STUDIES ON MYO-INOSITOL

I. EFFECT ON THE GROWTH OF RATS

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Woolley (1, 2) first observed a considerable delay of growth and that symptom of alopecia in mice fed on an inositol-deficient diet, but the vitamin action of inositol on higher animals remains still equivocal, although it was found to be the essential factor for human and mammalian cell cultures (3, 4) and some microorganisms (5—7). Recently, the biosynthesis of *myo*-inositol from hexose in mammalian tissues has been established (8—10).

However, *myo*-inositol is widely distributed in organs and tissues of animals in fairly large amounts (11—13), and is thought to be turned over with rather a rapid rate (14—16). The possible role of *myo*-inositol on cells in the protection against low humidity (17, 18) and of phosphoinositides on the cation transport (19, 20) and on the ATP-linked contraction of mitochondria (21) may stimulate the nutritional re-examination of this substance in detail. In the present paper will be described the effect of *myo*-inositol on the growth of rats that had never been proved to have any symptoms caused by its deficiency.

EXPERIMENTAL

Male rats of Wistar strain, 20 days old and weighing about 50 g, were used. The basal diet used throughout these experiments was essentially the same as that described by Forker and Morgan (22), and consisted of, in per cent, vitamin-free casein, 22; sucrose, 64.5; corn starch, 2; hardened oil, 9; and salts, 2.5 (the mixture of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 180 mg; $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 235 mg; K_2HPO_4 , 644 mg; $\text{Ca}_3(\text{PO}_4)_2$, 365 mg; calcium lactate, 879 mg; and ferrous citrate $\cdot 5\text{H}_2\text{O}$, 80 mg). Commercial casein must be thoroughly washed before use to remove contaminated *myo*-inositol. Namely, the commercial casein was dissolved in 1 % sodium hydroxide, and after standing for an hour at room temperature, the solution was adjusted to pH 4.6 by adding dropwise 20 per cent acetic acid. The precipitates formed were collected, washed three times with 60 per cent ethanol and dried in the dark. After these treatments, contaminated *myo*-inositol could be completely eliminated.²

The composition of vitamin B supplements (daily dose per rat) orally administered was composed of, in mg, 0.005 of thiamine, 0.01 of riboflavin, 0.005 of pyri-

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² Scherer's test (23) showed no *myo*-inositol in the acid hydrolysate of washed casein.

doxine, 0.005 of folic acid, 0.025 of pantothenic acid, 0.025 of *p*-aminobenzoic acid, and 1.25 of choline. Besides these supplements, definite amounts of *myo*-inositol were added as given in Table 1. The fat-soluble vitamins consisting of 10 i.u. of A, 1 i.u. of D and 0.1 mg of tocopherol were separately prepared, and also orally administered.

TABLE 1
Dosage of myo-Inositol to Each Experimental Group

Experimental group	Dosage of <i>myo</i> -inositol
	mg/day/rat
A (<i>myo</i> -inositol-deficient)	0
B (standard dosage)	0.5
C (excess I)	1.0
D (excess II)	2.0

Rats were housed in the individual cage, and divided into four groups as shown in Table 1. Each group consisted of 6 to 10 animals. For standard assay group (B), 0.5 mg of *myo*-inositol was administered daily. All the animals were grown in the room maintained at 23°. Water and basal diet were given freely. Vitamins were given compulsorily. The growth rate of experimental animals was measured by weighing every 24 hours, lasting for about 45 days in every experiment. In some cases, prolonged breeding was held for about three months.

RESULTS

1. Growth-Promoting Action of *myo*-Inositol

As indicated in Fig. 1, the animals fed on the *myo*-inositol-deficient diet still showed some appreciable increase in body weight during 45 days of breeding. The results were highly reproducible, and any symptoms of alopecia or spectacled eye (22) were not observed during these breeding periods. Even after continuing

FIG. 1 *Growth Curves of the Group Fed on a myo-Inositol-Deficient Diet (Group A)*

FIG. 2 Growth Curves of the Group Administered with 0.5 mg of *myo*-Inositol per Day (Group B)

the inositol deficiency for about 3 months, these symptoms were hardly perceived. Although we could find that only one case of all the 30 rats seemed to turn to so-called "spectacled eye" in four trials of examination, it is still doubtful whether such an indication could be attributed to the inositol deficiency or not. The fact seems to support the general view that any symptoms which had been observed in mice fed on the inositol-deficient diet (1, 2, 24), were scarcely seen in other higher animals.

However, it is necessary to compare the growth rates of the animals receiving *myo*-inositol with those of the animals without receiving no *myo*-inositol. As in

FIG. 3 Mean Growth Curves of Group A (dotted line) and Group B (solid line)

indicated in Figs. 1 and 2, there was a marked difference in the increase of body weight between the groups grown with and without *myo*-inositol. Four trials of experiments were repeated, and all the results showed similar tendency. Fig. 3

shows the typical example of the mean growth rate (Table 2, Exp. 2). From these curves, the mean increase in body weight per day was calculated to be 1.4 g for group A (inositol-deficient) and 2.5 g for group B (0.5 mg of inositol was dosed per day); thus indicating that the rate of increase in body weight of the rats grown with *myo*-inositol is fairly higher than that of those grown without the vitamin. Both groups showed no abnormal changes in their organs as far as they were examined by macroscopic observations.

Therefore, it might be reasonable to consider that *myo*-inositol, which actually promotes the increase in body weight of rats, is likely to have some beneficial effects on the growth of young animals.

2. Influence of the Increase of *myo*-Inositol Dosage

As shown in Fig. 4, no change occurred in the time course of body weight

FIG. 4 Growth Curves of the Group Administered with 2 mg of *myo*-Inositol per Day (Group D)

gain and in the appearance of the animals by increasing the inositol dosage from 0.5 to 2.0 mg. The result seems to indicate that the administration of *myo*-inositol in quantities of 0.5 to 2.0 mg produces desirable effect on the growth of rats without any harmful effects.³

TABLE 2
Effect of *myo*-Inositol on the Growth of Rats

	Mean increase in body weight per day			
	Group A	Group B	Group C	Group D
			<i>g/day</i>	
Exp. 1	1.9	2.9	—	—
Exp. 2	1.4	2.5	—	—
Exp. 3	1.7	2.7	2.7	2.4
Exp. 4	1.8	2.4	—	—

DISCUSSION

First and foremost information obtained in the present study is the fact that the

³ The effect of higher dosage is now under investigation.

rats fed on a *myo*-inositol-deficient diet showed unequivocal growth, though their growth rates were fairly lower than those of the *myo*-inositol-administered rats. In addition, we could scarcely find any symptoms of alopecia or spectacled eye which were common in mice fed on diets containing no *myo*-inositol (2, 24). So the facts seem to indicate that in the case of rats, *myo*-inositol could be in part supplied endogeneously (25), *e. g.*, from intestinal flora (26) and/or by biosynthesis in the tissues (8—10).

However, it is unlikely to ignore the nutritional value of *myo*-inositol only from the viewpoint of its endogeneous production. Although Fenton *et al.* (27) described that it is rather difficult to demonstrate the growth promoting action of dietary *myo*-inositol, the present results in fact indicate that the dietary *myo*-inositol may produce favorable influence upon the growth rate of young rats. The direct reason for such an effect of *myo*-inositol may be attributed to the following presumption that the total endogenous production of *myo*-inositol is rather insufficient to promote the cellular multiplication, probably due to the restriction of the net synthesis of cellular and subcellular constituents containing *myo*-inositol, and that it is necessary to supply the dietary *myo*-inositol for the favorable growth of young animals. At any rate, our results might be referred to the results of those who found relation between inositol and choline during the periods of rapid synthesis of cells such as the growth of a young animal (28), its vital action on the isolated tissues in culture (3, 4), the abnormal requirement for inositol during parturition (29), and so on.

Another but indirect reason for the favorable effect of *myo*-inositol may be attributed to its influence on the intestinal flora. As well known, *myo*-inositol is an essential factor for some microorganisms (5—7), and it is natural to suppose that the dietary *myo*-inositol may also stimulate the growth of intestinal microorganisms, which, as a result, lead to the secondary improvement of internal environments, such as vitamin balances. It is desired to clarify the real action of *myo*-inositol on the microorganisms living in the gastrointestinal tract of higher animals.

SUMMARY

The young rats fed on a *myo*-inositol-deficient diet showed no doubt the increase in body weight during the initial 45 days of breeding, and was scarcely found any symptoms of spectacled eye or alopecia in appearance, even after the prolonged breeding for more than 3 months. However, there was a marked difference in the growth rate between the animals fed on a *myo*-inositol-deficient diet and those fed on a *myo*-inositol-containing diet. The mean daily increase in body weight of rats fed on 0.5 to 2.0 mg of *myo*-inositol was fairly higher than that of those fed on a *myo*-inositol-deficient diet. Four trials of experiments were performed with high reproducibility.

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