The Quantitative Collection and Determination of Hydrogen Gas From the Rat and Factors Affecting Its Production (35749)

M. R. GUMBMANN AND S. N. WILLIAMS
(Introduced by A. N. Booth)
Western Regional Research Laboratory,[1] Albany, California 94710

Hydrogen production resulting from the action of microflora upon fermentable substrates in the intestine is known to occur in man and experimental animals. The generation of this gas has been associated with pathologic conditions resulting in carbohydrate malabsorption (1), with ingestion of specific carbohydrates known to be poorly digested (1, 2), and with ingestion of various foods generally considered to be gas- or flatulence-producing (3–5). The techniques used for studying hydrogen production have included, in man, either collection of flatus samples by rectal and peroral intubation or collection of respiratory gases (2, 5, 6); in animals, gas has been collected directly from the intestine of the rat after sacrifice (7) and from surgically prepared, isolated intestinal segments of the anesthetized dog (8). In none of the above methods, however, is total hydrogen production determined, and results are dependent upon the time gas analyses are performed. Presented below is a procedure by which the total hydrogen produced in the rat following a given feeding may be determined routinely. Also included are results of a preliminary investigation into some of the factors which appear to influence hydrogen production following bean consumption. This method should be of utility in studying bacterial metabolism in the intestine and its modification by various nutrients or drugs, the digestibility of different food materials, and the causative factors of flatulence. Since the technique is not destructive of the animal, repetitive testing of the same animal under various conditions is possible.

Materials and Methods. Animals. Sprague-Dawley male rats were used. Unless stated otherwise, an individual rat was tested for hydrogen production only after being maintained on a standardized diet (Purina[1] rat chow) for a period of at least 3 days prior to presentation of the test diet.

Diets. Test substances were fed mixed into the 40% casein diet described by Hedin (3) at the expense of an equal amount of the casein. In addition to casein,[2] this diet contained 60% basal mix consisting of 8% corn oil,[3] 5% salt mixture USP XIV,[2] 4% nonnutritive cellulose (Alphacel),[2] 1% vitamin mixture,[2] and 42% corn starch.[2] A standard bean diet was prepared from dried California small white beans that had been soaked, cooked, and then lyophilized. Dried lima beans which had been ground and autoclaved were also fed.

Life-support system. Figure 1 shows schematically the animal chamber and supporting system for collection of hydrogen. The chamber consists of a bowl desiccator (200 mm i.d. with tubulature in cover) which contains a wire-mesh floor and feed cup. A three-hole rubber stopper provides a water supply and an air inlet and outlet. Air is continuously circulated with a motor-driven diaphragm pump.[4] A gas-sampling port was constructed from a Swagelok stainless steel union tee[5] closed with a silicone rubber septum as used in injection ports on gas chromatography ap-

[1] Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[2] Nutritional Biochemicals Corp., Cleveland, Ohio.

[3] Best Foods, Englewood Cliffs, N.J.

[4] Neptune-Dyna-Pump, Model 4K, Neptune Products, Inc., Dover, N.J.

[5] Crawford Fitting Co., Solon, Ohio.

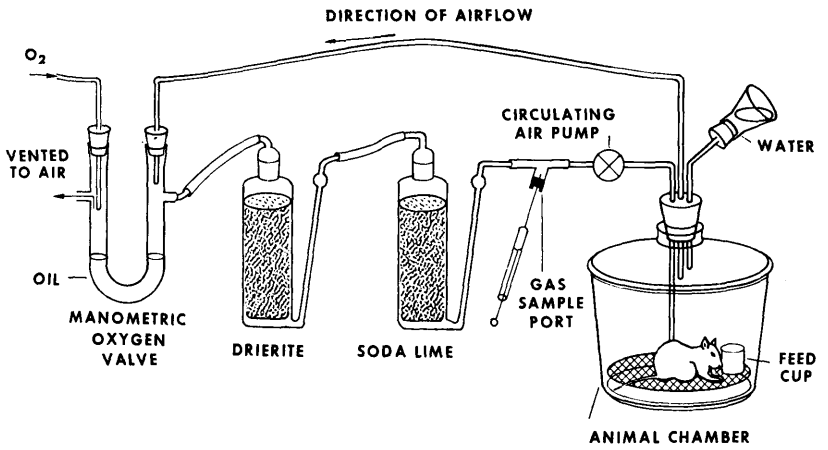

FIG. 1. Life-support system for the quantitative collection of hydrogen gas.

paratus. Absorption of carbon dioxide and excess water vapor was accomplished with 4–8 mesh soda-lime and Drierite, respectively, contained in gas washing bottles with fritted discs. A novel but simple manometric valve which permits entry of oxygen upon demand was constructed from a tubulated "U" drying tube containing a small amount of nonevaporating liquid such as light weight vacuum pump oil. The various pieces of apparatus were connected with Tygon tubing, and the lid of the animal chamber was sealed with silicone grease.

In operation, an excess flow of oxygen is passed continuously through the left arm of the "U" tube valve (Fig. 1) and out to the atmosphere. To begin, the rat is placed inside the chamber, the lid sealed and with the stopper on the right arm of the manometric valve removed, the pump is started. In a moment the stopper can be replaced without disturbance of the oil within the U-tube. Consumption of oxygen by the animal produces a slight negative pressure causing oxygen to bubble across the U-tube and enter into the otherwise closed system. The nitrogen and oxygen content of the system remain constant, and any hydrogen evolved is quantitatively trapped.

Measurement of hydrogen. One-ml gas samples were withdrawn with a gas tight syringe from the sampling port and analyzed by gas chromatography for hydrogen, oxygen, and nitrogen. Determination of the latter two gases was useful in indicating the proper functioning of the life-support system. The chromatographic unit contained a single stainless steel column (1/4 in. by 12 ft packed with molecular sieve, type 13×, 30/60 mesh) and a thermistor detector (9). The carrier gas was argon at a flow rate of 22 ml/min, and the operating temperature was 30°. Air pressure variations in the laboratory, which disturbed the detector, were effectively damped by passing the effluent gas into a 10-liter jar, which was vented to air through a 23-gauge hypodermic needle.

The ability of the life-support system to retain hydrogen without loss was verified by injecting pure hydrogen through the sample port and allowing the system to operate for an extended period. The average percentage loss from four such systems was 2.9% after 66 hr of continuous running (1% in 24 hr). For use with an animal in determining hydrogen production, a calibration curve was prepared for each system assembled which related peak height of hydrogen contained in a 1-ml sample of gas withdrawn from the system directly to an amount of pure hydrogen injected into the system. Based on the sensitivity of the detector and the volume of the systems, the limit of detection was approximately 0.2 ml of hydrogen.

Results and Discussion. Rate of hydrogen production. Figure 2 shows the cumulative production of hydrogen after ingestion of 10 g of 40% California small white bean diet.

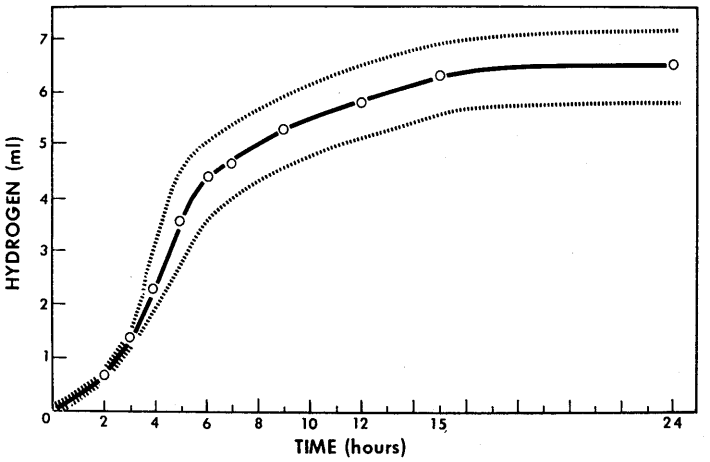

FIG. 2. Cumulative hydrogen production following ingestion of California small white beans: (—) each point represents mean results from 4 animals; (. . . .) the standard error of the mean.

The rats had been trained to eat in 1 hr. Hydrogen was detected 1 hr after the end of the feeding period and its rate of evolution reached a maximum between 4 and 5 hr. After 15 hr, further increase in the total amount produced was less than 4%. The period of maximum hydrogen production coincided with that reported by Hedin and Adachi (7) who measured peak hydrogen concentration in the gastrointestinal gases of the rat 4 to 8 hr after ingestion of a red bean diet. It is also similar to observations on breath hydrogen in the human after consumption of beans where the maximum concentration occurred between 5 and 7 hr (4).

Assay procedure. The time-consuming process of training rats to eat in a specified time interval for the determination of total hydrogen production following a given feeding was found to be unnecessary. Instead, a standard assay procedure was adopted which permits rats to eat normally (*i.e., ad libitum* and in the evening) while within the life-support system. In this procedure rats are fasted from 9:00 a.m. and placed within the chambers containing test diet at 4:00 p.m. To ensure ingestion of uniform quantities of test diet, approximately 80% of the usual feed consumption is generally offered. Gas sampling for hydrogen analysis is begun the following day, about 18 hr later. Repeated determinations up to 24 hr have shown consistently that essentially no increase in total hydrogen occurs after 17 hr.

Relation of hydrogen production to bean consumption. Using this assay procedure, the amount of hydrogen produced in relation to the quantity of beans ingested was investigated (Fig. 3). Feedings consisted of 12 g of

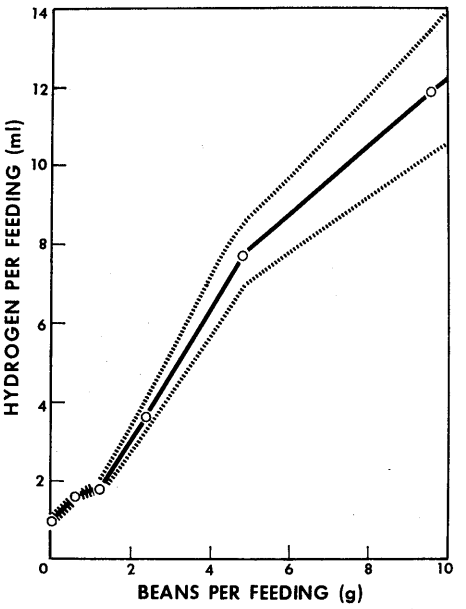

FIG. 3. The relationship of hydrogen production to quantity of beans ingested: (—) points represent mean results from 4 to 10 animals; (. . . .) the standard error of the mean.

TABLE I. Effect of Pretreatment on Hydrogen Production.

Expt.	Pretreatment (days)	Test diet (beans)	Feed eaten (g)	No. of feedings	H_2 (ml ± SD/ feeding)
1.	40% Casein, 3	40% Lima	10	6	5.90 ± 1.39[b]
	Purina Chow, 3	40% Lima	10	6	3.21 ± 1.90[b]
2.	40% Casein, 1	40% CSW[a]	12	10	7.70 ± 2.23[c]
	Purina Chow, 1	40% CSW	12	10	5.60 ± 4.48[c]
3.	40% Casein, 1	40% CSW	12	4	5.07 ± 2.63
	Fasted, 1	40% CSW	12	4	4.87 ± 3.51

[a] CSW (California small white).
[b] Means significantly different, $p < .01$.
[c] Variation significantly different, $p < .05$.

40% casein diet mixed with increasing amount of California small white beans, first at the expense of casein and finally at the expense of a portion of the entire casein diet. The response to the 40% casein diet alone (0 beans/feeding) resulted in slightly less than 1 ml of hydrogen. In the production range of about 2 to 8 ml of hydrogen/feeding, direct proportionality to bean consumption was obtained. Above 8 ml, relative hydrogen production decreased. In comparing the results of Hedin (3), it was found that the total hydrogen produced after feeding either 40% bean or casein diets was from 10 to 25 times greater than the quantity determined by them to be actually present in the gastrointestinal tract at the time of maximum concentration.

Effect of dietary regimen before testing. Characteristic of hydrogen production from a given diet was the occurrence of considerable variation both within the same rat upon repeated testings as well as among different rats. The effect that dietary regimen might have on such responses was investigated. Shown in Table I, Expts. 1 and 2, are the results of feeding either a commercial maintenance chow or 40% casein diet for 1 or 3 days prior to presentation of a test diet. In both cases, more hydrogen was produced after prefeeding with casein diet (significantly so in Expt. 1) and with less variation (significantly so in Expt. 2). Fasting the rats 24 hr prior to feeding the test diet (Expt. 3) resulted in neither increased response nor improved variation over that for prefeeding with casein diet. The hydrogen producing ability of Purina chow, itself, was tested and found to be approximately twice that of the 40% casein diet but considerably less than a 40% bean diet.

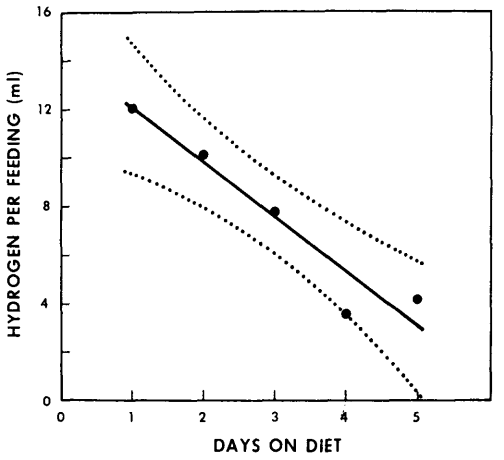

FIG. 4. Hydrogen production from consecutive feedings of 40% lima bean diet: Rats were prefed 40% casein diet for 3 days. Plotted are mean results from 7 rats, each fed 12 g diet daily, and the computed curve of linear regression with 95% confidence limits.

Effect of continued bean consumption. Since dietary conditions prior to the time of testing influence response, hydrogen production from consecutive feedings of a bean diet was investigated. Figure 4 shows mean hydrogen production resulting from ingestion of 40% lima bean diet over a period of 5 days. The amount produced dropped progressively for 3 days after which a minimum may have been reached. The least squares estimate of slope showed the average rate of decrease over the 5-day period to be 2.2 ml of hydro-

gen (18.6% of the initial value)/day. This decline most likely resulted from a dietary influence on intestinal microflora as opposed to an induction of adaptive intestinal enzymes which could increase the rate of breakdown of poorly digested carbohydrates. This latter mechanism appears to be completely incompatible with the immediate response to the bean diet when comparison is made to known examples of intestinal enzyme induction such as that of lactase which requires 5 to 8 weeks of exposure to high levels of lactose (10).

Summary. A life-support system for the rat is described in which hydrogen evolved after consumption of a given diet is quantitatively trapped and may be determined. The technique is not traumatic to the animal. The maximum rate of hydrogen production after feeding a dried-bean diet occurred between 4 and 5 hr. The quantity of hydrogen arising from such a diet increased with increasing consumption of beans and was directly proportional to the amount of beans eaten over a range which produced 2 to 8 ml of hydrogen/feeding. Dietary regimen of the animal before testing was found to influence response. With consecutive daily feedings of a bean diet, hydrogen production decreased by 18.6% of the initial value per day for approximately 4 days.

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