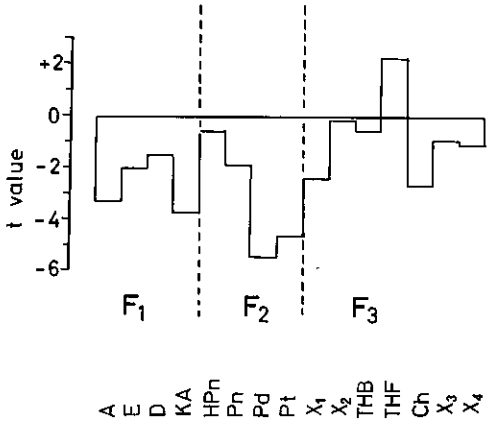

Fig. 4. The effect of a rice-rich diet on the excretions of 15 urinary steroids in female ICR mice. Dietary conditioning was continued for 6 months before urine collection. The numbers of mice in the rice diet and the standard diet groups were 15 and 16, respectively. The deviation of the former group from the latter was expressed in terms of the t value of Student's t test for each steroid. A t value with a minus sign denotes a decrease of the former as compared with the latter, and *vice versa*. If $|t|$ is larger than 2.10, the corresponding intergroup difference is statistically significant ($P<0.05$). The names, abbreviations and classifications of 15 urinary steroids are given below. Androgen family (F_1): androsterone (A), etiocholanolone (E), dehydroepiandrosterone (D), 11-ketoandrosterone (KA). Progestin family (F_2): 17-hydropregnanolone (HPn), pregnanolone (Pn), pregnanediol (Pd), pregnanetriol (Pt). Corticosteroid family (F_3): unknown substance No. 1 (X_1), unknown substance No. 2 (X_2), tetrahydrocorticosterone (THB), tetrahydrocortisol (THF), cholesterol (Ch), unknown substance No. 3 (X_3), unknown substance No. 4 (X_4).

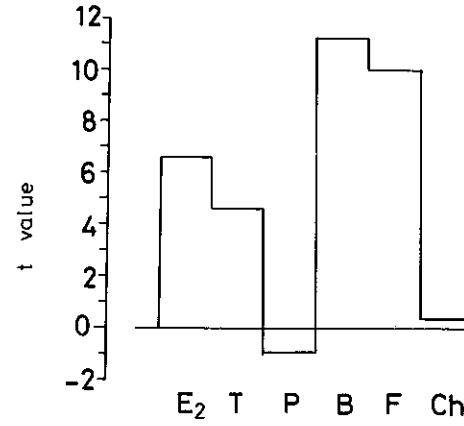

Fig. 5. The influence of a rice-rich diet on the plasma levels of 5 steroids and cholesterol of female ICR mice. Dietary conditioning was continued for 10 days before blood collection. Each diet group consisted of 20 mice. The deviation of the rice diet group from the standard diet group was expressed in terms of the t value of Student's t test. The names and abbreviations of steroid substances are given below: 17β-estradiol (E_2), testosterone (T), progesterone (P), corticosterone (B), hydrocortisone (F), cholesterol (Ch).

rice-rich diet on the hormonal environment of the host were assessed by estimating both urinary and plasma steroids in tumor-free mice. As can be seen in Fig. 4, a mouse fed a rice-rich diet was distinguished from a control mouse by a reduction of both androgen (A, KA) and progestin (Pd, Pt) in urine, and also by an increase of a glucocorticoid metabolite (THF). Figure 5 shows that a rice-rich diet

produced significant increases in the plasma levels of estradiol, testosterone, corticosterone and hydrocortisone. The proposed association of rice diet intake with glucocorticoid excess was supported by the data on both urinary and plasma steroids. The discrepancy of results with other steroids between the 2 experiments will be referred to in the discussion section.

Discussion

The proposed diet-hormone relationship in tumor-bearing mice may well be liable to 2 criticisms: 1) the beneficial influences of hydrocortisone and a rice-rich diet on the therapeutic efficacy of cyclophosphamide could have been produced by 2 different mechanisms; 2) the hormonal impact of a rice-rich diet, as investigated in tumor-free animals, might not be the same in tumor-bearing ones, in which the steroid-generating system is under the influence of constant tumor stress. It will be pertinent for our discussion to recall some results of our early investigations on the hormonal aspects of host-tumor relationship. a) The response of ascitic Ehrlich tumor (ip transplantation product) to hydrocortisone treatment was different from one tumor line to another, i.e., the hormone treatment prolonged the survival time of mice bearing Ehrlich 4N stock tumor, whereas it rather shortened the survival of mice bearing Ehrlich 2N stock tumor.[5] b) When tested with the solid form of tumor (sc transplantation product), the growth of Ehrlich 4N tumor was stimulated by testosterone, and was inhibited by hydrocortisone and estradiol.[13, 14] Castration experiments gave support to the proposed hormone-responsiveness of that tumor in its growth.[13] On the other hand, the growth of Ehrlich 2N tumor under similar conditions responded poorly to both gonadal and adrenal steroid hormones.[13] c) Ehrlich 2N tumor was distinguished from Ehrlich 4N tumor by an increased TTD_{50} (50% tumor take dose).[5] Hydrocortisone pretreatment in mice reduced the TTD_{50} of Ehrlich 2N tumor.[5] These findings indicate that the response of Ehrlich ascites tumor to a given hormonal milieu varies depending on the difference in weight between direct hormonal effect on tumor cells and indirect effect via the modulation of host immunity.[5] The former effect predominates in the 4N tumor system, and the latter effect comes to the foreground in the 2N tumor system. By the same token, a negative influence of hydrocortisone at an early stage of tumor development, as experienced in the present study, will be related to a suppression of transplantation immunity. In the case of a steroid-responsive tumor, a combination of 3 steroidal changes (androgen depression, hydrocortisone stimulation and estradiol stimulation) is a condition which is expected to induce tumor regression. It is therefore reasonable to assume that a rice-rich diet may exert a suppressive influence on tumor growth through the production of specific changes of androgen, estrogen and glucocorticosteroid. The hormonal impact of a rice-rich diet will be detailed later. The ineffectiveness of a salt- and rice-rich diet in either improving the drug effect or producing specific steroidal changes seems to exclude the possibility that rice contains a minor element that may *per se* stimulate transplantation immunity. The relation between a rice-rich diet and transplantation immunity will also be referred to in the later part of this section. So far, we have accumulated much experience with the hormonal environment of a cancer patient. A brief description of the results will be of use to rebut the 2nd criticism. A case-control study was attempted for each of 5 neoplasias (cancers of the breast, uterine cervix, endometrium, ovary and stomach) by estimating comparatively the urinary steroid excretions of patients and corresponding normal controls.[15] First, each of the 5 tested neoplasias was associated with a steroidal change that was specific to each tumor. Second, the observed change of urinary steroid excretions was not affected by the praxis of radical surgery. Third, the steroidal change of a cancer patient is very often reproducible in mice by long-term exposure to a suitable environment. For example, maintenance of mice on a rice-rich diet produces an atrophy of the glandular stomach together with a specific change of urinary steroids that are reminiscent of the steroidal disorder of human gastric cancer.[2, 16] It is only at the terminal stage of tumor development that a cancer patient is inclined to suffer from a non-specific glucocorticoid excess regardless of the origin of the tumor (unpublished data from our labora-

tory). In short, the steroidal change of a tumor-bearing subject at an early stage of tumor development can be a cause of tumor induction, but not a result.[15]

As early as 1969, Graff *et al.* noted that the gonads and adrenals exerted a suppressive influence on the immune response of a mouse to skin allorgafts.[17] An overview of results on the removal and transplantation of endocrine organs indicates that the testis is more powerful than the ovary and adrenals in suppressing allograft rejection. Castro in 1974 reported that prepuberal orchidectomy in mice induced hyperplastic changes in the lymphoid tissues (thymus, spleen and lymph nodes) together with an increased reactivity to a variety of antigens.[18] The development of murine lupus in NZB/NZW F_1 hybrid mice has also attracted the attention of both immunologists and endocrinologists, since there is strong evidence to indicate that sex hormones play a role in the onset and severity of the disease in those mice: the immune disorder progresses much earlier in females than in males,[19] and androgen therapy improves survival through rescue of impaired suppressor T cell function.[20] In accordance with the murine lupus, the incidence of human systemic lupus erythematosus is much higher in females than in males.[21] Endocrinologically, this disease is associated with a complex of androgen depression and estrogen excess.[22, 23] One could postulate the sequence of events to be as follows. 1) a candidate for this malady is predisposed to a predominance of estrogen over androgen, a condition which is expected to depress the function of suppressor T population without affecting the activities of helper T and B populations. 2) The progress of adolescent maturation of the gonad-adrenal axis aggravates both the hormonal and immunological imbalances so that a variety of auto-antibodies are produced and released into the circulation to bring into expression the clinical picture of systemic lupus erythematosus. In the *in vitro* system, progesterone and testosterone increased suppressor T cell activity,[24] and estradiol inhibited it.[25] Glucocorticoid at higher doses depressed both helper and suppressor T cell functions, while the same hormone at lower doses increased B cell function and preferentially depressed suppressor T cell function.[26] In the absence of estrogen receptors in helper T lymphocytes, estradiol does not seem to play a positive role in the stimulation of helper T cell function.[27] What interpretation is feasible for all these findings of the present investigation? Before answering this question, the discrepancy of results on urinary steroids, plasma steroids and weights of visceral organs (Table IV and Figs. 4 and 5) should be cleared up so as to obtain a unified picture of the hormonal effect of a rice-rich diet. The discussion will be started on the basis of our experience with salt intake as follows. 1) Hydrocortisone administration in mice remarkably increased the intake and output (into urine) of both water and NaCl.[2] 2) The enhancing effect of the hormone on the turnover of water and NaCl vanished promptly after cessation of hormone treatment.[2] 3) A salty diet increased the turnover of hydrocortisone by the glandular stomach epithelium without affecting the level of plasma hydrocortisone (unpublished data from our laboratory). We can learn 3 lessons from this study: a) the expression of hormonal function is associated with rapid turnover of a hormone at the target tissue. b) Interaction between a hormone and its target tissue is dependent on the activity of specific binding sites (receptors). c) The activity of a hormone at its target tissue is often unrelated to the plasma level of hormone. Therefore, estimation of hormone turnover rate or of hormone metabolites in urine is more reliable than plasma hormone assay in investigating a given hormonal milieu. Bearing in mind the above discussion as well as the data in Table IV and Figs. 4 and 5, one may consider that 4 steroidal changes are involved in the effect of a rice-rich diet: 1) androgen depression; 2) estradiol stimulation; 3) progesterone depression; 4) hydrocortisone stimulation. We are now ready to understand the whole situation in that all of these steroidal disorders are working together to create a relative predominance of helper T and B lymphocyte functions over suppressor T lymphocyte function or an increase of cellular and humoral immunities in a rice-fed mouse. If the proposed relationship between the intake of a rice-rich diet and an incresed immunity be correct, the incidence of systemic lupus erythematosus will be higher in Asians than in Caucasians. Serdula and Rhoads noted remarkable ethnic differences in

the prevalence rates of this disease in the inhabitants of Hawaii: age-adjusted prevalence rates per 100,000 at the end of 1975 were as follows: white 5.8, Chinese 24.1, Filipino 19.9, part-Hawaiian 20.4, and Japanese 18.2. There was also a heavy preponderance of females in each ethnic group.[28] It may not be a mere coincidence that AIDS, an antipode of the autoimmune disease, has spread in the Western world rather than in Japan. The striking difference between the 2 cultural spheres ragarding the spread of this immunodeficiency syndrome would be beyond the scope of our comprehension without taking into account the possible participation of specific immune responsiveness based on the traditional life style of each nation. Our suggestion that the diet-induced increase of transplantation immunity can be related to a shift of balance in the activities of 2 T cell populations still remains to be proved. It might also be intriguing to see whether or not a rice-rich diet improves the therapeutic efficacy of cyclophosphamide in a syngeneic tumor system.

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