Effect of Calcium Pantothenate on Isoniazid Toxicity in the Guinea Pig.*† (23235)

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The treatment of tuberculosis with high doses of isoniazid (isonicotinic acid hydrazide) is frequently limited by development of peripheral neuropathy in the patient(1). Central stimulation with or without convulsions results from acute toxic doses of isoniazid in man as well as in animals but chronic administration of low doses does not produce significant toxic side reactions(2). Peripheral neuropathy characterized by muscle spasticity and abnormal gait has been produced in rats maintained for several months on a diet containing 0.25% isoniazid(3). In the present study repeated administration of subconvulsive doses of isoniazid produced in the guinea pig a toxic reaction which in certain aspects resembles the peripheral neuropathy reported in man. Daily administration of calcium pantothenate to these animals prevented development of this reaction.

Materials and methods. Guinea pigs of either sex and of mixed breed weighing 100-200 g were housed in individual cages and maintained on Purina rabbit chow checkers plus cabbage. Animals were weighed each morning and isoniazid or a combination of isoniazid and ethanol was administered in 0.9% saline by intraperitoneal injection 3 times a week. Experiments were conducted for 4 to 6 weeks after which the animals were sacrificed and examined for gross pathological changes. Except for local necrosis at the injection site in a few of the animals receiving alcohol, no such changes were observed.

Results. Within one hour after an injection of 50-75 mg/kg of isoniazid the guinea pig becomes restless, easily startled and excitable and after another one or 2 hours behaves normally. However, in about 25% of the animals which have received 3 to 5 injections of isoniazid on alternate days, the period of hyperexcitability is followed by muscle tremors but overt convulsions do not develop. By 2 hours after injection the animal squeals frequently and makes obvious efforts to remain off his feet. Finally, coordinated control of the hind limb musculature is lost and the guinea pig lies on his side. His reflexes are hyperactive and an increased sensitivity to pain in the hind limbs is apparent. Merely touching any hind limb joint during this period causes the animal to withdraw his leg violently. This hyperesthesia persists for an hour or more. Atonia, initially evident only in the hind limbs, may progress forward until a flaccid paralysis of all of the body musculature is present. (This total flaccid paralysis was used as the criterion for the response throughout the remainder of the study.) Respiratory muscles, however, are not affected since respiration continued normally except in a few cases in which fluid accumulated in the respiratory tract after several hours of paralysis. The majority of animals appear normal within 5 hours after injection of the isoniazid. The guinea pigs usually lose weight on the day of injection but regain their weight on the following day and are able to maintain a normal growth curve over the experimental period. The majority of guinea pigs did not react positively to every subsequent injection of isoniazid but to only every third or fourth test with this drug when given on alternate days.

Injection of a 15% solution of ethanol in a dose of 1.2 g/kg together with the isoniazid produced a significant increase ($p(\chi^2) < 0.001$) in incidence of response in the guinea pigs (Table I). This dose of alcohol alone did not produce any observable reaction when injected 3 times a week into control animals. However, when given in combination with

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TABLE I. Toxic Response to IP Injection of Isoniazid and Alcohol in Guinea Pigs.

Drug and dose	No. animals responding/No. animals tested	% animals responding
Isoniazid 50-75 mg/kg	10/39	25.7
Idem + alcohol 1.2 g/kg	22/27	81.5
Alcohol 1.2 g/kg	0/6	0.

isoniazid, not only did alcohol cause more animals to respond but the responses appeared earlier in the sequence of injections and with greater regularity thereafter. In many cases a response to the combination of isoniazid and alcohol was seen on initial injection of these drugs. This never occurred with isoniazid alone. Some animals responded to each injection of isoniazid and alcohol while others did not respond to the first injection given at the beginning of the week but only to the second and third injections.

The symptomatology of the response to the combination of isoniazid and alcohol was in every way similar to that seen after isoniazid alone. An increase in the incidence of response to isoniazid was also obtained in a limited number of animals in which the alcohol was given orally. Other agents given along with isoniazid but which did not significantly increase the incidence of toxic response included sodium salicylate, cortisone and acetaldehyde.

Since the response to the combination of isoniazid and alcohol was more consistent than that to isoniazid alone, these 2 drugs were used to test ability of certain vitamins to protect against the toxic response. These vitamins were injected daily in guinea pigs that had previously given 2 consecutive responses to isoniazid and alcohol. The vitamins were always given some 18 hours prior to the challenging doses of isoniazid and alcohol in order to decrease likelihood of protection being achieved by a direct chemical interaction in the bloodstream.

While control animals showed continuing positive responses (total flaccid paralysis) to test doses of isoniazid and alcohol, animals treated with calcium pantothenate no longer responded (Table II). A single injection of 20 mg/kg of the vitamin blocked the reaction in most cases on the following test day. With 10 mg/kg of calcium pantothenate several daily injections of the vitamin were required to prevent the reaction from appearing in all of the guinea pigs. However, in these animals there was a noticeable diminution of intensity of response even after the first injection of the vitamin. All animals on pantothenate therapy showed the initial stages of excitement after receiving the isoniazid and alcohol, but the spasticity, joint sensitivity and flaccidity seen previously did not appear. The decrease in incidence of total paralysis in pantothenate treated animals as compared to controls is statistically significant ($p(\chi^2) < 0.01$). When the pantothenate therapy was discontinued, responses to the isoniazid and alcohol combination again appeared after 3 to 5 injections. In a separate experiment, 2 guinea pigs giving repeated responses to only isoniazid were similarly protected by pantothenate.

Calcium ion equivalent to that present in the calcium pantothenate was administered as the gluconate to 4 guinea pigs responding to isoniazid and alcohol. These animals continued to respond to succeeding challenges of isoniazid and alcohol until they ultimately died during a period of prolonged paralysis.

Daily administration of 20 mg/kg of pyridoxal HCl in a similar series of experiments was without effect on either incidence or duration of responses to isoniazid plus alcohol. Pyridoxal administered together with or shortly after the isoniazid-alcohol combination, however, noticeably decreased severity of the responses.

Discussion. The initial hyperreflexia, the

TABLE II. Antagonism of Toxic Action of 75 mg/kg Isoniazid plus 1.2 g/kg Ethanol (IP) in Guinea Pig.*

Treatment and mg/kg	Response ratios on alternate days 1	2	3	4	5	6
Control	6/6	4/6	5/6	6/6	5/6	6/6
Ca pantothenate 10	8/9	1/9	2/9	1/9	0/9	0/9
Idem 20	1/6	0/6	0/6	0/6	0/6	0/6
Pyridoxal HCl 20	4/4	4/4	3/4	4/4	4/4	4/4
Ca gluconate 18	4/4	4/4	4/4	3/3	3/3	3/3

* Control and treated animals selected only after they had given 2 consecutive responses to isoniazid-ethanol.

hyperesthesia particularly evident in all the hind limb joints, and to some extent the muscle flaccidity present in these guinea pigs were all indicative of peripheral neuropathy. However, the extensive flaccid paralysis noted as well as the transient nature of the response are in general not characteristic of drug-induced polyneuritis. Hence, the toxic response from isoniazid observed in these guinea pigs differs in these aspects from the peripheral neuropathy produced in patients by isoniazid therapy.

The potentiation of toxic response from isoniazid by alcohol in these studies may have some significance in therapeutics. Oestreicher *et al.*(4) noted that 7 out of the 8 cases of peripheral neuropathy found in a series of patients on isoniazid therapy occurred in chronic alcoholics. Although isoniazid may merely have activated a sub-acute polyneuritis in such patients, it is possible that their alcohol intake may have directly contributed to the increased incidence of toxic response. Since, in the present studies, orally administered alcohol gave essentially the same reaction as injected alcohol, the increase in toxicity noted with alcohol probably did not result from local irritation by the injected alcohol. Negative results with acetaldehyde indicated that alteration in the metabolism of alcohol was also probably not an essential factor in causing the response. Since neither sodium salicylate nor cortisone altered the incidence of response, it is not likely that the response was mediated through adrenal stimulation by isoniazid.

The peripheral neuropathy seen in isoniazid treated patients has been associated with alteration in vit. B_6 activity by the drug(1). Pyridoxine HCl has been shown to relieve isoniazid induced polyneuritis in patients(5). Concomitant administration of pyridoxal HCl and isoniazid in the present studies did noticeably decrease the severity of response in the guinea pigs. However, when the pyridoxal was given some 18 hours prior to the isoniazid, no protection was noted. Thus it is likely that the protection noted in the former experiments was dependent on some effect of the high blood level of pyridoxal present. This suggests that the isoniazid toxicity observed in these studies is not directly a result of vit. B_6 inactivation in the tissues.

A peripheral neuropathy similar to that resulting from isoniazid therapy in patients has been associated with a pantothenate deficiency in man(6). Pantothenate deficiency is known to cause a peripheral neuropathy characterized by abnormal gait in swine(7). However, since a sustained peripheral neuropathy was not seen in the present studies, it is not likely that the response to isoniazid in the guinea pig is dependent on a drug induced tissue deficiency of pantothenic acid. Since a high concentration of pantothenate blocked the transient response to isoniazid in these guinea pigs it is, however, possible that the utilization of pantothenic acid was inhibited by the isoniazid.

Summary. 1. Administering 50-75 mg/kg of isoniazid 3 times a week to the guinea pig produces a transient peripheral neuropathy characterized by initial hyperreflexia and hyperesthesia particularly evident in limb joints. An extensive muscle flaccidity of 3-4 hours duration also occurs during this reaction to isoniazid. 2. Addition of 1.2 g/kg of ethanol to the isoniazid injection significantly increases incidence of the toxic response to isoniazid. 3. The response is prevented by daily administration of 20 mg/kg of calcium pantothenate but is not prevented by daily administration of 20 mg/kg of pyridoxal HCl.

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