Spectrophotometric Determination of Nicotinaldehyde Thiosemicarbazone in Blood. (19091)

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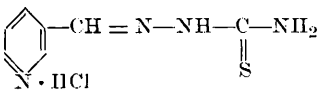
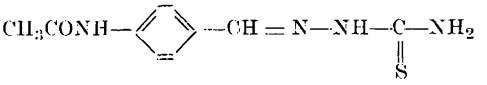
The effectiveness of nicotinaldehyde thiosemicarbazone in the treatment of hematogenous murine tuberculosis was recently reported by Levaditi *et al.*(1). Independently, this compound was synthesized by Gardner *et al.*(2) and studied by Grunberg and Leiwant(3) in the Roche Research Laboratories. The latter workers, in addition to noting the high degree of effectiveness of nicotinaldehyde thiosemicarbazone in overcoming tuberculosis of mice when infected by the intravenous route, also observed a high degree of activity of this compound in intranasally infected animals. This is of considerable importance because the latter technic of infection leads to the generally more resistant bronchogenic form of the disease.

In contrast to the insoluble *p*-acetylaminobenzaldehyde thiosemicarbazone (Tibione), the nicotinaldehyde molecule is basic in reaction and forms a hydrochloride which is readily soluble. Because of this characteristic, it was considered desirable to determine its degree of absorption in comparison with Tibione. Whereas Tibione may be measured in biological fluids by the Bratton-Marshall technic(4), nicotinaldehyde thiosemicarbazone cannot be coupled with diazonium salts. Therefore a spectrophotometric method, using hexanol for extraction, suggested by Burke and Titus(5) as an alternative technic for measuring Tibione appeared to offer the desired specificity, speed and precision. The method is based on the original work by Spinks(6), who used chloroform. The chemical formulae of these compounds are as follows:



Nicotinaldehyde thiosemicarbazone hydrochloride



p-acetylaminobenzaldehyde thiosemicarbazone (Tibione)

The determination is based on the measurement of ultraviolet absorption maxima of the bands; for nicotinaldehyde thiosemicarbazone at 321 mμ, $E^{1\%}_{1\,cm} = 1570$ and for Tibione at 331 mμ, $E^{1\%}_{1\,cm} = 1810$. The ratio E(320 mμ)/E(342 mμ) = 1.05 for Tibione.

Procedure. Two ml of heparinized whole blood were added to 2 ml of distilled water in a glass stoppered centrifuge tube. To this was added approximately 350 mg of powdered K_2HPO_4 and 5 ml of normal hexanol. The mixture was mechanically shaken for 10 minutes and then centrifuged at 2,000 rpm for 5 minutes. The supernatant layer of hexanol was then withdrawn and the ultraviolet absorption was measured in a Beckman quartz spectrophotometer directly or after suitable dilution. In all but a few isolated runs, the measured extinction was between 0.2 and 0.8. Blanks were prepared in an

TABLE I. Percent Recovery of Thiosemicarbazones in Hexanol Extraction of Blood.

Blood conc. (γ/ml)	1	3	5	6	8	10	15	25	250
% recovery									
Nicotinaldehyde thiosemicarbazone	93	75	71	73	64	60	72	64	67
Tibione	137	92	88	89	93	92	98	88	—

1. Levaditi, C., Girard, A., Vaisman, A., and Ray, A., *Compt. rend.*, 1950, v231, 1174.

2. Gardner, T. S., Smith, F. A., Wenis, E., and Lee, J., *J. Org. Chem.*, 1951, v16, 1121.

3. Grunberg, E. and Leiwant, B., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 47.

4. Bratton, A. C. and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, v128, 537.

5. Burke, J. C., and Titus, E., Minutes 9th Vet. Admin. Streptomycin Conf., St. Louis, Mo., 1950.

6. Spinks, A., *Brit. J. Pharmacol.*, 1949, v4, 254.

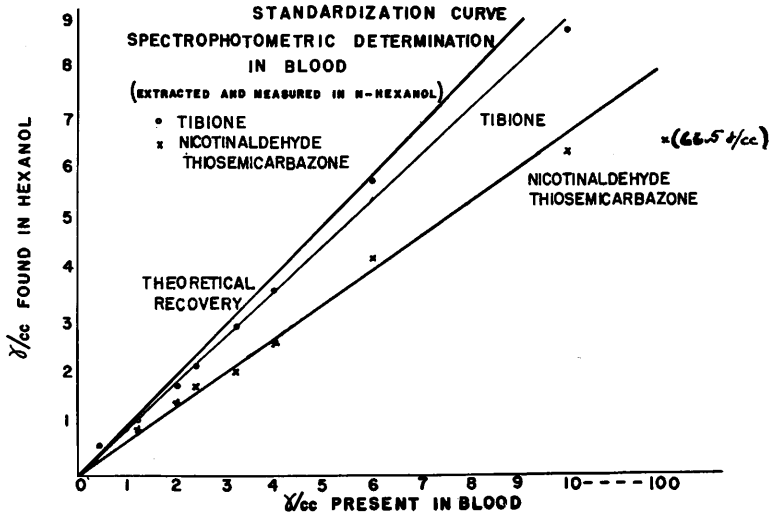


FIG. 1.

identical manner from blood free of the drugs. These were subtracted from the test readings.

Standardization. For the purpose of standardization, 2 ml of normal blood were added to 2 ml of aqueous solutions of the thiosemicarbazones ranging in concentration from 0.1 to 2.5 mg per 100 ml. Nicotinaldehyde thiosemicarbazone was also run at 25 mg/100 ml. These mixtures were then treated as described above. The results are summarized in Fig. 1 and the recoveries in Table I. All solutions were measured against blanks. For dog blood the blanks were found to be: at 321/2 mμ (4 solutions) *avg .067* ± .009 (2 S.D. = *.017*); at 331 mμ (4 solutions) *avg .054* ± .009 (2 S.D. = *.017*). For rat blood the blank was very low (.005). The best range for the analysis (largest slope of per cent absorption vs. concentration) was found to be:

Sample	Hexanol, γ per ml	Corresponding conc (γ per ml) in blood
Nicotinaldehyde thiosemicarbazone	2 -7.5	5-20
Tibione	1.2-5	3-12

The average recovery factors are as follows:

Nicotinaldehyde thiosemicarbazone .68 ± .036 (Stand. error P=.05)
Tibione .91 ± .026 (Stand. error P=.05)

These factors were calculated leaving out the value found in the first experiment, where the concentration of the drugs in blood was below the sensitive range.

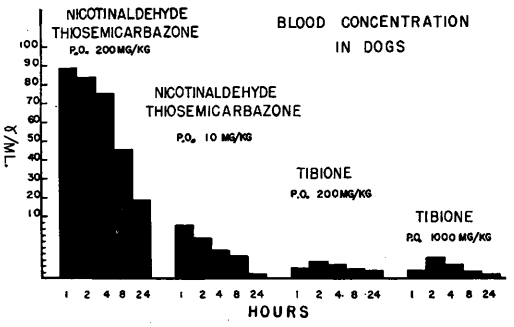


FIG. 2.

The precision of this measurement is estimated to be about 5%, including the uncertainty of the blank. The error is considerably larger outside the best range indicated. This can be avoided easily for higher concentrations by using a suitable dilution but not in the case of too low concentrations without a substantial change in the analytical technic. Beer's law is followed in the range investigated.

Blood concentration in dogs. Nicotinaldehyde thiosemicarbazone and Tibione were administered in single oral doses to dogs. At varying time intervals following dosage, blood was obtained by jugular venipuncture. The results of the spectrophotometric determinations on these specimens are presented in Fig. 2. The highest concentration of Tibione is still below the limit of sensitive determina-

TABLE II. Concentration in Hexanol Extracts of Blood of Rats on Feeding Experiment.

	Concentration in diet:							
	Nicotinaldehyde thiosemicarbazone				Tibione			
	.005%		.05%		.05%		.5%	
	No.	γ/ml found	No.	γ/ml found	No.	γ/ml found	No.	γ/ml found
	1	.19	11	3.40	21	.079	32	.13
	3	.12	12	4	22	.48	33	.84
	4	.076	14	1.55	23	.22	34	1.58
	5	.24	15	3.30	24	.31	35	2.23
	6	.18	16	2.60	25	.44	36	2.48
Avg in hexanol		.16		3		.31		1.45
blood		.4		7.5		.77		3.6
2 stand. error		± .14 (35%)		± 1.4 (19%)		± .037 (48%)		± 2.2 (60%)

tion; consequently the limits of error of these measurements are relatively wide, *i. e.*, of the order of 30%. Since the difference between the blood levels achieved is so great, the large error in the determination of Tibione does not influence the comparison.

With nicotinaldehyde thiosemicarbazone (200 mg/kg P.O., Fig. 2) only the last point at 24 hours carries a large error; the 8-hour point has an error of about 10%. In the dog receiving 10 mg/kg the 8 and 24-hour points have larger errors, particularly the latter, so that the 24-hour values in Dogs 2, 3 and 4 are not significantly different. The apparent lack of proportionality between the blood levels in the dogs receiving 200 and 10 mg/kg may be significant, but this conclusion can be only tentative since the differences between the ratios in the two series are at present too close to the estimated limits of error. On the other hand, as judged from the series of standard, the reproducibility of the technic appears to be good, providing measurements are made in the sensitive range.

Blood concentration in rats. Nicotinaldehyde thiosemicarbazone and Tibione, incorporated in ground diet, were administered to weanling, male Sprague-Dawley rats for a period of 13 weeks. At the end of this time, the animals were sacrificed by decapitation and blood was drawn from 5 of each group of 10 animals receiving the drug. The dosage levels were 0.005 and 0.05% of nicotinaldehyde thiosemicarbazone and 0.05 and 0.5% of Tibione. The values, presented in Table II, vary widely. This may be due in large part to differences in the feeding habits of the individual animals.

Discussion. Estimations of p-acetylaminobenzaldehyde thiosemicarbazone have been carried out by various workers using different technics. A colorimetric method using Grote's reagent ($Na_2[Fe(CN)_5H_2O]$) has been described by Mingoja and Moscovici(7). The same authors described an iodometric titration method. Haugas and Mitchell(8) reported a gravimetric procedure based upon precipitation of the thiosemicarbazone by silver nitrate. Colorimetric procedures based on the diazotization and coupling technic of Bratton and Marshall(4) were described by Moss(9) and by Heilmeyer and Heilmeyer(10).

As can be expected from their structures, the recovery of nicotinaldehyde thiosemicarbazone was less favorable than that of Tibione. The recoveries were 68% and 91% respectively.

The present data indicate that nicotinaldehyde thiosemicarbazone hydrochloride, was absorbed to a much greater extent by the animal body than Tibione. The low solubility in body fluids of the latter compound had also been demonstrated by Francis, Spinks and Stewart(11). These workers noted a maximum average blood concentration of 0.37 mg per 100 ml (3.7 γ/ml) of blood with single oral doses of 500 mg/kg to dogs. In

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8. Haugas, E. A., and Mitchell, B. W., *J. Pharmacy and Pharmacol.*, 1950, v11, 759.

9. Moss, D. G., *Lancet*, 1950, v2, 570.

10. Heilmeyer, I., and Heilmeyer, L., *Arch. f. exp. Path. u. Pharmak.*, 1950, v210, 424.

11. Francis, J., Spinks, A. and Stewart, G. T., *Brit. J. Pharmacol.*, 1950, v5, 549.

one of our animals, Dog 3, which received 1,000 mg/kg of Tibione, a maximum blood concentration of 0.33 mg per 100 ml (3.3 γ/ml) was found. This level was even less than that achieved by 1/100 the dose of nicotinaldehyde thiosemicarbazone, *i.e.*, 10 mg/kg. At the end of eight hours after the latter dosage, the level still remained above the maximum level obtained with Tibione. (Fig. 2).

Our results are substantially in agreement with those of a recent work by Spinks(12), who found blood levels of Tibione of the order of 3 γ/ml in the first one to two hours in therapeutic applications. His value (.4 γ/ml) in dogs using a dose of 5 mg/kg is the same as our value for nicotinaldehyde thiosemicarbazone suggesting that at this dosage level the low solubility of Tibione does not yet limit absorption.

The photostability of the compounds in n-hexanol in diffuse daylight can be expected from Spinks' data and appears confirmed by our results. No erratic readings were observed with our standards and all spectrophotometric readings, which were routinely checked back after the absorption curves were recorded, were stable within .005 of the density scale of the Beckmann spectrophotometer. Our recovery factor for Tibione using n-hexanol is .91 as compared to about .80 using chloroform reported by Spinks.

In rats having received comparable amounts of nicotinaldehyde thiosemicarbazone hydrochloride and Tibione, *i.e.*, 0.05% in their diets for 13 weeks, the concentration in the blood of the former agent (7.5 γ/ml) was roughly 10 ten times that of the latter agent (0.77 γ/ml). It is thus apparent from these studies in rats as well as those in dogs that nicotinaldehyde thiosemicarbazone hydrochloride is absorbed to a very great degree as compared with Tibione.

Summary. (1) A spectrophotometric method has been developed for the determination of nicotinaldehyde thiosemicarbazone in blood. (2) Following single oral doses of 200 mg/kg in dogs, the maximum blood concentration achieved by nicotinaldehyde thiosemicarbazone was ten to forty times greater than that of p-acetylaminobenzaldehyde thiosemicarbazone. (3) Following the chronic daily oral ingestion of 0.05% of these drugs in the diet of rats, the average blood concentration attained by nicotinaldehyde thiosemicarbazone was approximately 10 times that attained by p-acetylaminobenzaldehyde thiosemicarbazone.

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Changes in Plasma and Tissue Water and Electrolytes After Epinephrine Administration in Rats. (19092)

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The great difference in the concentrations of potassium in the extracellular and intracellular fluid compartments has often raised the question of hormonal regulation of the "balance" between these two compartments. The adrenal cortex has been particularly considered as the secretory source of hormonal regulation of potassium balance in the organism because of the changes that occur in adrenocortical deficiency and when desoxycorticosterone is used. However, this view has not been generally acceptable(1). The effect of desoxycorticosterone on the renal excretion of potassium has been shown by Harrison and Darrow(2) to be sufficient to explain the change in potassium balance. It is of interest

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2. Harrison, H. E., and Darrow, D. C., *Am. J. Physiol.*, 1939, v125, 631.