dissolved and 2% starch was suspended.

The control animals lived as long as the experimental animals (353 to 647 days). There were no spontaneous tumors throughout the colony of over 500 rats from which most of the experimental animals were taken.

One experimental animal was sacrificed after 362 days and the tumor found carried through 6 generations of transplants.

Discussion. It is believed that these results definitely indicate the presence of a carcinogenic substance in crude ether-extracted wheat germ oil. Because of the presence of adhesions within the abdomen it might be argued that the malignancy was caused solely by irritation. But, if one considers that while all of the crude wheat germ oils used produced similar adhesions, yet the percentage of actual malignancy varied widely with different oils, this conclusion appears unwarranted. Indeed, this variation of tumor growth with different batches of crude wheat germ oil made by extraction with ether, lends support to our belief that a carcinogenic substance is present in some oils and absent in others. We are, however, unable to state as yet all the conditions essential to reproduce consistently these results with a wheat germ of any source.

Summary. Malignant tumors have been produced in, on an average of, 32% of Wistar rats injected with crude wheat germ oil made by extraction with ether. With one batch of oil, tumors resulted in 66% of the injected rats. The tumors were preponderantly sarcomas but carcinomata were induced in 3 rats.

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Effect of Pseudopyridoxine on Rat and Yeast Growth.*

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In a recent report Snell, Guirard, and Williams[1] demonstrated the presence of a substance in natural products which surpasses pyridoxine in its physiological activity on *Streptococcus lactis* cultured on a pyridoxine-free medium. The substance had properties similar to pyridoxine, hence was called pseudopyridoxine. Tissues and urine were found to contain pseudopyridoxine, the amount depending on the quantity of pyridoxine ingested. These workers suggested that the compound is a metabolite of pyridoxine. Snell[2] showed that pseudopyridoxine was formed when pyridoxine was autoclaved with the basal culture medium for *S. lactis*. The amount formed increased as the period of heating was increased, and was due to the interaction of pyridoxine with amino acids during autoclaving.

Before the paper of Snell *et al.*[1] appeared it had been observed in this laboratory that the recovery of pyridoxine after various chemical treatments was very high as measured by the *Lactobacillus casei* method of Landy and Dicken.[3] Since this test organism responds to pseudopyridoxine in a manner

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1 Snell, E. E., Guirard, B. M., and Williams, R. J., *J. Biol. Chem.*, 1942, **143**, 519.

2 Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 356.

TABLE I.
Pyridoxine and Pseudopyridoxine Content of Preparations.

Preparation	*L. casei* assay value (pyridoxine plus pseudopyridoxine)	Yeast assay value (pyridoxine)	Pseudopyridoxine content
	Micrograms per cc or g		
Acid autoclaved yeast extract	1617	17	1600
Pyridoxine hydrochloride, cystine treated, equivalent to 5 γ B_6 per cc	9.4	4.6	4.8
Pyridoxine hydrochloride, peroxide treated, equivalent to 5 γ B_6 per cc	24	4.6	19.4

similar to *S. lactis*[2,4] the active substance formed on chemical treatment was probably pseudopyridoxine.

It is apparent that pseudopyridoxine has some physiological significance in the metabolism of microörganisms. It seemed important to decide as definitely as possible whether it would also promote growth of higher animals, and of the strain of yeast used for pyridoxine assay.[5] Experiments designed to provide this information were therefore undertaken.

Experimental. Pseudopyridoxine determinations were made by a differential assay procedure to be described in detail elsewhere.[4] This method involves a preliminary estimation of both pyridoxine and pseudopyridoxine with *L. casei* followed by determination of pyridoxine alone by the yeast method.[5] For convenience, pseudopyridoxine is expressed in the terms used by Snell *et al.*, namely, microgram equivalents of pyridoxine.

Solutions containing pseudopyridoxine were prepared by the following 3 methods: (a) Cystine treatment. A solution of 250 μg of pyridoxine hydrochloride, 50 mg cystine hydrochloride, and 250 mg sodium acetate in 50 cc of water at pH 7.2 was autoclaved for 1 hour at 15 lb pressure. (b) Peroxide treatment. Two hundred fifty micrograms of pyridoxine hydrochloride in 40 cc of water were treated with 10 cc of 3% hydrogen peroxide for 4 hours at room temperature. The excess peroxide was removed with manganese dioxide, the solution filtered, and the filter paper washed thoroughly. (c) Heat treatment. Fifteen grams of Difco yeast extract dissolved in 50 cc of 0.1 N hydrochloric acid were autoclaved at 15 lb pressure for 1 hour. The solution was neutralized for assay.

The pyridoxine and pseudopyridoxine content of the 3 preparations are listed in Table I. Although some uncertainty attaches to the high pseudopyridoxine value listed for autoclaved yeast extract,[4] it seems very probable that a large fraction of this value actually represents pseudopyridoxine. Since the last 2 preparations were made from synthetic pyridoxine and 92% of the pyridoxine was recovered as determined by the yeast assay method, it is apparent that the pseudopyridoxine also present in the solutions did not stimulate yeast growth, and that most of the pyridoxine was unchanged by these treatments.

The effect of pseudopyridoxine on the growth of rats was determined by assaying the above preparations by the method of Conger and Elvehjem.[6] The pyridoxine content of the unheated yeast extract was found by the rat assay to be 15 μg per gram (Table II) while that of the acid autoclaved material was essentially the same. Both of these results are in good agreement with the yeast assay value (Table I). Acid autoclaved yeast extract, therefore, did not cause enhanced growth in the rat.

That pseudopyridoxine does not stimulate rat growth is more clearly demonstrated by

[3] Landy, M., and Dicken, D. M., *J. Lab. Clin. Med.*, 1942, **27**, 1086.

[4] Carpenter, L. E., and Strong, F. M., *Arch. Biochem.*, in press.

[5] Atkin, L., Schults, A. S., Williams, W. L., and Frey, C. N., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 141.

[6] Conger, T. W., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, **138**, 555.

TABLE II.
Effect of Pseudopyridoxine on Rat Growth.

No. rats	Supplement to basal	Amt per day	Avg gain per wk, g	Pyridoxine hydrochloride content, γ/ cc or g
3	None		7.8	
3	Pyridoxine hydrochloride	5 γ	24.0	
3	Difco yeast extract	0.33 g	24.0	15
3	Difco yeast extr. acid autoclaved	≏ 0.33 g	26.0	*ca.* 16
3	Pyridoxine hydrochloride, cystine treated	≏ 5 γ	20.0	3.8
3	Pyridoxine hydrochloride, peroxide treated	≏ 5 γ	22.0	4.2

TABLE III.
Effect of Pseudopyridoxine on Rat Growth.

No. rats	Supplement to basal	Amt per day	Avg gain per wk g	Pyridoxine hydrochloride content, γ/cc or g
5	None		6.8	
5	Pyridoxine hydrochloride	5 γ	23	
5	Difco yeast extract	0.33 g	27	*ca.* 18
5	Difco yeast extr., acid autoclaved	≏ 0.33 g	26	*ca.* 18
5	Pyridoxine hydrochloride, cystine treated	≏ 5 γ	19	3.8
5	Pyridoxine hydrochloride, peroxide treated	≏ 5 γ	20	4.1

the growth response of rats fed the cystine and peroxide treated pyridoxine preparations. Both preparations contained pseudopyridoxine, but the rats failed to show stimulated growth (Table II). The rat assay values for these preparations showed that 76 and 84% of the pyridoxine in the cystine and peroxide treated solutions respectively was unchanged by the chemical treatment.

The rat experiment was repeated using a larger number of animals per group and employing new pseudopyridoxine preparations. The hydrogen peroxide and cystine treated pseudopyridoxine preparations this time contained 22.0 and 12.8 μg equivalents of pseudopyridoxine and 4.7 and 4.9 μg of pyridoxine respectively. The results listed in Table III corroborate the previous findings.

Discussion. The above results indicate that pseudopyridoxine as found in natural materials or as prepared from pyridoxine by chemical agents does not stimulate yeast or rat growth. Since attempts to prepare solutions of pseudopyridoxine free from pyridoxine were unsuccessful,[4] it was impossible to determine whether or not pseudopyridoxine had any pyridoxine activity at all for these organisms.

Recently Robbins and Ma[7] have demonstrated that pseudopyridoxine does not affect the growth of certain molds that require pyridoxine for normal development. The fact that the determination of pyridoxine in natural materials by *Neurospora sitophila* gives results of the same order of magnitude as found by yeast and rat assay[4] suggests that this organism also does not respond to the pseudo form of the vitamin. From present evidence, therefore, it seems that the physiological activity of pseudopyridoxine is limited to the lactic acid bacteria.

Summary. Synthetic pyridoxine was converted to pseudopyridoxine by treatment with hydrogen peroxide and by autoclaving in a cystine solution. Pseudopyridoxine formed in this manner and that found in autoclaved yeast extract did not stimulate yeast or rat growth.

[7] Robbins, W. J., and Ma, R., *Proc. Nat. Acad. Sci., U. S.*, 1943, **29**, 172.