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Effect of Oxythiamine on Infection of Mice with the Lansing Strain of Poliomyelitis Virus.*

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It was reported from this laboratory[1] that a dietary deficiency of thiamine increased the resistance of albino mice to the Lansing strain of poliomyelitis virus. A few months later Rasmussen, *et al.*[2] reported similar observations. Using Theiler's virus. Lichstein and associates in the same laboratory[3] found that deficiencies of some other dietary constituents afforded a similar degree of protection to mice.

However, none of the several other dietary deficiencies studied at The Children's Hospital of Philadelphia showed a degree of protection against the Lansing strain, which is considered a true poliomyelitis virus, equalling that of a deficiency of thiamine, although these included some of those reported by Lichstein as giving protection against Theiler's virus.

Because of the superior effect of a deficiency of thiamine in mice inoculated with the Lansing strain, a study of the influence of inhibitory analogues of thiamine on the resistance of mice to this virus was indicated.

The first thiamine analogue thoroughly studied in this laboratory was oxythiamine.† It is produced by replacing the NH_2 group on the pyrimidine ring of thiamine by an OH group. Some of the physiological properties of this analogue have been studied by Soodak and Cerecedo.[4] They found that mice receiving 1 mg of thiamine per day and given 25, 50 or 100 mg of oxythiamine daily, lost weight rapidly and died in about 2 weeks. They also showed that the enzyme of carp which destroys thiamine was inhibited by oxythiamine, but they did not determine the minimum amount of thiamine which would just off-set a given quantity of oxythiamine.

Several experiments were performed in the authors' laboratory studying the effect of this inhibitor of thiamine upon the resistance of mice to the virus of poliomyelitis. All of these gave the same general results. It is sufficient, therefore, to present in this paper the data obtained in the largest and most complete of the experiments.

Experimental. Virus. The virus and the technics employed were described in an earlier paper.[5] A recent test in this laboratory showed that the virus was still infectious for rhesus monkeys.

Animals. The Swiss white mice used in these studies came from the same colony that has been maintained in this laboratory for 14 years, and has produced mice for all of the resistance experiments conducted here.

The usual precautions described elsewhere[6] and essential for this type of experiment were

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1 Foster, C., Jones, J. H., Henle, W., and Dorfman, F., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 215.

2 Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Bact.*, 1943, **45**, 85.

3 Lichstein, H. C., McCall, K. B., Kearney, E. B., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 279.

† We gratefully acknowledge the advice and cooperation of Dr. Gustav J. Martin of the National Drug Company of Philadelphia, Pa., who supplied the oxythiamine and provided the data on its structure, purity and microbiological assay.

4 Soodak, M., and Cerecedo, L. R., *J. Am. Chem. Soc.*, 1944, **66**, 1988.

5 Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, **79**, 221.

6 Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, **80**, 257.

taken. These included split litter technic for distribution, temperature and humidity control of the experimental rooms, and even a random distribution of the animals in the room to avoid the effect of any environmental differences. The mice were weighed daily, and following inoculation, each animal was removed from its cage for examination every 6 hours, day and night.

Diet. The basal complete diet had the following composition expressed in %: Casein, crude 25.0, cellulose, 2.0, salt mixture,[7] 4.0, linseed oil, 1.5, wheat germ oil, 1.0, glucose (cerelose) 66.5, carotene concentrate in oil, 5 drops, (Wyeth - 275 U.S.P. units pro-vitamin A per drop) Drisdol, 0.4 drop (Winthrop-250 U.S.P. units vitamin D_2 per drop). The B vitamins were supplied in the following quantities (mg per 100 g of diet.): Thiamine chloride 0.2, riboflavin 0.5, pyridoxine 0.5, calcium pantothenate 5.0, nicotinic acid 10.0, inositol 10.0, *p*-aminobenzoic acid 10.0, choline chloride 100.

This diet was given to the complete-diet control groups and to all of the oxythiamine groups. It was modified in the two low-thiamine groups only in so far as the thiamine content was concerned. Before the experimental regimens were initiated, all the animals were put on the complete diet for 2 days after they had been distributed into their individual experimental jars, in order to accustom them to the purified diet.

Inhibition Index.‡ Preparatory to the experiment, the inhibition index of oxythiamine was evaluated at 3 levels of thiamine: 3, 6, and 12 μg per mouse per day. In these titrations, both the thiamine and the oxythiamine were administered by mouth from a pipette. The inhibition index for the sample of oxythiamine used in the experiment reported here was found to be about 3 at all 3 levels of thiamine. Using *Lactobacillus fermentum,* the index in Dr. Martin's Laboratory was found to be 10, which is of the same general order as that obtained with the mice.

In the actual experiment this inhibition index was used as a guide, although the conditions were not quite comparable since the thiamine was given as an integral part of the diet, while the oxythiamine, on the other hand, was dissolved in saline and administered by pipette as above. In order to calculate, on the basis of the inhibition index, the amount of oxythiamine necessary to establish and maintain a given state of deficiency, the following assumptions had to be made. If the food intake for a normal mouse is 3 g per day, then each animal would receive 6 μg of thiamine. As the deficiency state advances under the influence of oxythiamine, the accompanying anorexia leads to a lower intake of diet and correspondingly a lower intake of thiamine. The oxythiamine dosage then has to be decreased by an estimated amount. It is worth commenting that with the pure samples of the analogue, severe signs of deficiency can be quickly terminated by reducing the oxythiamine dosage or by adding a suitable amount of thiamine.

Experimental Groups. The experimental animals were divided into 12 groups as presented in Table I. The mice of 6 of the groups were inoculated with a suspension of mouse brain infected with the Lansing strain of poliomyelitis virus (V), and for each of these there was a control group, the animals of which were injected with a suspension of uninfected ("normal") mouse brain (N).

The first pair of groups (1-V and 1-N) were fed the complete basal diet; the next 4 pairs of groups (2-V and 2-N, 3-V and 3-N, 4-V and 4-N, 5-V and 5-N) received the same complete basal diet and in addition were given oxythiamine; the last pair of groups (6-V and 6-N) were maintained on a low-thiamine diet, in which the intake of thiamine was varied between 20 and 60 μg per 100 g of diet. The amount of thiamine was regulated in an effort to maintain a level of deficiency that

[7] Jones, J. H., and Foster, C., *J. Nutr.*, 1942, **24,** 245.

‡ The inhibition index is defined as the quotient of the molecular amount of analogue divided by that molecular amount of metabolite which just counteracts the analogue. For example, if the inhibitory action of 1.0 millimole of analogue were just offset by 0.001 millimole of metabolite, then the index would be 1.0/0.001 or 1000 (see D. W. Woolley, *Advances in Enzymology,* 1946, **6,** 129.)

TABLE I.

Group No.	No. of mice	Thiamine, mg per 100 g diet	Oxythiamine	Time of beginning oxythiamine in respect to day of inoculation	No. of mice in N-groups dying during experiment
1-V*	56	.2	—	None	—
1-N†	6	.2	—	"	0
2-V	57	.2	+	9 days before	—
2-N	21	.2	+	9 " "	2
3-V	57	.2	+	2 " "	—
3-N	21	.2	+	2 " "	1
4-V	60	.2	+	day of inoculation	—
4-N	21	.2	+	" " "	1
5-V	60	.2	+	4 days after	—
5-N	22	.2	+	4 " "	1
6-V	58	.02-.06	—	None	—
6-N	21	.02-.06	—	"	0

* Groups with "V" in number received virus-infected brain.
† " " "N" " " " uninfected brain.

would keep the average weight more or less constant.

The 4 pairs of groups that received the oxythiamine differed among themselves in respect to the day the administration of the analogue was begun. An attempt was made with the first pair of these groups (Group 2-V and 2-N) to produce the deficiency gradually, and to time it so that the animals would be in the same state of deficiency on the day of inoculation as the mice on the diet low in thiamine. To that end 32 μg of oxythiamine, or about twice the amount that would be counteracted by the thiamine in the diet, were given per animal per day, starting 9 days before inoculation. As that was insufficient to produce a deficiency state in the desired time, the oxythiamine was increased to 64 μg 3 days later, but after 5 days on this amount it was necessary to reduce it to 8 μg and then to 4 μg in order to stop the rapid loss of weight of the animals. After 2 days on 4 μg and 2 additional days on 8 μg, their weights could then be held about stationary on 16 μg per mouse per day.

Groups 3-V, 4-V, and 5-V and their controls were given oxythiamine starting respectively 2 days before inoculation, on the day of inoculation, and 4 days after inoculation. In each of these cases, the dose of oxythiamine was 128 μg per mouse for the first and second days. This large dose produced an almost immediate fall in weight. From the third day on the dose was gradually reduced, and in each case finally levelled off at 16 μg per day.

The experimental animals were observed until the 28th day after inoculation when the experiment was terminated.

Results. Table I shows that there were very few deaths among the groups of mice receiving the "normal" brain. This indicates that the "deficiencies" were not responsible for many deaths in the groups inoculated with virus. This assumption is substantiated by the fact that paralysis or some other sign of poliomyelitis was seen in nearly all animals of the infected groups that died. The following discussion of data is concerned, therefore, only with the animals that were given virus.

The results are presented in Chart I. The curves show the daily cumulative deaths in per cent as ordinates plotted against days after inoculation, as abscissae. It can be seen that, except for a few days at the beginning of the experiment and again at the end, the curve for the animals on the complete diet was above that for any of the "deficient" groups. The curves for Groups 2-V, 3-V and 4-V are very much alike. In these groups, the maximum difference in percentage deaths between Group 1-V and each of the "deficient" groups came at about the 14th day after inoculation. At this point deaths in the "deficient" groups were roughly 50% of those in the group on the complete diet. The death rate in Group 5-V (animals given initial dose of oxythiamine 4 days after inoculation) was nearly equal to

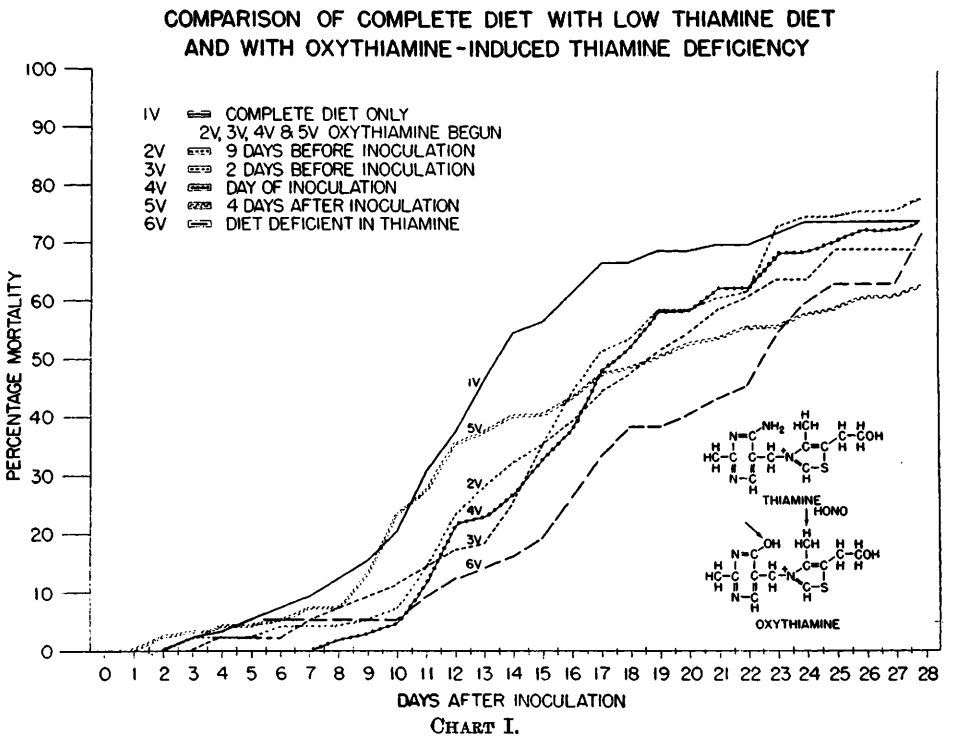

CHART I.

that of Group 1-V for the first 12 days. From then until the end of the experiment the death rate in Group 5-V was considerably decreased, so that even when the experiment was terminated, the percentage deaths was below those of the controls (63 to 74).

None of the groups of mice receiving the oxythiamine were protected to the extent of the animals on the low-thiamine diet. The cumulative percentages of deaths of each virus-inoculated test group were compared by the Chi-square method with the corresponding values for the animals of the virus-inoculated control group for each day of the experiment. The days after inoculation (inclusive) on which the differences were significant are as follows: Group 2-V, 10 to 15; Group 3-V, 12 to 18; Group 4-V, 6 to 17; Group 5-V, 17 to 20; and Group 6-V, 10 to 23.

Discussion. It seems to be possible to induce in mice a state of comparative resistance to poliomyelitis by means of an agent that can be given by mouth, the dose of which can be regulated within certain fairly narrow limits, and the deficiency signs quickly obliterated by suitable doses of thiamine. The protection resulting from oxythiamine treatment appeared to be of the same general order as that resulting from a diet deficient in thiamine, and presumably was associated with the presence of this induced deficiency. As in other deficiency studies, the protective action manifested itself mainly by a slowing of the death rate—a delaying rather than an outright protective action. However, as the animals of group 5-V were given some protection to the end of the experiment, it suggests that if the proper conditions could be found, it is possible that the degree of protection might be increased. It is of particular interest that in this same group (5-V), protection was afforded even though oxythiamine was not administered until 4 days after inoculation with the virus.

The difference between the greater protective effect of thiamine deficiency resulting from a low-thiamine diet, in contrast to the lesser protection afforded by the thiamine deficiency induced by oxythiamine, is not entirely explicable. One of the factors may have

been the difference in the weight of the mice in the two groups. The animals of oxythiamine groups were not given the analogue until a varying number of days after the low-thiamine group had been started on the diet deficient in the vitamin. During this interval, the mice which later received oxythiamine were growing at a normal rate, while the vitamin-deficient animals were growing subnormally. The growth-rate differential resulted in a difference of about 3 g between the thiamine-deficient and the oxythiamine-fed animals at the time of inoculation, which may indicate a difference in the degree of deficiency not otherwise recognizable.

Summary. Mice from a genetically controlled colony and under controlled environmental conditions were used to examine the effect of oxythiamine, an inhibitory analogue of thiamine, on resistance against the Lansing strain of poliomyelitis.

A significant degree of protection was induced in all the groups on the oxythiamine as compared with those on the normal diet. This protection was not quite as marked as in the mice on the low-thiamine diet.

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Comparison of Penicillin G and A Biosynthetic Penicillin with Regard to Diffusion into Cerebrospinal Fluid.

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Under natural conditions *Penicillium notatum* or *Penicillium* chrysogenum produces a mixture of penicillins G, F, dihydro-F, K and X. The predominant form of the penicillin elaborated can be influenced by the addition of precursors to the nutritive media. Similarly, the addition of precursors that do not occur in nature has resulted in the formation of penicillins that do not occur naturally. Such penicillins are formed by reason of the ability of the mold to utilize these precursors and incorporate portions of them into the penicillin molecule.[1] Penicillins produced by the addition of precursors not occurring in nature may be referred to as biosynthetic penicillins. BT penicillin* is such a biosynthetic penicillin and will predominate if the precursor used is one containing the n-butylthiomethyl grouping in available form.

The widespread use of crystalline penicillin G (benzyl penicillin) has established the fact that many patients show the usual phenomena of drug sensitivity. In addition to the possible advantage of having a penicillin in which a substitution had been made for the benzyl group, it was thought that perhaps such a penicillin would have distinctive properties not possessed by benzyl penicillin. Preliminary observations made in animals suggested that BT penicillin might diffuse into the cerebrospinal fluid more readily than benzyl penicillin.[2] The investigation here reported was carried out to test this hypothesis.

Materials used. The water-soluble potassium salt of BT penicillin with a potency of 2900 units per mg was employed throughout. The material contained a small amount of penicillin G as a contaminant, for the media upon which the mold was grown during the preparation of this material contained naturally occurring precursors which permitted the formation of penicillin G. The sodium salt of crystalline penicillin G was used for comparison with the BT penicillin.

Patients studied. Eleven patients with central nervous system syphilis were investigated.

[1] Biosynthesis of Penicillins, *Science*, 1947, **106**, 503.

* n-butylthiomethyl penicillin supplied through the courtesy of Dr. N. P. Sullivan of Eli Lilly & Co.

[2] Sullivan, N. P., personal communication.