

minimum stimulation dose of prolactin in 0.05 ml thus has its apparent effectiveness increased 4 times by a dilution of 10 times. No simple explanation of this result is apparent. Micro-assays of prolactin by intracutaneous injections over crop-sacs must utilize a constant volume of fluid to be of much quantitative value.

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Use of Illuminating Gas to Check Metabolism Apparatus.

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Combustion of alcohol, ether or acetone is standard procedure to check the operation of metabolism apparatus (Carpenter, *et al.*¹). We have found it impossible, however, to devise a burner by which the combustion of any of these would proceed evenly for metabolically significant periods at rates comparable to the respiratory metabolism of the rat. On the other hand, small validity would seem to attach to a test several times more severe than planned capacity; failure would be no indication of inability of the apparatus to do what it was designed to do; nor would success be any guarantee that it could perform the more delicate task for which it was made. A micro-balance is not checked with kilogram weights.

As a result, recourse has been had to combustion of gas which can be successfully controlled at almost any desired rate. It was originally intended to use a pure, commercial preparation of one of the lower hydrocarbons in order to eliminate the necessity of control determinations. Preliminary work with ordinary illuminating gas from the city mains was so satisfactory, however, that it has been adhered to; especially since equipment was at hand for the necessary control determinations which involve only slightly additional work.

Since the only difficulty in the application of this principle is accurate measurement of the small volume of gas burned, description of an apparatus which has been found accurate and simple to operate and is easily assembled from odds and ends about any laboratory may be of interest.

This apparatus is shown diagrammatically in Fig. 1.

¹ Carpenter, T. M., Fox, E. L., and Sereque, A. F., *J. Biol. Chem.*, 1929, **82**, 335.

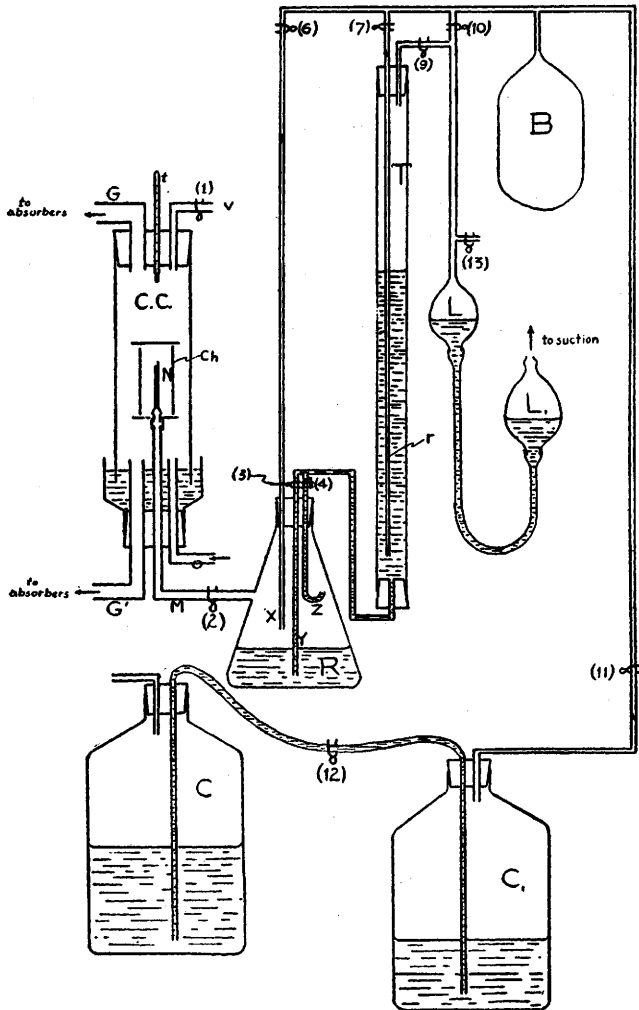


Fig. 1

The gas sample. Since the composition of the city gas supply is not absolutely constant, gas is not burned directly from the supply line. Instead, a sufficient sample for several runs and their attendant control determinations is secured in the 18 liter carboy (C_1) by water displacement into the similar carboy (C). Conversely, as needed, the gas is displaced from (C_1) into the rubber football bladder (B) by siphoning of water from (C) to (C_1).

The combustion chamber, (C-C) 5.5 cm in diameter and 25 cm long, takes the place of the animal chamber of the metabolism

apparatus (Schwabe and Griffith²). The base of the chamber (S) is the upper part of a wide-mouth bottle and contains mercury for an air-tight seal. Tube (O) connects with the oxygen measuring device of the metabolism apparatus and admits pure oxygen as needed. Tubes (G) and (G₁) connect with the carbon-dioxide absorbers of the metabolism apparatus, by which, also, circulation of air within the chamber is maintained. (t) is a thermometer and (V) a tube which is open during assembly of the chamber to equalize

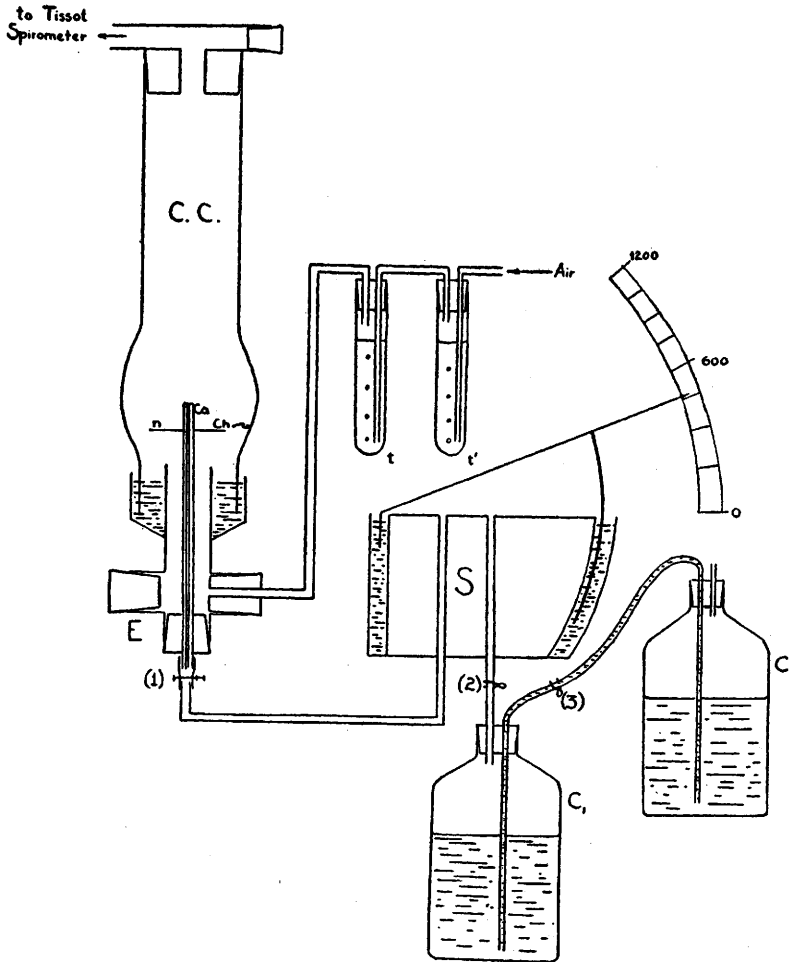


Fig. 2.

² Schwabe, E. L., and Griffith, F. R., Jr., *J. Nutrition*, 1938, **15**, 187.

pressure. The illuminating gas is admitted through (M) to whose end, within the chamber, a 26-gauge hypodermic needle (N) which serves as burner, is attached by rubber tubing. The burner (and flame) is protected from air-currents by the chimney (Ch).

Gas measurement is made by the assembly consisting of the one-liter suction flask (R) and the tube (T) which is 2.5 cm in diameter and 100 cm long. It is merely an adaptation of the "microspirometer" of Hanan³ similar to that used by Schwabe and Griffith to measure the rate of oxygen utilization in the metabolism apparatus. At the beginning of a run (T) is full of water and (R) has been filled with gas to be burned through tube (X) from the bag (B). During a run, due to the fact that the end of (r) in (T) is 15 cm above the opening of (Z) in (R), water flows from (T) to (R) through (Z), and gas enters (T) from (B) through (r) (Mariotte principle), thus establishing a slight, constant, positive pressure on the gas in (R). This gas is forced from (R) through (M) to (N) at the tip of which it is burned. By proper adjustment of the screw-clamp (4) water can be made to flow from (T) to (R) and gas from (R) to (N), at any desired rate. (T) is carefully calibrated so that the amount of water siphoning into (R) and therefore the volume of gas which has been burned, can be accurately known.

Procedure. At the beginning all clamps are closed except (11) and (12). Since there is positive pressure on the gas sample in (C₁) this will fill the football bladder (B). Also with clamps (6) and (2) open and the combustion burner (N) open to the room, (R) is thoroughly flushed out to make sure it contains no trace of foreign gas. Clamps (2) and (6) are then closed, as well as (11) and (12).

To set (R-T) for a run, *i.e.*, to get the water from (R) to (T) a negative pressure is established by the leveling bulbs (L) and (L₁). Open (13) and raise (L₁) until (L) is full; close (13). Lower (L₁) below (L); this establishes a negative pressure in (L) which upon opening clamps (9), (3) and (6) will draw water from (R) to (T). Actually, since (T) is 100 cm long, sufficient negative pressure to effect this transfer is obtained with the help of a water-faucet suction pump attached to the upper, open end of (L₁). When this transfer is complete (9) is closed, the suction is stopped and (3) and (6) are closed.

By operating the unit (T-R) as a closed system as is done here and as explained by Schwabe and Griffith who used a similar modification of Hanan's device for measurement of oxygen admitted

³ Hanan, E. B., *Science*, 1929, **70**, 582.

to the metabolism chamber, the water that is employed is kept in constant contact and equilibrium with the gas that is used, thereby preventing error due to solution of the gas in the water.

At this time all clamps are closed.

To prepare for a run, clamps (7), (4) and (2) are opened in this order. Due to the slight positive pressure between (T) and (R), water will immediately begin to siphon from (T) to (R); gas will enter (T) from (B) at atmospheric pressure and will escape from the burner (N) where it is lit. The chimney (Ch) is put in place, the line to the oxygen supply opened, and with clamp (1) open the combustion chamber is put in place. Before closing (V) slight suction is applied to it to draw enough pure oxygen into the chamber to make sure the flame will burn; clamp (1) is then closed.

Control determination is effected by the set-up shown in Fig. 2. A sample of the same gas is transferred from the carboy (C₁) to the small one-liter, Krogh spirometer (S) and burned at the tip of the 1 mm capillary glass tube (Ca) in the combustion chamber (CC). The flame is protected by the baffle (n) from the current of air (from the compressed air supply) which enters the combustion chamber after being washed through NaOH (t-t₁) and

TABLE I.
Results.

		Rate gas burned, cc/min	R.Q.	Ratio CO ₂ /gas	Ratio O ₂ /gas	Rate of O ₂ utilization, cc/min
1/9/40	E*	5.0	.51	.82	1.60	8.00
	C†		.49	.79	1.60	
1/9/40	E	10.0	.52	.82	1.58	16.00
	C		.49	.79	1.60	
1/22/40	E	5.5	.51	.84	1.68	9.25
	C		.52	.87	1.66	
2/5/40	E	5.1	.49	.79	1.57	8.01
	C		.50	.83	1.68	
2/13/40	E	5.2	.49	.85	1.72	9.15
	C		.50	.85	1.71	
4/22/40	E	6.5	.52	.84	1.62	10.53
	C		.50	.86	1.73	
Average	E		.51	.83	1.63	
	C		.50	.83	1.66	

*Refers to results obtained when a small flame was burned in the Schwabe-Griffith Metabolism Apparatus.

†Refers to results obtained when the products of combustion of a larger flame were determined with the Tissot spirometer and gas analysis.

on leaving (at the top of CC) is collected and measured in a 100-liter Tissot spirometer. This air is then analysed in Haldane apparatus.

Comparison of the results obtained in a few of the more recent "experimental" and "control" determinations is shown in Table 1.

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Studies on Intercostal Nerve Physiology.

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Pathological cases of processes involving the parietal pleura and the diaphragmatic pleura innervated by the lower intercostal nerves, and the attendant referred pain and muscular rigidity in the abdominal region (lower quadrant), have long suggested neural associations between the regions involved. Capps¹ competent studies upon such clinical cases have yielded valuable information relating the portion of pleural membrane or diaphragm stimulated to site of referred pain.

It is a well established fact known to clinicians that:² "The abdominal symptoms of thoracic disease are often so misleading that needless or harmful surgery may be carried out. A pleurisy may give referred pain over the distribution of the 6 lower intercostal nerves, with fever may simulate appendicitis."

In attempting to establish a physiological basis for such phenomena, we have investigated the intercostal nerves of dogs. The effects of our various manipulations upon blood pressure and respiration were kymographically recorded, the blood pressure obtained from the carotid artery, and respiratory movements recorded by the usual pneumograph-tambour method. In cases where a pneumo-thorax was unavoidable, artificial respiration was administered. Isolation of various intercostal nerves and their branches was facilitated by appropriate rib resection. A tetanizing current was used for nerve stimulation.

Stimulation of the central end of any intercostal nerve results in a transient lowering of blood pressure (approximately 25 mm Hg.)

¹ Capps, J. A., *An Experimental Study of Pain*, Macmillan, 1932.

² Nelson, *Living Medicine*, Thomas Nelson and Sons, 1920-1937, 5, 222.