On the other hand, 2,4-D apparently specifically enhances liver retention of thyroxine. The effect of 2,4-D on biliary secretion of thyroxine was not studied but, by other methods, no increase of 'free' thyroxine was found in the blood.[5]

The present results with 'Atromid-S' confirm that it resembles 2,4-D in enhancing liver retention of thyroxine. But there was no evidence that the rate of biliary excretion of radioactive thyroxine was influenced by the drug. This, and the absence of any direct evidence (by paper electrophoresis studies) of displacement of radioactive thyroxine from TBP, in our opinion fail to support the hypothesis, in this species, that 'Atromid-S' might resemble salicylates and DNP in producing certain pharmacological effects by the displacement of thyroxine from its binding-sites on plasma proteins. In this connection, it may be of significance that little or no alteration of binding of tri-iodothyronine by red cells is detectable in the blood of humans treated with 'Atromid-S'.[9]

The primary effect of 'Atromid-S' on the liver may be reflected in the increase of serum glutamic oxaloacetic and serum glutamic pyruvic transaminases which occurs with continued dosage in experimental animals[11] and in man.[12] It has also been observed[13] that changes in the structure of the liver cell, consisting of an increase of mitochondria and lysosomes occurs in rodents given the drug. Whether these functional and structural changes arise as a result of a continuing effect on the metabolism of the liver cell from the increased concentration of thyroxine in the organ brought about by the drug (with a largely incidental effect on the turnover of lipids) or whether Atromid also exerts a direct effect on enzyme systems concerned with intrahepatic lipid metabolism cannot be established from the evidence at present available.

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The effect of vitamin B_6 deficiency and of isoniazid on the pyridoxal phosphate content of rat brain

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Convulsions in animals fed vitamin B_6-deficient diets have been thought to be caused by a deficiency of the coenzyme, pyridoxal phosphate (PALP),[1, 2] in the brain. The object of the present study was

to find whether a vitamin B_6 deficiency would reduce the PALP content of the brains of rats; and whether isoniazid (INH), known to inhibit the action of some PALP-dependent enzymes, would reduce the amount of PALP when fed to rats living on B_6-adequate and B_6-deficient diets. Lyon *et al.*[3] found the PALP content of the brains of mice fed a B_6-deficient diet decreased.

METHOD

Thirty-two male Holtzman rats, 39 days of age, with a mean weight of 124 g, were divided into two groups of equal weight. The food for both groups contained 2 mg/pyridoxine-hydrochloride (PIN-HCl) per kg; 1 g INH/kg was added to the diet fed to one of the groups. All rats were fed 12 g of food per day. After 3 weeks both groups were divided into two sections: one to be continued on a modified diet and one to be sacrificed. The latter were killed at the rate of a pair (one fed INH, one not fed INH) a day. Since the rats were fed in three lots of 8, 12, and 12 at a time, the longest interval any animals were left on the original diets was 3 weeks plus 3 days. The half of each group that was continued on experimental diets for 2 weeks beyond the 3 weeks was fed a diet containing neither PIN-HCl nor INH. They were killed at the rate of a pair a day.

At the time of sacrifice the rats were etherized and the entire brain removed, dissected longitudinally, and frozen immediately. The procedure of Boxer *et al.*[4] was used. The brains were defrosted, homogenized with water, and aliquots of the homogenates were heated in a boiling water bath for 5 min after the addition of sodium hydroxide. HCl was added until the protein precipitated; the precipitate was removed by centrigufation. Aliquots of the supernatant were used for the determination of PALP by the tyrosine decarboxylase method.

RESULTS AND DISCUSSION

The removal of vitamin B_6 from the diets of the rats caused a decrease in the amount of PALP in the brains, whether the previous diet had contained INH or not. After PIN-HCl had been excluded for 2 weeks the mean PALP content of the brains of 8 rats was significantly lower according to group *t* tests than the mean content of PALP in the brains of 8 rats fed a vitamin B_6-adequate diet ($P<0.02$). After both PIN-HCl and INH had been removed for 2 weeks the mean content of PALP was significantly lower than that for rats fed both compounds ($P<0.02$; Table 1).

TABLE 1. THE PYRIDOXAL PHOSPHATE CONTENT OF THE BRAINS OF RATS DEPRIVED OF VITAMIN B_6 AND OF RATS FED ISONIAZID (INH)

No. of rats on each diet	For three weeks	
	PIN-HCl* (μg PALP/g)	PIN-HCl + INH* (μg PALP/g)
8	1·86 ± 0·09†	1·85 ± 0·06
	For 2 more weeks	
	PIN-HCl removed	PIN-HCl + INH removed
8	1·43‡ ± 0·10	1·58§ ± 0·06

* PIN-HCl, pyridoxine-hydrochloride; INH, isoniazid.
† Standard error.
‡ Significantly lower than PIN-HCl by group *t* test ($P< 0.02$).
§ Significantly lower than PIN-HCl + INH by group *t* test ($P< 0.02$); not significantly higher according to a paired *t* test ($P< 0.10$) than rats from which PIN-HCl had been removed.

Isoniazid did not cause a decrease in the PALP content of the brains, whether vitamin B_6 was in the diet or had been removed for 2 weeks. At the end of 3 weeks of treatment with INH, the PALP content of the brains of 8 rats was 1·85 ± 0·06 μg/g, a value almost identical with the value for 8 rats not given INH. The mean value for 8 rats given INH for 3 weeks then given neither INH or PIN-HCl for 2 weeks was 1·58 ± 0·06 μg/g, a value not significantly different according to paired

t tests from 1·43 ± 0·10 ($P < 0·10$), the value for rats which never had INH in their diets but had had PIN-HCl removed for 2 weeks (Table 1).

The object of the present study was to find whether long-continued oral therapy with INH would lower the PALP content of the brain. Bain and Williams,[5] who have also determined the PALP content of brain after administration of INH, had a different object, which was to find whether the brains of mice in convulsion caused by the parenteral injection of a single large dose in INH contained less PALP than those of control animals. Under these conditions they found a decrease in PALP content. Uchida and O'Brien,[6] who used hydrazines rather than INH and injected just less than a convulsive dose, found no increase in the PALP content of the brains of rats.

Tests were made in our laboratory to find whether the method used in the present study determined the nicotinyl hydrazone of PALP as well as the free PALP. The process of heating PALP-nicotinyl hydrazone, synthesized by the method of Gonnard,[7] with sodium hydroxide caused a liberation of PALP so that three fifths of it was determined by the tyrosine decarboxylase method. However, whether a hydrazone was formed or not may be of little importance, because several groups of workers[8–11] have demonstrated that the hydrazone of INH does not prevent the activation of PALP-dependent apoenzymes. They synthesized the hydrazone and compared its activity with that of free PALP for several PALP-dependent apoenzymes and found that the hydrazone gave the same amount of activity as PALP.

In short, when 1 g INH/kg was added to a diet containing 2 mg PIN-HCl/kg and fed to rats for 3 weeks, the brain content of free PALP plus the PALP in such PALP-hydrazone as was present and measurable by the tyrosine decarboxylase method, did not decrease. The failure of INH to produce a decrease under these conditions may have been caused by the development of a tolerance to INH. Also, the amount of PIN-HCl in the diet possibly was sufficient to protect against the effect of INH in the brain, although the amount of PIN-HCl was insufficient to prevent a decrease in PALP of plasma and muscle.[12] Two weeks after the removal of B_6 from the diet, the PALP content of the brains had decreased.

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