

COMPARISON OF THE EFFECTS OF ISONIAZID DERIVATIVES ON THE DEVELOPMENT OF YOUNG ANIMALS ON A DIET DEFICIENT IN VITAMIN B₆

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Despite the large volume of work done on the synthesis of various derivatives of isonicotinic acid hydrazide (isoniazid), the comparative study of the effects of these compounds on various aspects of metabolism has so far received insufficient attention. We therefore considered that it would be useful to investigate the effect of isoniazid and several of its derivatives (phthivazid, saluzid soluble and metazid*) on vitamin B₆ metabolism. The metabolism of this vitamin was selected as an index of the comparative effect of these preparations on the body because, from reports in the literature, isoniazid has a well-marked effect on vitamin B₆ metabolism.

In about 40% of patients isoniazid causes side effects which are due, in the opinion of several authors, to interference with vitamin B₆ metabolism.

In animal experiments vitamin B₆ prevented the development of polyneuritis and other phenomena which are characteristic of the toxic action of isoniazid [8-11].

In addition to the compounds listed above (phthivazid, saluzid soluble and metazid) we studied acetylated isoniazid and glucuronyl phthivazid, isolated from the urine of rats receiving phthivazid. Both these compounds are of great interest, since they are the detoxication products of phthivazid and isoniazid in the body. For this reason it was interesting to discover how these preparations would act on readministration to the animal.

EXPERIMENTAL METHOD

Acetylated isoniazid is the main conversion product of isoniazid in the body and is far less toxic and 500 times less active than isoniazid [3, 5]. Glucuronyl phthivazid is also the main conversion product of phthivazid in the body, but it retains almost completely the antituberculous activity of phthivazid [1].

Experiments were performed on 76 young male white rats weighing 135-140 g. The rats were kept on a basic diet [2] containing 20% of casein, carbohydrates, fats, mineral substances and vitamins (with the exception of vitamin B₆). To this basic diet were added the following components for the groups of animals (which were kept in cages of six – see scheme).

Rats in all groups not receiving vitamin B₆ received throughout the experiment 10 g of basic diet, and animals receiving vitamin B₆ were given 12 g of food daily, starting on the tenth day of the experiment. The preparations for testing were added to the food in amounts equivalent to 50 mg/kg of isoniazid. The animals were weighed every three days. From 20 to 25 days after the beginning of the experiment the animals were examined

*Isonicotinoyl-(3-methoxy-4-oxybenzal) hydrazone, the diethylammonium salt of isonicotinoyl-(2-carboxy-3,4-dimethoxybenzal) hydrazone and 1-1'-methylene-bis-isonicotinoyl hydrazone respectively – Translator's note.

post-mortem; the blood sugar and hemoglobin were estimated.

EXPERIMENTAL RESULTS

The results of the weight changes of the rats are shown in Table 1 and in Figures 1 and 2; the morphological and biochemical findings in Table 2.

The data in Table 1 suggest that phthivazid and isoniazid inhibit growth of the animals to roughly the same extent. When vitamin B₆ is added to the diet, these preparations have no apparent action (i.e., animals receiving and those not receiving phthivazid and isoniazid developed equally).

Scheme of the Experiments and Composition of the Diets

Group	Number of rats	Diet	
		basic	additions to the basic diet
I	12	basic diet	Without additions - control
II	6	"	+ 50γ vitamin B ₆
III	12	"	+ phthivazid
IV	6	"	Phthivazid + 50γ vitamin B ₆
V	12	"	+ isoniazid
VI	6	"	+ isoniazid + 50γ vitamin B ₆
VII	6	"	+ glycuronyl phthivazid
VIII	4	"	+ acetylated isoniazid
IX	6	"	+ saluzid soluble
X	6	"	+ metazid

All the remaining preparations for testing also caused retardation of growth of the animals. The only noteworthy feature was the more pronounced action of acetylated isoniazid.

TABLE 1

The Effect of Phthivazid and Other Derivatives of Isoniazid on the Growth of Young Rats on a Diet Deficient in Vitamin B₆ (weight of the animals in percentages of the original)

Experimental group	Additions to the basic diet	No. of rats	Day of experiment									
			0	3rd	6th	9th	12th	15th	18th	21st	24th	
I	Without additions (control)	12	100	104	105	110	114	118	122	122	125	
II	+50γ vitamin B ₆	6	100	105	110	111	116	122	131	139	143	
III	+ phthivazid	12	100	103	106	108	110	112	115	114	114	
IV	phthivazid + 50γ vitamin B ₆	6	100	106	109	119	123	128	135	141	145	
V	+ isoniazid	12	100	104	107	111	112	113	113	112	113	
VI	" + 50γ vitamin B ₆	6	100	105	108	112	115	119	127	134	139	
VII	+ glycuronyl phthivazid	6	100	103	107	109	112	114	116	117	—	
VIII	+ acetylated isoniazid	4	100	103	105	107	109	109	109	110	—	
IX	+ saluzid soluble	6	100	103	105	107	109	111	113	111	114	
X	+ metazid	6	100	104	107	111	116	117	121	120	119	

In their outward appearance the animals of the various groups differed slightly from each other. Rats receiving phthivazid and isoniazid, from the sixth to ninth day became unwilling to eat their food and lethargic, and their fur became dishevelled. In rats receiving saluzid soluble and glycuronyl phthivazid poor appetite was observed after the twelfth to fifteenth day. Rats receiving metazid ate all their food until the eighteenth day of

TABLE 2

Biochemical and Morphological Changes Resulting from the Action of Phthivazid and Other Derivatives of Isoniazid on Rats on a Diet Deficient in Vitamin B₆

Experi- mental group	Additions to the basic diet	No. of rats	Wt. of rats be- fore kill- ing	Blood hemo- globin (% of normal)	Blood sugar in mg %	Weight					
						of thymus gland		of testicles		of testicular appendages	
						in mg	in % body wt. of animal	in g	in % body wt. of animal	in g	in % body wt. of animal
I	Without addition (control)	10	173	102	115	272	0.16	2.32	1.34	0.64	0.37
II	+ 50 γ vitamin B ₆	6	207	100	125	357	0.17	2.40	1.16	0.92	0.44
III	+ phthivazid	10	151	93	116	164	0.11	2.02	1.34	0.49	0.32
IV	phthivazid + 50 γ vitamin B ₆	6	212	101	113	303	0.14	1.99	0.94	1.12	0.53
V	+ Isoniazid	10	151	91	110	162	0.11	2.15	1.42	0.47	0.31
VI	" + 50 γ vitamin B ₆	6	214	101	119	387	0.18	2.46	1.15	1.02	0.48
VII	+ glycuronyl phthivazid	6	160	93	105	230	0.14	2.08	1.30	0.62	0.39
VIII	+ acetylated Isoniazid	4	145	101	92	242	0.17	2.22	1.53	0.47	0.32
IX	+ saluzid soluble	4	149	98	105	155	0.10	2.01	1.35	0.50	0.34
X	+ metazid	4	160	97	104	134	0.08	2.35	1.47	0.63	0.39

the experiment and looked better than the animals of the other groups. Rats of the control group ate all their food throughout the experiment: outwardly they appeared normal, but they differed from the animals receiving the vitamin by their slow growth and lack of movement.

The results shown in Table 2 show that the blood sugar and hemoglobin were only slightly abnormal; only a slight fall in the hemoglobin content of the blood of rats receiving phthivazid, isoniazid (without vitamin) and glycuronyl phthivazid could be observed, and a tendency for the blood sugar to fall in rats after administration of all the test preparations (and without vitamin B₆).

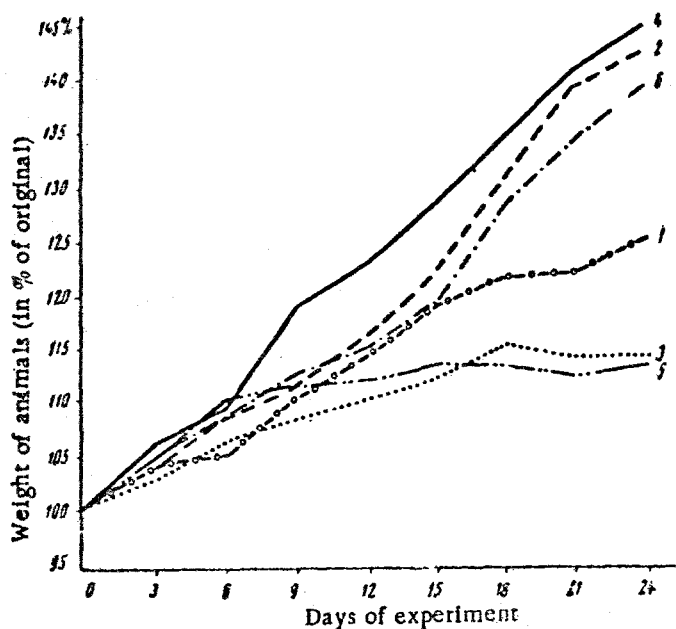


Fig. 1. The effect of phthivazid and isoniazid on the growth of young rats on a diet with and without vitamin B₆: 1) basic diet; 2) basic diet + 50γ vitamin B₆; 3) basic diet + phthivazid; 4) basic diet + phthivazid + 50γ vitamin B₆; 5) basic diet + isoniazid; 6) basic diet + isoniazid + 50γ vitamin B₆.

The characteristic changes found in the organs were diminution of the thymus gland and testicular appendages (in absolute figures and as a percentage of the body weight) and increase in the weight of the adrenals (in percentages of the body weight of the animal). In all the killed animals the thymus gland was prepared, weighed and examined histologically.

Animals of the control group and also of groups IV and VI, which were on a balanced diet containing vitamin B₆ and at the same time received phthivazid or isoniazid did not differ in their outward appearance. All these rats were in a satisfactory nutritional state, the fur was smooth and shining. The average weight of these animals was 210 g. The thymus gland was large in size, milky white in color and of a firm consistency. On microscopic examination no structural changes could be found in the gland in either the control or experimental animals. The lobules of the gland were large and the cortical and medullary layers well defined, with an abundance of cells. The interlobular connective tissue septa were moderately well developed.

Rats kept on a diet lacking in vitamin B₆ and receiving phthivazid, isoniazid, metazid and saluzid were very different in their outward appearance from the rats of groups II, IV and VI. The nutritional state of the animals was depressed; the fur dull and dishevelled. The average weight of these animals was 151.6 g. The thymus gland was greatly reduced in size — less than half the weight of the gland in the control rats. Histological examination showed reduction in the size of the lobules, and narrowing of the cortical zone on account of diminution of the number of lymphoid cells. In the cells of the cortical zone degenerative changes were observed in the form of karyorrhexis or pyknosis. Marked proliferation of the interlobular connective tissue and fatty tissues were observed.

Animals receiving glycuronyl phthivazid and acetylated isoniazid and a diet lacking in vitamin B₆ were identical in weight with the animals of groups III, V, IX and X. The thymus gland of these rats was almost the same weight as that of the control animals on a diet lacking in vitamin B₆ (group I). Microscopic examination of the thymus gland showed reduction in the size of the lobules, narrowing of the cortical zone on account of a fall in the number of cells, and absence of degenerative changes in the cells of the cortical layer.

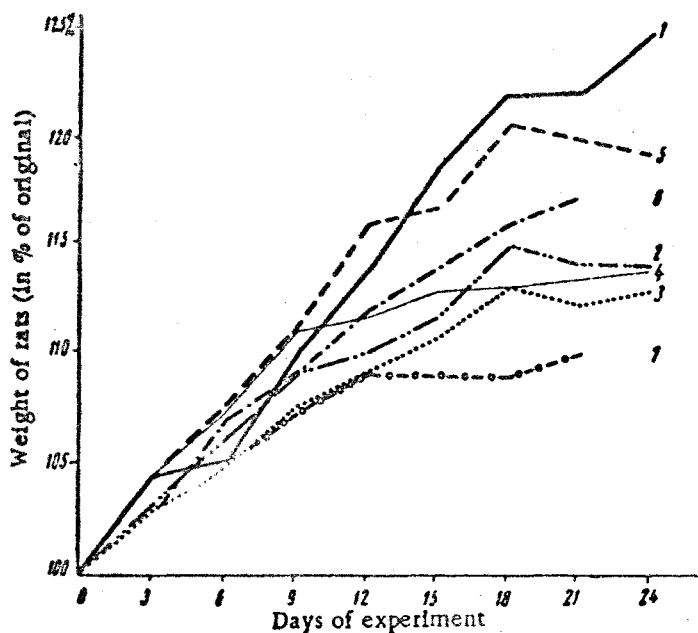


Fig. 2. The effect of derivatives of isoniazid on the growth of young rats: 1) basic diet; 2) basic diet + phthivazid; 3) basic diet + isoniazid; 4) basic diet + saluzid soluble; 5) basic diet + metazid; 6) basic diet + glycuronyl phthivazid; 7) basic diet + acetylated isoniazid.

On the basis of our work it may thus be considered that the effect of all the antituberculosis preparations examined on the young rat is similar in principle and is shown by inhibition of growth and by morphological changes in the organs of the endocrine system — reduction in the weight of the thymus gland and the testicular appendages and increase in the weight of the adrenals. The most pronounced effect on the growth of the animals was shown by isoniazid.

The addition of vitamin B₆ to the diet of the animals removes the inhibiting effect of isoniazid and phthivazid on the growth and the morphological changes in the endocrine system of the experimental animals.

SUMMARY

The effect of the following compounds was studied with regard to their effect on the growth of young rats and on the morphological changes in their organs when kept on a B₆-deficient diet: hydrazide of isonicotinic acid, phthivazid, saluzid soluble, metazid, acetylated hydrazide of isonicotinic acid and glucuronide of phthivazid. All these preparations had an enhancing effect on the signs of B₆-avitaminosis in animals kept on vitamin B₆-deficient diet. The most pronounced effect on the retardation of rats' growth was noted under the action of acetylated HINA, while the least marked action was due to the influence of phthivazid and metazid.

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