about 0.1 ml of the sample was placed in a small platinum container in a combustion oven. The vapor passed through zinc, heated just below its melting point. The resulting hydrogen and deuterium were accumulated in a sampler and fed through a manifold into the tube of the spectrometer. The resulting data on mass 3/mass 2—that is HD/H_2—were converted into the excess in mole % deuterium. The averages of the results are shown in Table 1. The results indicate

TABLE 1

Sample	Excess (mole % D)	Increment (% D)
M	0.0025	17
M_2	.0025	17
S	.0041	28
D	.0031	21
L	.0044	30
Wax	0.0028	19

that the natural abundance of deuterium in honey, as determined through densimetric measurements by Dole (*6*), seems to be verified by these mass spectrometer data. The error in the measurements with the mass spectrometer is in general below ± 5%.

The differences in the various samples are distinct, and, compared with the average of deuterium in rain and lake water, as given by Bleakney and others (*12, 2*), they are significant.

The values obtained in this table are based upon our standard containing 0.0148 mole% D, and we can assume that buckwheat plants in the Finger Lakes region have taken up water with the same average deuterium content.

The increment of the isotope deuterium in Sample M might indicate that the moisture in the nectar of flowers is coming partially from anabolic degradation of sugars, and is probably enriched through repetitive processes.

The fact that honey contains more levulose than dextrose, and that the levulose has in this sample about two times higher deuterium content, could point to a specific deuterium affinity of the invertase in the stomach of the bees, which could build deuterium atoms into sugar molecules at some point in the hydrolytic process. There are a few indications in the literature that deuterium has an influence on enzymatic systems (*13*): inhibitory action at higher concentrations (*14*), stimulating at low concentrations (*15*), even raising the resistance of certain bacteria toward strong disinfectants (*16*).

It should be mentioned that the dispersion coefficient—namely, $\frac{\text{mass 3/mass 2 in sugar}}{\text{mass 3/mass 2 in water}}$—is quite negligible by such low concentrations as it is present in living tissues, and the dispersion coefficient of fructose, which is about 10% higher than that of glucose (*17*), cannot account for the excess of deuterium as found in our experiments.

These data and the preliminary conclusions drawn need, of course, further work and confirmation. Besides the repetition of above experiments with other honey samples it would be interesting to have the data on the water from the geographical micro-unit where the honey was gathered; it would also be important to feed plants with excess heavy water and analyze the nectar for its deuterium content, or feed the bees with deuterium oxide and determine the deuterium in the sugars of the honey produced by them. Beyond the apicultural significance of this question it is enormously interesting for our general concept of biochemical reactions.

References

1. WASHBURN, E. W., *et al. Science,* **79**, 188 (1934).
2. KIRSHENBAUM, I. *Physical Properties and Analysis of Heavy Water.* New York: McGraw-Hill (1951).
3. STETTEN, D., JR. *J. Biol. Chem.,* **165**, 147 (1946).
4. HEVESY, G. *Naturwissenschaften,* **23**, 775 (1939).
5. STETTEN, D., JR. *J. Biol. Chem.,* **165**, 147 (1946).
6. DOLE, M. *J. Chem. Phys.,* **2**, 337 (1934).
7. BARBOUR, H. G., *et al. J. Biol. Chem.,* **69**, 625 (1926).
8. LINDENSTRØM-LANG, D., *et al. Compt. rend. trav. lab. Carlsberg. Sér. chim.,* **23**, 17 (1938).
9. RICHARDS, T. W., *et al. J. Am. Chem. Soc.,* **34**, 599 (1912).
10. LAMB, A. B., *et al. Ibid.,* 35, 1666 (1913).
11. NIER, A. O. *Phys. Rev.,* **52**, 933 (1937).
12. BLEAKNEY, W., *et al. Ibid.,* **44**, 265 (1933).
13. FARKAS, Z. *Proc. Roy. Soc.* (London), **115**, 373 (1934).
14. BRANDT, G. *Klin. Wochschr.,* **14**, 1597 (1935).
15. CASTELLANI, M. *Boll. sez. ital. soc. intern. microbiol.,* **7**, 396 (1935).
16. LOCKEMAN, G., *et al. Ber. deut. chem. Ges.,* **67**, 1299 (1934).
17. KOIZUMI, M., *et al. Bull. Chem. Soc. Japan,* **13**, 427 (1935).

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Competitive Action of Isonicotinic Acid Hydrazide and Pyridoxal in the Amino Acid Decarboxylation of *Escherichia coli*

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We have previously shown that the inhibitory effect of isonicotinic acid hydrazide (INAH) on the growth of *Escherichia coli* significantly decreases in broth media (more than 10 mg/ml), compared with that of INAH (0.6–1.2 mg/ml), in a synthetic medium (Anderson's *M*-9 medium) (*1, 2*).

Thus it is possible to postulate the existence of certain substances that may be contained in broth and that are able to inhibit the action of INAH on the growth of *E. coli*. In further investigations of these postulated substances, we found that one of them can, to some extent, be replaced by pyridoxine hydrochloride (*1, 3, 4*); hence, it may be suggested that there is an intimate connection between INAH and pyriodoxine and its derivatives.

It has been generally agreed that pyridoxine derivatives play an important part in some enzyme systems of *E. coli*—i.e., decarboxylase (*5, 6*), tryptophanase

[1] We express our thanks to Dr. Egami, of the Department of Biochemistry, Nagoya University, for his kind adyice.

(*7*), and transaminase (*8, 9*)—as their cofactor. Therefore it is not impossible to predict the inhibitory effect of INAH and also the competitive effect of pyridoxine and its derivatives on INAH in these enzyme systems.

While attempting to investigate the action of INAH on the amino acid (arginine, orithine, and glutamic acid, etc.) decarboxylating activity of washed suspensions of *E. coli*, evidence has been obtained that the cell suspension treated with INAH remarkably decreases its decarboxylating activity, more or less in proportion to the amounts of INAH added, and that the activity recovers when pyridoxal is added to the reaction system. Typical results showing the effect of INAH on the arginine decarboxylating activity of *E. coli* communior (stock) are given in Fig. 1 (Expt. 1); the results showing the recovery effect of pyridoxine and pyriodoxal are given in Table 1 (Expt. 2).

In order to obtain the activity of arginine decarboxylation, a culture of *E. coli* communior (stock) was grown at 25° C in 2% glucose broth containing 10 mg/liter of arginine for 16 hr (*10, 11*). At the end of the incubation period the organism was centrifuged out of the growth medium, washed in saline three times, and then made up into suspension in McIlvaine's citric acid–phosphate (pH 4.2) buffer (*12*). The dry weight of organism/ml suspension was determined in each case by a photoelectric nephelometer.

The evolution of CO_2 from arginine under the action of washed organisms in suspension was studied in a Warburg manometer. The following quantities were used: In Expt. 1, in the main compartment of the flasks, 0.5 ml washed suspension, 0.6 ml of 0.1 *M*, 0.3 ml of 0.1 *M*, 0.05 *M*, 0.025 *M*, and 0.0125 *M* INAH, respectively, and in the side bulb, 0.5 ml *M*/30 solution of arginine; in Expt. 2, in the main compartment of the flasks, 0.5 ml washed suspension, 0.3 ml of 0.1 *M* INAH, and in the side bulb, 0.3 ml of 0.1 *M* pyridoxine hydrochloride and pyridoxal hydrochloride, respectively, 0.5 ml *M*/30 solution of arginine.

The quantity of the liquid in the flask was then made up to a volume of 3.0 ml with McIlvaine's citric acid (0.1 *M*)-phosphate (0.2 *M*) buffer (pH 4.2) in each experiment. The gas phase was air. In order to obtain the effective action of INAH on the cell suspension, these flasks were agitated at 37.5° C without reagents for 30 min, and then the reagents in the side bulb were instantaneously mixed in each main compartment. The experiments were run at 37.5° C, and the gas output during the first 5 min was disregarded.

The Q values were calculated as follows:

$$Q_{CO_2}^{air} = \begin{pmatrix} X_{CO_2} \text{ evolved in} \\ \text{experimental flask} \end{pmatrix} - \begin{pmatrix} X_{CO_2} \text{ in correspond-} \\ \text{ing control flask} \end{pmatrix} / \text{dry weight of cell (mg)/hr}$$

As may be observed in Fig. 1 from the results of Expt. 1, INAH considerably, though not completely, inhibits the arginine decarboxylase activity of the washed coli-cell suspension, and the inhibitory effect of various amounts of INAH is as follows:

Final conc of INAH (*M*/liter)	Inhibitory effect (%)
0.02	70
.01	65
.005	47
.0025	34
0.00125	12

From the results of Expt. 2 (Table 1), it will be seen that pyridoxal (final conc, 0.01 *M*) recovers the arginine decarboxylase activity of the washed suspension inhibited with 0.01 *M* (final conc) INAH by more than 60% for the first 30 min.

Therefore, considering that the coenzyme of amino acid decarboxylase has been determined as pyridoxal-

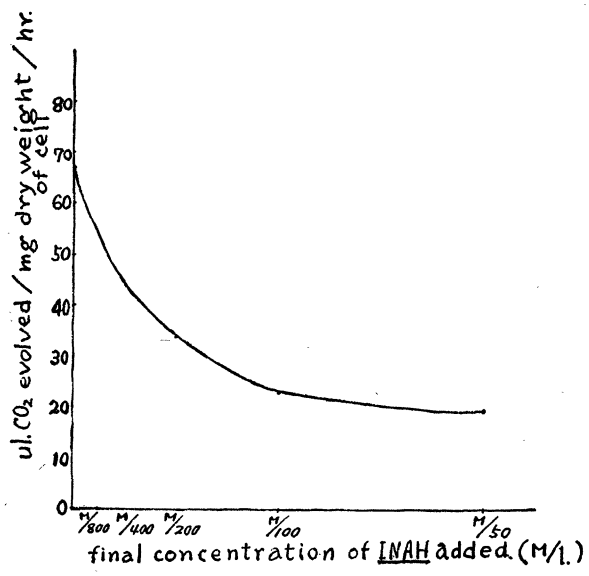

FIG. 1. Expt. 1: Inhibitory activity of INAH on the arginine decarboxylase of the washed suspension of *E. coli* communior (stock). Temp, 37.5° C; substrate, *M*/180 arginine (final conc); INAH was in contact with the washed suspension of cell at 37.5° C for 30 min.

TABLE 1

RECOVERY EFFECT OF PYRIDOXINE AND PYRIDOXAL*

(Experiment 2)

No.	Final conc INAH (*M*/liter)	Substance added	Q_{CO_2}/mg dry wt of bacteria cell	Recovery effect (%)
1	0.01	—	20.5	—
2	0.01	Pyridoxine (0.01 *M*)	30.5	20.0
3	0.01	Pyridoxal (0.01 *M*)	51.0	63.0
4	—	—	70.5	—

* Temp., 37.5° C; substrate, *M*/180 arginine (final conc); pyridoxine and pyridoxal were added, respectively, at the end of INAH-contact with cell. Recovery percentage was calculated from the following equation:

Recovery percentage = $[Q_{CO_2}\ (2 \text{ or } 3-1)/Q_{CO_2}\ (4-1)] \times 100$.

5-phosphate, these results indicate that the substantial recovery effect by pyridoxal is due to its competitive activity on INAH. Whatever the mode of the competitive action of INAH and pyridoxine derivatives may be, one important implication of these experiments is that INAH can act on amino acid decarboxylase of *E. coli* as an inhibitor, possibly being an antimetabolite against pyridoxine derivatives. These results are consistent with those obtained in experiments on the inhibitory action of INAH on the indole formation of *E. coli* (*4*).

References

1. YONEDA, M. 63rd Nagoya Med. Assoc., May 1952.
2. ANDERSON, T. *Proc. Natl. Acad. Sci., U. S.*, **32**, 120 (1946).
3. ATA, S., *et al. Current Medicine* (Japan.), **2**, (3), (1952).
4. YONEDA, M., *et al. Ibid.*
5. GUNSALUS, I. C., BELLAMY, W. D., and UMBREIT, W. W. *J. Biol. Chem.*, **155**, 685 (1944).
6. BELLAMY, W. D., UMBREIT, W. W., and GUNSALUS, I. C. *Ibid.*, **160**, 461 (1945).
7. WOODS, D. D., GUNSALUS, I. C., and UMBREIT, W. W. *Ibid.*, **170**, 313 (1947).
8. SCHLENK, F., and SNELL, E. E. *Ibid.*, **157**, 425 (1945).
9. LICHSTEIN, H. C., GUNSALUS, I. C., and UMBREIT, W. W. *Ibid.*, **161**, 311 (1945).
10. GALE, E. F. *Biochem. J.* (London), **34**, 392, 846, 853 (1940).
11. ———. *Bacteriol. Rev.*, **4**, 135 (1941).
12. MCILVAINE, *Biochem. J.*, (London), **49**, 183 (1951).

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Determination of Specific Activities of Tritium-labeled Compounds with Liquid Scintillators

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Recently several articles (*1–4*) have appeared on the determination of specific activity of compounds labeled with low energy β-emitting isotopes using liquid scintillation techniques. This procedure, which provides both ideal geometrical conditions and a relatively rapid technique of measurement, consists of dissolving and counting the labeled material in a liquid scintillator, such as terphenyl-xylene, terphenyl-dioxane, or 2,5-diphenyloxazole-toluene.

The following communication describes preliminary results obtained from the measurement of tritium-labeled materials using liquid scintillators and an experimental arrangement which permits satisfactory counting efficiency. The method may be applied to counting both tritium-labeled organic and inorganic compounds.

In general, present techniques for the determination of tritium are experimentally involved, especially for an organic material. Briefly, these methods are:

1) Ionization chamber measurement of hydrogen gas obtained by the combustion of sample to water and reduction of this water to hydrogen (*5*).

[1] We are pleased to acknowledge the aid of Winifred Bennett and Richard Cotton for the ionization chamber analyses and of Alice McCarthy for the preparation of the scintillation solutions.

2) Geiger tube measurement of a tritiated hydrocarbon obtained by the combustion of sample to water and reaction of water with Grignard reagent (*6*) to give tritiated hydrocarbon.

3) Measurement of solid samples with windowless, methane-flow-type counter operated in the proportional region (*7*).

TABLE 1

COUNTING OF TRITIUM-LABELED COMPOUNDS USING LIQUID SCINTILLATORS

Compound	Liquid scintillator	Expected cps from ionization chamber analysis	Measured cps from scintillation analysis*	Counting efficiency for scintillation method
Tritiated stilbene	Phenylcyclohexane, diphenylhexatriene, terphenyl	10,650	790	7.4%
Tritiated water	Dioxane, terphenyl	10,000	380	3.8%

* Average of several readings.

The two methods employing gas counting have high efficiencies (70–100%) but involve quite complicated preparation techniques; and that using solid samples, although simple, has fairly low counting efficiencies (~2–3%).

The tritium-labeled materials that we have counted are approximately 3% by weight of tritiated water in a solution of dioxane containing 5 g/liter of terphenyl and tritiated stilbene (*8*) in a solution of phenylcyclohexane containing 3 g/liter of terphenyl and .01 g/liter of diphenylhexatriene (*9*). The *p*-dioxane was purified by distillation over sodium at atmospheric pressure, and the phenylcyclohexane was distilled at reduced pressure through a fractionating column.

The experimental arrangement used for this set of measurements was similar to that described in a previous article (*3*), and all data were taken at room temperature. However, here the integrating time constant at the output of the photomultiplier tube was 1 μsec. Because of significant phosphorescence of the liquid scintillators, all solutions were allowed to dark-adapt for at least 30 min before a measurement was taken.

The preliminary results thus far obtained are summarized in Table 1. The ionization chamber analyses were accomplished by combustion of the stilbene on a semimicro scale and conversion of the tritiated water to hydrogen gas (*5*), which was then counted in an ionization chamber with a Lindemann-Ryerson electrometer (*10*). The equivalence of rate of discharge of the electrometer (mv/sec) per millicurie of tritium gas was established with aliquots of tritium obtained by manometric dilution of several tritium ampules of given activity, as supplied by Oak Ridge National Laboratory.