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## Prevention by Vitamin $B_{12}$ of Protein Catabolic Action of Cortisone.* (20875)

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Large doses of vit. $B_{12}$ partially counteract certain catabolic effects of cortisone in young rats fed diets deficient in this vitamin(1-3). Reductions in body, hair and thymus growth caused by cortisone were partially overcome by feeding 10 times the normal requirement for this vitamin. The beneficial effects were invariably accompanied by increases in food intake and by greater efficiency in converting food into body weight gains. It was suggested that (a) large doses of cortisone may increase requirements for vit. $B_{12}$ and (b) administration of the vit. may enhance the availability of protein. The first was partially corroborated by Wahlstrom and Johnson(4), who reported that large doses of cortisone injected into baby pigs increased urinary excretion of vit. $B_{12}$. The results of the present experiments demonstrate that when young rats are fed *ad libitum*, vit. $B_{12}$ can prevent an increase in urinary nitrogen losses produced by injecting large doses of cortisone.

*Methods.* Two similar experiments were performed in 90 rats, but since the results were essentially the same in both, only one will be reported. Fifty young male Carworth rats were divided into 5 uniform groups of 10 each, and fed the vit. $B_{12}$-deficient, corn-soybean diet previously described(1) for 20 days. After this depletion period, each group was treated as follows for 30 days: 1) no vit. $B_{12}$; 2) 200 $\mu$g vit. $B_{12}$‡/kilo of diet; 3) cortisone‡; 4) cortisone and 200 $\mu$g vit. $B_{12}$/kilo of diet; 5) same as 4 but pair-fed to group 3. Groups 3, 4 and 5 received one mg of cortisone acetate daily by subcutaneous injection for the first 10 days, 2 mg daily for the second 10 days, and 4 mg daily for the third 10 days. During the initial depletion period, three 24-hour urine specimens were collected at approximately weekly intervals and analyzed for total nitrogen by the micro-Kjeldahl procedure(5). After this, urinary nitrogen was determined on all rats of each group on the 5th and 10th days of each 10-day treatment with a particular dose of cortisone. Measurements of body weight and food consumption were made every 2 days. The rats were housed in metal cages with raised screen bottoms in an air-conditioned room at a temperature of 78 $\pm$ 1°F.

*Results.* The 50 rats averaged 58.3 g each in body weight at the beginning and 94.9 g each at the end of the 20-day depletion period. Body growth trends after this period are shown in Fig. 1. Rats which were continued on the vit. $B_{12}$-deficient diet (group 1) reached a final average body weight of 140.0 g as compared to 192.4 g each for the rats fed vit. $B_{12}$ (group II). Growth of rats given cortisone but no vit. $B_{12}$ (group III) was completely suppressed by one and 2 mg of hormone daily, and loss of body weight re-

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‡ Crystalline vit. $B_{12}$ and cortisone acetate (Cortone) were furnished through the kindness of Dr. L. Michaud of Merck and Co., Rahway, N. J.

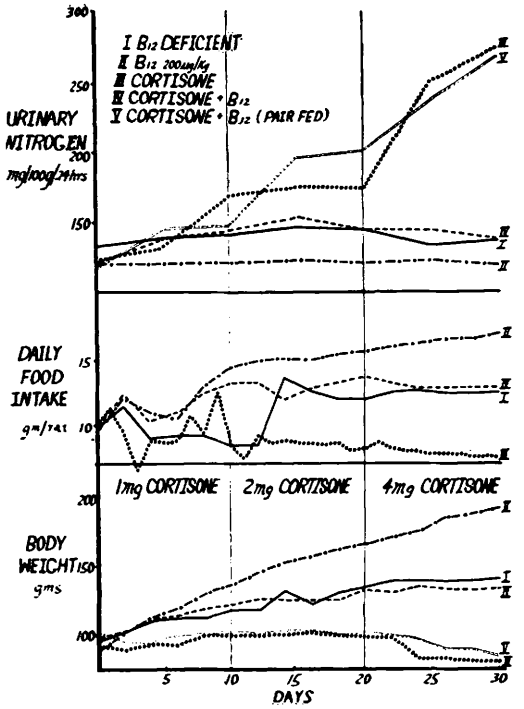


FIG. 1. Effects of vit. $B_{12}$ and cortisone on body wt, food intake and urinary nitrogen of young male rats.

sulted when 4 mg were injected daily. When vit. $B_{12}$ was added to the diet of cortisone-injected rats (group IV), growth inhibition was prevented although it did not reach the level of group II. Vit. $B_{12}$ did not prevent growth inhibition by cortisone when food intake was limited (group V) to that of the rats given cortisone without vit. $B_{12}$ (group III). Food intake and efficiency of utilization for body growth (Table I) were greatest in rats treated only with vit. $B_{12}$ (group II) and least in rats given cortisone without vit. $B_{12}$ (group III). Food intake was increased when vit. $B_{12}$ was given to the cortisone-treated rats (group IV), but efficiency of food utilization was well below that of either group I or II.

The average daily excretion of urinary nitrogen per 100 g of body weight (Table I) during the vit. $B_{12}$-deficiency period apparently was not altered after the vitamin was added to the diet (group II). Although nitrogen values of group I are slightly higher than those of group II, these differences are not believed to be statistically significant. All levels of cortisone significantly increased urinary nitrogen losses in vit. $B_{12}$-deficient rats, and the largest dose of hormone doubled the initial nitrogen values (group III). The addition of vit. $B_{12}$ to the diet largely prevented any increase in urinary nitrogen excretion by cortisone under *ad libitum* feeding conditions (group IV), but was completely ineffective when food intake was limited (group V) to that consumed daily by the rats of group III.

*Discussion.* These results demonstrate that in young rats fed *ad lib.*, vit. $B_{12}$ can largely counteract the protein catabolic action of large doses of cortisone. This aids in explaining the ability of vit. $B_{12}$ to overcome partially the cortisone-induced inhibition of body, hair and thymus growth(1-3). Vit. $B_{12}$ apparently was unable to alter normal urinary nitrogen values, an observation in accord with others who have failed to demonstrate a specific effect of this vitamin on protein metabolism(6-9). These workers have suggested that vit. $B_{12}$ may be involved primarily in utilization of carbohydrate and its transformation to fat, rather than in protein metabolism. The effectiveness of vit. $B_{12}$ in preventing an increase in urinary nitrogen loss by cortisone may be explained by the observations of Engel(10), who noted that administration of glucose or amino acids to fasted nephrectomized rats prevented the usual increase in blood urea elicited by injecting adrenal cortical extracts. It is probable therefore, that vit. $B_{12}$, by increasing appetite and food intake, increases the availability of glucose or amino acids to the organism and thus overcomes the protein catabolic action of cortisone.

Vit. $B_{12}$ failed to prevent cortisone from increasing urinary nitrogen losses when food intake was limited to that consumed by cortisone-treated rats on the vit. $B_{12}$-deficient diet. This is in agreement with Rupp and Paschkis(11), who similarly noted that vit. $B_{12}$ was ineffective in counteracting protein catabolic effects of large doses of cortisone in rats limited in their food allowance. Apparently vit. $B_{12}$ does not exert any growth effect in young rats when food intake is reduced to that of rats on a vit. $B_{12}$-deficient diet(12,13).

TABLE I. Effects of Vit. $B_{12}$ and Cortisone on Urinary Nitrogen and Food Intake.

| Treatment | Avg food intake, g | | Avg mg N/100 g/24 hr | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Treatment period in days | | | | | |
| | Total | Per g gain body wt | Before treatment | 5 | 10 | 5 | 10 | 5 | 10 |
| I. No V‡ | 327.6 | 7.47 | 132.56* ± 5.52† | 139.51 ± 4.68 | 141.92 ± 4.87 | 146.87 ±12.5 | 144.01 ± 2.02 | 133.87 ± 9.70 | 136.16 ± 8.50 |
| II. V | 386.0 | 3.71 | 120.01* ± 5.45 | 120.71 ± 2.50 | 121.28 ± 2.94 | 123.58 ± 2.75 | 120.96 ± 8.46 | 122.72 ± 2.34 | 119.23 ± 1.20 |
| | | | | 1 mg cortisone | | 2 mg cortisone | | 4 mg cortisone | |
| III. C, no V | 251.9 | — | 123.34* ± 4.39 | 132.68 ±19.43 | 169.42 ±30.71 | 175.69 ±37.38 | 169.85 ±22.19 | 251.43 ± 4.65 | 276.78 ± 2.64 |
| IV. C and V | 357.0 | 9.92 | 120.64* ±10.77 | 137.64 ± 7.17 | 144.44 ± 6.14 | 154.09 ± 6.51 | 144.34 ± 9.15 | 144.81 ± 2.03 | 137.63 ±16.8 |
| V. C and V (pair fed to group III) | 251.9 | — | 118.14* ± 8.00 | 145.34 ± .18 | 148.46 ± .10 | 192.24 ±11.45 | 201.59 ± 6.00 | 238.74 ±10.5 | 270.70 ± .47 |

* Avg of 3 figures. † Stand. error of mean. ‡ V = Vit. $B_{12}$; C = cortisone.

These observations, however, cannot be interpreted as indicating that a mere increase in food consumption in the absence of adequate intake of vit. $B_{12}$ would suffice to overcome the protein catabolic action of large doses of cortisone.

It will be noted (Fig. 1) that although vit. $B_{12}$ prevented growth inhibition by cortisone (group IV), it did not increase growth to the level of the rats given vit. $B_{12}$ without cortisone (group II). This was true even though the rats were fed approximately 10 times their normal requirement for this vitamin. In explanation, it can be seen (Table I) that despite the return to practically normal urinary nitrogen and food intake levels, the efficiency of food utilization for body weight gains was only about 1/3 as great in the former as in the latter group. Thus, the doses of cortisone employed antagonized one of the normal actions of vit. $B_{12}$ and induced a considerable wastage of food. This emphasizes that there are limits to the ability of vit. $B_{12}$ to overcome the inhibitory effects of cortisone on growth processes, and that the hormone may increase requirements for other factors than vit. $B_{12}$. In this connection, previous work has shown that a combination of vit. $B_{12}$ and aureomycin is more effective in counteracting the inhibitory actions of large doses of cortisone in young rats than vit. $B_{12}$ alone(1-3).

*Summary.* 1. Fifty young male rats were divided into 5 uniform groups and were fed a vit. $B_{12}$-deficient diet for 20 days. For the following 30 days, one group received 200 $\mu$g of vit. $B_{12}$/kilo of diet, another was continued on the deficient diet, and the other 3 groups were injected with large doses of cortisone acetate with or without vit. $B_{12}$. 2. Vit. $B_{12}$ alone did not alter daily urinary nitrogen excretion/100 g body weight, but increased appetite and body weight gains. Without vit. $B_{12}$, cortisone significantly increased daily urinary nitrogen losses and reduced body growth and food consumption. Vit. $B_{12}$ largely prevented these protein catabolic actions of cortisone under *ad lib.* feeding, but was ineffective when food intake was reduced to that of rats given cortisone without vit. $B_{12}$. 3. It is concluded that (a) vit. $B_{12}$ can prevent increased urinary nitrogen losses induced by cortisone, probably by increasing appetite and thereby enhancing the availability of carbohydrate or protein to the organism (b) large doses of cortisone can markedly inhibit the ability of vit. $B_{12}$ to transform food into body weight gains. Other implications of these findings are discussed.

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## Intracerebral Adaptation to Mice of the Leon Strain of Type 3 Poliomyelitis Virus.* (20876)

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The adaptation to mice of the Leon Strain of type 3 poliomyelitis virus employing the intraspinal route has been reported by Li and Habel(1). Their results have been confirmed by Casals, Olitsky, and Brown(2). In a more recent paper Li and Schaeffer(3) have described a further modification of the mouse adapted line of the Leon Strain; continued mouse passage increased the virulence for mice but decreased intracerebral infectivity for the monkey. Both Li and Schaeffer(3) and Habel (ref. cited in 3) noted that this strain would occasionally infect mice by the intracerebral route. The present report gives details of procedures whereby this virus has been adapted to mice resulting in a line that is pathogenic via the intacerebral route as well as the intraspinal.

*Methods and materials. Technic of criss-cross passage.* In the adaptation experiments to be described criss-cross passages were used between tissue cultures and mice, injecting tissue culture fluid intracerebrally in the mouse and recovering, in tissue cultures, the virus from the cords of animals thus paralysed. The 2nd tissue culture passage was again inoculated intracerebrally and the criss-cross passages repeated in the same way. The tissue culture fluids of various passages were then titrated in mice by the intracerebral and intraspinal routes. *Viruses.* Drs. Li and Schaeffer, to whom we express our thanks, were kind enough to send to us their line of the Leon Strain, adapted for intraspinal infection of mice. The material consisted of 10% cord suspension and was taken from the 81st mouse passage. During our experiments, this strain was typed frequently in tissue cultures and identified as type 3 poliomyelitis virus. A second strain (Lansing, type 2), which is well adapted for the intracerebral route in mice, was used for comparative titration both intraspinally and intracerebrally in mice. *Test in mice.* CFW mice (15-20 g) were used throughout these experiments and a standard inoculum of 0.025 ml was employed for both the intracerebral and intraspinal route. The technic of the intraspinal inoculation was the same as described by Habel and Li(4). Average loss of mice paralysed by trauma was about 10% in the present studies. Animals found dead or paralysed after 12 to 24 hours were eliminated from the final results. Only cords of animals found paralysed after cerebral inoculation were pooled and used as 10% tissue suspension for inoculation into tissue cultures. It was felt that the ability to invade the cord might be one characteristic of a pathogenic virus. The cord suspension was prepared in distilled water and clarified with streptomycin according to a

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