Abderhalden (1) discovered that 2-methyl-4-amino-5-hydroxymethyl-pyrimidine produced a typical severe convulsion preventable by yeast and rice bran. Makino and Sasaki (2) also observed similar effects and gave the name "toxopyrimidine (TXP)" to this pyrimidine and the name "atoxopyrimidine (ATXP)" to substances which inhibit the convulsion. We have found that a group of vitamin B₆ shows the action of ATXP (3). It was assumed from the similarity of chemical structures of the vitamin B₆ group and TXP that there would be a biological antagonism between them.

The purpose of the present study is to demonstrate whether TXP has an antivitamin B₆ activity in both animal experiments and bacterial growth. The experiments, the outline of which has been reported in a preliminary communication (4), are described in detail in the present paper.

**EXPERIMENTAL**

*Accelerating Effect of TXP upon Vitamin B₆ Deficiency Syndrome*

DeRenzo and Cerecedo (5) found that in mice the antivitamin B₆ activity of desoxypyridoxine manifested itself both by its effect on growth and by its ability to produce the typical vitamin B₆ deficiency syndrome. Emerson (6) also observed that desoxypyridoxine remarkably decreased the time required for the appearance of acrodynia in deficient rats.

An observation was made by the author to find the possibility of TXP of producing a severe deficiency symptom in rats. Young albino rats weighing 40–50g were used as test animals. Deficiency control animals were fed on a synthetic vitamin B₆-deficient diet presented in Table I. Two other groups (I and II) received the same deficient diet mixed with 2 and 5 mg TXP per rat, respectively (TXP diet). As reference, another group (III) was fed on the vitamin B₆-deficient diet containing 5 mg niazid (INAH diet).

As shown in Fig. 1, the animals of both groups (I and II) did not increase in body weight at all, while the deficiency control animals stopped to grow after two weeks. The growth curve of the INAH group (III) mediates between those of the deficiency control and experimental groups.

A typical acrodynia occurred after three weeks in group I (as illustrated in
Fig. 7) and after two weeks in group II as in Fig. 8, while the deficiency control rats showed a slight deficiency syndrome as in Fig. 6. The vitamin B₆ deficiency syndrome was also noted by the increase in the urinary xanthurenic acid (XA) following L-tryptophan administration and confirmed by the fact that the acrodynia was recovered after two weeks when the TXP diet was switched to the vitamin B₆ diet (50 mg per animal), while riboflavin showed no effects upon the recovery of the deficiency syndrome (Fig. 2).

**Table I Composition of Synthetic Vitamin B₆-Deficient Diet.**

<table>
<thead>
<tr>
<th>Basal diet</th>
<th>Vitamin supplement (per kg basal diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified casein</td>
<td>Thiamine hydrochloride</td>
</tr>
<tr>
<td>Salt</td>
<td>Riboflavin</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Niacin</td>
</tr>
<tr>
<td>McCollum salt</td>
<td>Calcium pantothenate</td>
</tr>
<tr>
<td>Cod-liver oil</td>
<td>2-Methyl-1, 4-naphthoquinone</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>Folic acid</td>
</tr>
<tr>
<td></td>
<td>Choline chloride</td>
</tr>
<tr>
<td></td>
<td>Inositol</td>
</tr>
</tbody>
</table>

An examination was made to find whether TXP suppresses the growth activity of pyridoxine in vitamin B₆-deficient rats.

Weanling rats were fed on a synthetic vitamin B₆-deficient diet given in Table I for four weeks till they have showed apparent weight loss. The four control groups were fed on vitamin B₆-deficient diet supplemented with 5, 10, 25 and 50 mg per animal of pyridoxine hydrochloride (PIN), respectively.

One of the two experimental
groups received the vitamin B6-deficient diet containing 10\( \gamma \) PIN and 2.5 mg TXP. The other group received the same diet containing 25\( \gamma \) PIN and 5 mg TXP.

As shown in Fig. 3, the four control groups increased in body weight on respective averages of 24.5, 32.0, 46.0 and 74.0 g after three weeks of feeding. In the former experiment in which 10\( \gamma \) PIN and 2.5 mg TXP were added to the vitamin B6-deficient diet the body weight of the rats increased by 12 g after three weeks, while that of the corresponding control animals receiving 10\( \gamma \) PIN increased by 32 g. In the latter experiment with 25\( \gamma \) PIN and 5 mg TXP, the gain in weight during three weeks was on almost the same level as that of the corresponding control, but after the fourth week their body weight stopped to increase and vitamin B6 deficiency syndrome became apparent.

Effect of TXP on Tryptophan Metabolism in Rats

Glazer et al. (7) reported that in human beings the vitamin B6 deficiency caused by the administration of desoxypyridoxine promotes XA production as a result of a disturbance of tryptophan metabolism. Porter et al. (8) suggested that the study of excretion of tryptophan metabolites would offer a rapid method for screening compounds having antivitamin B6 activity.

In the present experiment, the effects of the short term administration of TXP upon the excretion of XA into urine were observed using rats. A part
of the animals in the preceding experiment were housed in metabolic cages at the end of the second week of the experimental period as shown in Fig. 3. They were groups A, B and C given in Table ‡U. The amount of XA in 24 hour urine was determined by the method described by Rosen, Huff and Perlzweig (9) using Beckmann Model B Spectrophotometer. The experiment was carried out according to the schedule shown in Table ‡U: The amount of XA was measured in the same animal after the administration of tryptophan alone (day 2), tryptophan plus desoxypyridoxine (day 5), tryptophan plus TXP (day 10) and tryptophan plus pyridoxine and TXP (day 12). Each compound was given by a stomach tube in combined doses.

As shown in Fig. 4, XA excretion significantly increased by administering tryptophan in both deficient (A) and experimental animals (C) but remained on the same level in the control animals (B). The XA value became higher after the administration of desoxypyridoxine, particularly in the control animal owing to the antivitamin B6 activity of desoxypyridoxine. The administration of 2 mg of TXP showed the tendency to increase the excretion of XA, and 5 mg of TXP enhanced the excretion of the metabolite in the control animals. The higher value of XA due to TXP decreased by simultaneous administration of pyridoxine.

It has thus been observed that TXP showed an antivitamin B6 activity in excreting abnormal tryptophan metabolites.

**Antivitamin B6 Activity of TXP in the Growth of Saccharomyces carlsbergensis**

The antivitamin B6 activity of TXP, which has been proved by the above

| Table III |
| Atkin’s Medium for B6 assay |

| Glucose | 10 g | FeCl3 | 500 µ |
| Sodium citrate | 2 µ | MnSO4 | 500 µ |
| Casein hydrolysate (Difco) | 1 µ | Inositol | 5000 µ |
| Citric acid | 400 mg | Ca pantothenate | 500 µ |
| KH2PO4 | 110 µ | Thiamine HCl | 50 µ |
| KCl | 85 µ | Biotin | 1.6 µ |
| CaCl2·2H2O | 25 µ | | in 100 ml (double strength) |
| MgSO4·7H2O | 25 µ | | |
animal experiments, was tested on microorganism. For this purpose, Saccharomyces carlsbergensis (IFO 0565), a widely used microorganism for assaying pyridoximers, was used for the determination of the growth inhibitory activity of TXP. It was incubated in Atkin’s medium (Table III) for 24 hours at 37°C, and the turbidity was determined with Colemen Junior Spectrophotometer at 650 mμ. According to Robinowitz and Snell (10), the organism grows well at the limiting concentration of pyridoxine, if thiamine is added to the culture medium. Hence, the minimal inhibition of TXP was determined in the culture medium with or without thiamine.

As shown in Table IV, TXP inhibits the growth of S. carlsbergensis and the inhibitory grades are different according to the presence or absence of thiamine. The minimal inhibitory concentration of TXP was found to be 16 and 400 μ/ml, respectively.

The reversal effects of pyridoxine on the growth inhibition caused by TXP was then determined. Fig. 5 shows that the growth inhibition by varying amounts of TXP was overcome by increasing amounts of pyridoxine. The relationship between TXP and PIN was competitive in a certain range of the concentration tested, as shown in Table V.
FIG. 6 B6-Deficient Control Rat Without Any Remarkable Sign of B6 Deficiency Syndrome.

FIG. 7 Experimental Rat, Fed on B6-Deficient Diet with 2 mg TXP per Rat for 3 Weeks.

FIG. 8 Experimental Rat, Fed on B6-Deficient Diet with 5 mg TXP per Rat for 2 Weeks. Note the remarkable B6-deficiency syndrome (in Figs 7 and 8).

FIG. 9 Recovered Stage of the Extremely B6-Deficient Rat (Fig. 8), after Feeding 50 µg Pyridoxine-Supplemented Diet for 2 Weeks.

TABLE V
Relation between the Concentration of Toxopyrimidine added to the Media and the Concentration of Pyridoxine Necessary for the 50 Per Cent Maximal Growth of S. carlsbergensis.

<table>
<thead>
<tr>
<th>TXP concentration added to the medium</th>
<th>PIN concentration necessary for 50 per cent maximal growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ/ml</td>
<td>γ/ml</td>
</tr>
<tr>
<td>50</td>
<td>0.0113</td>
</tr>
<tr>
<td>100</td>
<td>0.0251</td>
</tr>
<tr>
<td>500</td>
<td>0.2280</td>
</tr>
</tbody>
</table>

DISCUSSION

The above-mentioned data offer the proof that TXP has an antivitamin B6 activity. Such a relationship between TXP and vitamin B6 may be explained by the structural similarity. A somewhat similar situation has been
proved in an enzymatic experiment. According to Koike and Makino (11),
the coenzyme action of pyridoxalphosphate can be competitively inhibited by
TXP-phosphate in the tyrosine decarboxylase of Lactobacillus. It is very in-
teresting to note that both INAH and thiosemicarbazide are also metabolite
antagonists to vitamin B₆ in the microorganism and cause the severe convul-
sion similary as TXP (12 15). Furthermore, it was reported that the fits
of an epileptiform natures were observed in rats after long deprivation of
vitamin B₆ (16).

SUMMARY

It was proved both by animal experiments and by bacterial growth that
TXP showed an antivitamin B₆ activity under conditions similar to those
employed in the case of 4-desoxypyridoxine, a known antimetabolite of pyrid-
oxine. That is, a typical symptom of vitamin B₆ deficiency (acrodynia) was
produced in rats with the aid of TXP. It was shown that TXP inhibited
the growth action of pyridoxine in vitamin B₆-deficient rats, whose body
weight had stopped to increase. TXP was found to possess an antivitamin B₆
action characterized by the abnormal metabolism of tryptophan. TXP
inhibited the growth of S. carlsbergensis (IFO 0565), especially in the culture
medium containing thiamine. The relationship between TXP and pyridoxine
was competitive in a certain concentration range tested.

ACKNOWLEDGEMENT

The author wishes to express his heartfelt thanks to Mr. Kiyogi Okami,
Chief of the Osaka Plant and Dr. Satoru Kuwada, Chief of the Takeda Re-
search Laboratory, for their continued encouragement, and to Dr. Atsusi
Watanabe and Dr. Yoshitomo Aramaki for helpful suggestions and advice.
The author is indebted to Dr. Taizo Matsukawa for supplying toxopyrimidine
and 4-desoxypyridoxine, and to Professor Takeo Sakan, Osaka University, for
his kind gift of xanthurenic acid. The author's thanks are due to Mr. Minoru
Kawashima for carrying out the growth analysis of bacteria. Acknowl-
edgement is also made to Mr. Eiichi Yamazaki, Chief of the Testing Depart-
ment of the Osaka Plant for permission to publish this paper.

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