sesame oil suspension. The doses used are described in the table. Control animals received appropriate volumes of vehicle. Blood samples were obtained from the marginal ear vein with a heparinized syringe in rabbits. In the other 4 species, blood samples were obtained from the posterior vena cava with a heparinized syringe while the animals were under ether anesthesia. In all 5 species, blood samples were collected at 3 days after administration. Plasma glucose was determined by the glucose oxidase method[4] with kits supplied by Wako Pure Chemical (Osaka, Japan). Plasma triglyceride was determined by the enzyme method of Spayd et al.[5], using a kit (Wako). Pancreatic tissue for light microscopic examination was fixed in buffered, pH 7.4, 10% formalin. Tissue was embedded in paraffin and sections were stained with either hematoxylin and eosin or Gomori's aldehyde fuchsin. The statistical significance of the differences between sample means was assessed by Student's t-test.

Results and discussion. Our findings are given in the table. TPTOH produced marked hyperglycemia and hypertriglyceridemia in rabbits and hamsters after a single oral administration of the compound. In contrast, the administration of TPTOH to mice, rats and guinea-pigs showed no evidence of diabetes although the depression in weight gain indicated that the compound had an effect in these species. Although the TPTOH-treated hamsters showed marked hyperglycemia and hypertriglyceridemia (table), morphological examinations showed no abnormality in the islet tissue of the animals (fig.). These results are the same as those in triphenyltin fluoride-treated rabbits[3]. No hypertriglyceridemia was seen in species in which no elevation of blood glucose occured after TPTOH administration. These data suggest that TPTOH-induced hypertriglyceridemia is due to insulin deficiency as suggested previously[3].

The reasons for the difference between species are unclear. The species specificity could reflect a difference in the disposition of TPTOH or a difference in the fundamental mechanisms of insulin secretion. Another possible explanation would be differences in absorption or metabolism of the compound in the gastrointestinal tract. Furthermore, it may be necessary to consider the influence of the stage of animal development or/and dose of the chemical on the different sensitivity of the various species. Studies designed to elucidate the mechanisms involved in this variability are in progress.

Effect of triphenyltin hydroxide (TPTOH) administration on plasma glucose, triglyceride and body weight in 5 species[a]

Species	TPTOH (mg/kg)	N[b]	Glucose (mg/100 ml)	Triglyceride (mg/100 ml)	Percentage change from initial weight
Rabbit	0	5	104 ± 6	36 ± 3	1.4 ± 3.1
	100	5	337 ± 55*	1538 ± 603*	− 3.3 ± 1.0
Hamster	0	5	142 ± 13	224 ± 28	0.9 ± 1.1
	100	5	417 ± 75*	671 ± 181*	− 9.5 ± 2.1*
Rat	0	6	151 ± 6	44 ± 6	12.4 ± 6.2
	200	6	147 ± 7	53 ± 7	− 14.4 ± 8.9*
Mouse	0	8	237 ± 8	77 ± 9	1.6 ± 0.4
	100	8	210 ± 11	56 ± 7	− 11.0 ± 1.2*
Guinea-pig	0	5	147 ± 4	32 ± 3	2.6 ± 0.3
	100	5	150 ± 8	35 ± 5	− 4.4 ± 0.8*

[a] Values are the mean ± SE at day 3 after the administration of TPTOH. [b] N is the number of animals studied.
* Indicates a significant difference ($p < 0.05$) from control.

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Isonicotinic acid hydrazide: Early effects on peripheral nerve conduction velocity

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Summary. This report describes the effects of short treatments with isonicotinic acid hydrazide (isoniazid), 300 mg/kg/day, on conduction velocity in the rat tail dorsal nerve trunk. After 6 days of continuous treatment, conduction velocity falls significantly for measurements made at 35 °C. After 10 days it falls significantly at both 25 °C and 35 °C. This appears to be the first electrophysiological corroboration of the early neuropathological changes recently observed in isoniazid treated rats and seems to provide evidence that the temperature at which the experiments are made is important in determining conduction velocity changes.

The neurotoxic effects of isonicotinic acid hydrazide (INH), initially reported in humans by Pegum[2], were further studied experimentally by Zbinden and Studer[3] who found that rats show degeneration of peripheral nerves after 15 days of continuous treatment but functional disturbances do not appear for several months. Subsequently a great number of studies have been published, mainly from the histological and biochemical points of view, and only a few papers deal with the effect of INH on electrophysiological parameters such as conduction velocity[4-6]. In the first of these, only a few animals were tested and the method used did not allow proper temperature control, nerve length mesurement or serial in vivo determinations. In the others, both performed in humans, the first is only a case report and the second presents effects involving long treatments. Since it seems to have been clearly established in recent works[7,8] that short periods of administration are sufficient to produce axonal degeneration, we thought it interesting to

Individual changes in body weight, peripheral nerve conduction velocity (PNCV) and temperature coefficient (Q_{10}) after 6 and 10 days of continuous INH oral administration (300 mg/kg/day)

	Before treatment		After treatment		Individual variation	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
a) 6-day treatment (8 rats)						
Weight (g)	(271, 300)	286.8 ± 12.9	(192, 278)	241.0 ± 31.7	(−79, −26)	−45.9 ± 19.7
PNCV (25 °C) m/sec	(20.0, 26.1)	23.26 ± 2.26	(18.8, 24.0)	22.06 ± 2.47	(−3.2, 0.0)	−1.19 ± 1.26
PNCV (35 °C) m/sec	(31.6, 35.3)	33.76 ± 1.56	(28.6, 35.3)	31.72 ± 2.37	(−4.8, −1.6)	−2.75 ± 1.41
Q_{10} (25–35 °C)	(1.35, 1.57)	1.46 ± 0.09	(1.31, 1.57)	1.44 ± 0.09	(−0.07, −0.02)	−0.03 ± 0.03
b) 10-day treatment (8 rats)						
Weight (g)	(275, 304)	287.9 ± 11.0	(204, 253)	217.1 ± 16.2	(−81, −51)	−72.0 ± 10.1
PNCV (25 °C) m/sec	(20.0, 26.1)	23.27 ± 2.32	(18.2, 24.0)	20.90 ± 2.34	(−3.3, −1.0)	−2.21 ± 0.91
PNCV (35 °C) m/sec	(31.6, 37.5)	34.23 ± 1.93	(27.3, 33.3)	29.86 ± 2.50	(−6.1, −2.9)	−4.36 ± 1.36
Q_{10} (25–35 °C)	(1.35, 1.57)	1.48 ± 0.08	(1.31, 1.55)	1.46 ± 0.10	(−0.10, −0.05)	−0.07 ± 0.02

carry out in vivo electrophysiological studies in the early stages of INH-induced neuropathy. The aim of this study was to determine whether, by using a precise experimental technique, changes in peripheral nerve conduction velocity (PNCV) could be found in rats subjected to short INH treatments.

Material and methods. Since PNCV is related to chronological age and not to body size[9] the rats used were within a strict range of age (24–26 weeks) and the body weight (270–305 g) was representative of this age. All animals were maintained in uniform conditions of room temperature and light-dark photoperiod and were given a standard diet and water ad libitum. In treated rats, a daily dose of 300 mg/kg b.wt of INH was given orally for a period of 3, 6 or 10 days respectively.

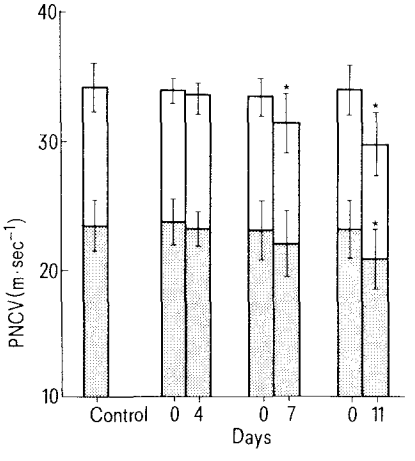
Determinations of PNCV in rat tail dorsal nerve trunk were performed with the electrophysiological technique originally proposed by Hegmann[10] in mice, which permits serial in vivo measurements, and modified by us in order to obtain a strict control of the most important factors of error present in in vivo determinations[11,12]. To minimize the probable circadian modulation of conduction velocity[13] and/or the depth of the barbiturate anesthesia[14] all the measurements were made at the same time (14.00–16.00) each day. Prior to the determinations, the tail of the anesthetized (45 mg/kg i.p. sodium pentobarbital) and conveniently restrained rat was placed in a thermostated paraffin bath in order to obtain an effective temperature control. In spite of the results of other authors[12,15] showing that s.c. temperature becomes stabilized after 5 min of inmersion in the bath, we found a period of 15 min necessary for the stabilization of conduction velocity when the rat tail was changed from room temperature (20 ± 2 °C) to the temperatures used in this study. Measurements were taken at 25 °C and 35 °C in order to study the temperature coefficient Q_{10}(25–35 °C).

Values of PNCV were obtained using a standard technique. Supramaximal stimuli of 0.1 msec duration were delivered by bipolar needle electrodes inserted in the base of the tail and compound action potentials picked up by 2 pairs of distal recording electrodes were displayed simultaneously on a double-beam oscilloscope screen and the velocity, calculated by the indirect method (latency differences), was determined in m/sec. Two types of controls were used: a general control with statistical comparison (Student's t-test) of the mean values between controls and treated rats and individual controls evaluating each treated rat before and after the treatment. This has the advantage that individual variations may be followed. Values are expressed as mean ± SD.

Results and discussion. 1. Body weight decrease. One of the earliest changes shown in treated rats is a body weight decrease (table) which could be interpreted as the result of nutritional deficiency, since it has been noted that INH given p.o. can inhibit the amino acid uptake by the gut wall[16]. It is not clear whether the weight decrease is related to the observed decrease in PNCV. In s.c. INH-treated rats, the peripheral nerves degenerate without significant body weight decrease[17]. Nevertheless, in order to obviate any nutritional/digestive effect of INH, alternative ways of administration are being studied.

2. Conduction velocity changes. PNCV was determined in a control group of 35 normal rats and used for statistical comparison with the mean values obtained for each group of treated rats (fig.). The control values obtained were 23.55 ± 1.95 m/sec at 25 °C and 34.47 ± 1.96 m/sec at 35 °C, with a Q_{10}(25–35 °C) of 1.46 ± 10.

In treated rats, the effect of INH on PNCV was also investigated at the 2 temperatures. As can be seen in the figure, after 6 days of continuous treatment conduction velocity falls significantly at 35 °C but not at 25 °C, and after 10 days a decrease also occurs at 25 °C but less than at 35 °C. No significant changes in Q_{10} were obtained at either time. Thus it appears that small initial damage can be better visualized at higher temperatures. Similar effects have been described for experimental neuritis[18] and multiple sclerosis[19]. These are demyelinating neuropathies and the explanation given so far has been a loss of myelin resistance. However, INH produces a non-demyelinating neuropathy and therefore this explanation cannot be given here. Our results, showing early disfunction in conduction



Peripheral nerve conduction velocity (PNCV) in 35 control rats and in 21 INH-treated rats measured at 25 °C (shaded) and 35 °C (unshaded) the day before the beginning (day 0) and after the end (days 4, 7 and 11) of the treatment. (*) Bars labeled with this symbol are significantly different ($p < 0.01$) from the controls.

phenomena, could point to a more direct effect of INH on the axon. The time-course of onset of PNCV changes obtained in this study is consistent with the described Wallerian type of axonal degeneration[17,20]. The demyelinating neuropathies do not give such an early and small (less than 20% of control values) decrease in PNCV. Moreover, recent work[7,8] clearly shows that a large dose of this compound, administered either in one single dose or in several smaller doses (quantitatively similar to those employed by us), produces a multifocal localized action on the axon itself indicating a special susceptibility of the axon to this drug.

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Lipid peroxidation in rabbit reticulocytes[1]

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Summary. Lipid peroxides in rabbit erythrocytes and plasma were determined while anemia was induced by daily bleeding. They increased as reticulocytes increased and returned to normal with the morphological transformation to mature cells.

Peroxidation of polyunsaturated fatty acids of the cell membrane is considered to be mediated by active oxygen species, and the rate of malondialdehyde formation has been used as an index of this reaction[2]. In the course of maturation, young erythrocytes undergo a number of metabolic changes including loss of membrane components, such as total lipid, cholesterol and phospholipids[3,4], a gradual decrease in a number of enzymes[3,5-8], and a decline in mitochondrial activity[9]. Reticulocytes are also known to consume much more oxygen than mature cells[10], with a resultant production, presumably, of larger amounts of oxygen metabolites. It is postulated that a peroxidation reaction may be working during the decomposition of the membrane structures, and that elevated malondialdehyde levels may be associated with reticulocytes. The purpose of this article is to demonstrate such changes in reticulocytes and in plasma during the course of anemia due to blood loss in rabbits.

Materials and methods. The method of induction of reticulocytosis in rabbits and the preparation of reticulocyte specimens have been described elsewhere[11]. To determine lipid peroxide, erythrocyte suspensions in saline were adjusted to contain 10 g of hemoglobin/100 ml and treated in ice by sonication with a Heat Systems Cell Disruptor Model W-225R (Ultrasonics, Inc., Plainview, NY) for 60 sec at 60 W. $^1/_{10}$ ml of the hemolysate was used without further centrifugation in a final volume of 0.5 ml of 17 mM N-hydroxyethyl-piperazine-N'-ethanesulfonic acid, pH 7.4 (HEPES) buffer. Plasma and erythrocyte lipid peroxides were measured by the fluorometric method of Yagi[12], using thiobarbituric acid, and expressed in terms of malondialdehyde (nmol/ml plasma or g hemoglobin) using tetraethoxypropane as a standard. As the average percent difference in erythrocyte counts in relation to hemoglobin was small, i.e., 470 ± 21 (mean $\pm$ 1 SD) $\times 10^4$ RBC/mm^3 throughout the experiment, the lipid peroxide value was expressed only as nmol MDA/g hemoglobin. The procedure of sonication itself did not affect the MDA value which was the same before and after this treatment. Every specimen was assayed in triplicate.

Results. The general trend of changes in reticulocyte count during the course of daily bleeding was as follows: the count, which usually remained less than 2% for the first 4-5 days, rose rather abruptly on days 6-8 to 30-35%, then fell to below the original level during the next 3-4 days.

Figure 1 illustrates the changes of lipid peroxide levels in erythrocytes and plasma during the course of daily bleeding for 7 days and for the next 8-10 days. As is clear from figure 1, changes of the lipid peroxide values in both erythrocytes and plasma paralleled those of the reticulocyte count, showing peak values on days 7 und 8 and subsequent return to their initial levels after daily bleedings were discontinued. The peak values were 1.34 and 1.52 times the initial value for erythrocytes and plasma, respectively. The lipid peroxide values on days 7 and 8 were the only ones significantly different from the original values. Although