verse axis.

It is proposed to call this transverse axis of heart level the *phlebostatic axis* and any of the horizontal planes passing through this axis used as the reference level the *phlebostatic level* in order to avoid confusion with the many different "heart level" landmarks described previously in the literature.

Summary. It has been found that the reference level or heart level for the measurement of venous pressure is an axis which runs transversely through the thorax at the point of junction of a plane passing cross-sectionally through the fourth intercostal space adjacent to the sternum with a frontal plane passing midway between the posterior surface of the body and the base of the xiphoid process of the sternum. Horizontal planes passing through this axis are the reference levels or heart levels to be used for that particular position of the patient. It is proposed to call the axis the *phlebostatic axis* and the horizontal planes passing through the axis the *phlebostatic level.*

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Biological Activity of N-Methylnicotinamide and Nipecotic Acid.*

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The use of microbiological vitamin assays always involves an uncertainty in that the activity of certain related compounds may be different for the test organism than for the animal. In the case of nicotinic acid, no derivative has been found, to our knowledge, which is active for *L. arabinosus* but inactive when ingested by an animal. In fact, it has been established that a number of precursor compounds readily elicit an active response from the animal, but must first be hyrolyzed to be active for *L. arabinosus*.[1-3] It is now generally accepted that autoclaving samples with 1N alkali gives a degree of potency with *L. arabinosus* which agrees closely with the biological vitamin activity for animals. The results given in two recent reports may raise some question about this assumption.

Von Euler *et al.*[4] have reported that hydrogenated nicotinic acid (nipecotic acid) is active for *Staph. aureus* and *Proteus vulgaris.* This is of interest because nipecotic acid has been found inactive for the dog[5] and dysentery bacilli.[6] Najjar *et al.*[7] have reported that N-methylnicotinamide chloride (nicotinamide methochloride) is active in preventing and curing nicotinic acid deficiency in dogs and produces a growth response with *E. coli.* This compound has previously been found inactive for the dog,[5] and *L. arabinosus*.[3] Furthermore, many investigators have tested trigonelline, which is chemically related to N-methylnicotinamide as nicotinic acid is to nicotinamide, and have found it inactive for the dog, man and all microörganisms tested.[1]

In this paper, we wish to present further studies on the activity of N-methylnicotin-

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7 Najjar, V. A., Hammond, M. M., English, M. A., Worden, M. B., and Deal, C. C., *Bull. Johns Hopkins Hosp.*, 1944, **74**, 406.

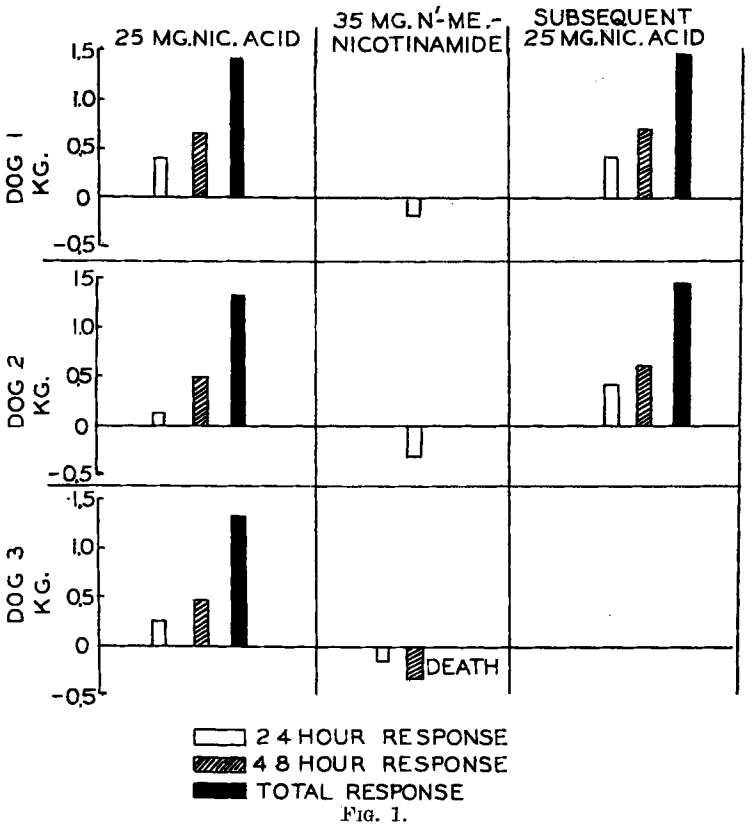


FIG. 1.
Weight response of blacktongue dogs to nicotinic acid and N'-Me-Nicotinamide.

amide and nipecotic acid.

Experimental. Nipecotic acid was tested with *L. arabinosus* and found to be only 1/10,000 as active as nicotinic acid. The procedure used has been described in detail[8] and acid production was measured after 48 hours incubation. The very slight but definite activity was probably due to impurities.

With the improved synthetic ration containing "folic acid,"[9] N-methylnicotinamide chloride was tested for its activity in the dog. It has been the experience of this laboratory that in an individual dog on this diet, nicotinic acid deficiency may be repeatedly produced and cured, with uniformly rapid responses to nicotinic acid administration. Three animals were standardized by producing nicotinic acid deficiency and feeding a sub-optimum dose of 25 mg of nicotinic acid. The dogs were again brought down with a severe nicotinic acid deficiency and nicotinamide methochloride, equivalent to 25 mg nicotinic acid on the molar basis, was administered orally. The dogs continued to lose weight during the next 24 hours. Twenty-five mg nicotinic acid were then fed and the dogs began eating and drinking within 12 hours, and made a weight gain which checked very well with the response in the standardization. This indicates that there was no supplemental action due to N-methylnicotinamide. In the case of the third dog, no further supplement was given for 48 hours after the nicotinamide methochloride administration. At this point the animal was in extremely poor condition and died before a cure could be effected with nicotinic acid. The results are presented graphically in Fig. 1.

Discussion. It should be pointed out that in

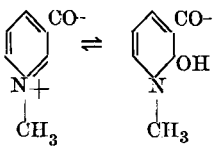
[8] Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 471.

[9] Krehl, W. A., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

Najjar's experiments, high levels of nicotinamide methochloride were employed. In the case of the *E. coli* tests, 20γ-100γ/cc of medium were used. These amounts seem very high in view of the nicotinic acid requirements of other bacteria that have been studied, *e. g.*, 0.1γ of nicotinic acid/cc produces maximum growth with *L. arabinosus*. It has been our experience that almost any preparation of a compound chemically related to nicotinic acid contains enough impurities to show activity if it is added to bacterial media at very high levels.

The amounts of nicotinamide methochloride administered to dogs by Najjar *et al.* were also very high. In their prophylactic experiments, 50 mg and 25 mg were fed per day, which is in great excess of the nicotinic acid requirement. In the curative treatments, 250 mg were given within 12 hours and 50 mg per day thereafter, which also greatly exceeds the amount of nicotinic acid required to cure black-tongue. We have consistently obtained cures with 25-50 mg of nicotinic acid.

We have no proof that N-methylnicotinamide is completely devoid of biological activity, but since we have used levels of this compound which are comparable to amounts of nicotinic acid that have been found effective, we are convinced that if it is biologically active, its potency must be extremely low. Najjar *et al.* suggest that the following structure is essential for biological activity



regardless of the radical that is attached to the carboxy group. If this theory is correct, trigonelline and N-methylnicotinamide should be at least as active, if not more active, than nicotinic acid. N-methylnicotinamide has already been discussed. In the case of trigonelline, there is overwhelming evidence that it has no biological activity whatsoever. Finally, von Euler *et al.*[4] have found guvacin (1-2-5-6 tetrahydro nicotinic acid) active for *Staph. aureus* and *Proteus vulgaris*. When this compound was converted to the N-methyl derivative (arecaidine), there was no activity whatsoever.

Najjar is of the opinion that the correlation between N-methylnicotinamide excretion and nicotinic acid deficiency supports his view. It seems more likely, however, that methylation is merely the usual treatment of pyridine-type compounds in the body, since pyridine itself is methylated.[10] It is entirely logical that with low nicotinic acid intake, the excretion of N-methyl derivatives would drop more or less proportionately.

Summary. 1. Nipecotic acid was found to be only 0.01% as active as nicotinic acid for *L. arabinosus*. 2. N-methylnicotinamide chloride was found to be ineffective in curing black-tongue in dogs. 3. The theory of Najjar *et al.* as to the relation of chemical structure to anti-blacktongue activity is discussed.

[10] Harrow, B., *Textbook of Biochemistry*, 3rd Ed., W. B. Saunders Co., 1943, p. 252.