

opposite end and was over five feet long. The stump appeared to be bulbous but showed nothing of the attachment of the roots. Being completely decorticated it showed no surface characters. Several other large pieces of wood were scattered over the floor of the quarry, and according to the owner the largest specimen, measuring about three by eight feet, had been blasted to pieces and used in the construction of a bridge foundation.

Preliminary investigations show that the wood from the New Albany shale is *Callixylon*. This genus is also known from south Russia, the Hunton formation of Oklahoma, the Chattanooga shale of Kentucky, the black shale of Ohio and in great abundance throughout the lower half of the upper Devonian formations of central and western New York. It has also been found in the glacial drift of Michigan. In New York

only fragments of small branches, twigs and roots are known, while in Indiana only large trunks have been seen so far. Specific determinations of the Indiana material have not been made.

Although the wood is widely scattered, it appears to occur mostly near the top of the New Albany formation. While formerly considered as belonging to the upper Devonian and of the same age as the Genesee shale of New York, the New Albany shale is now viewed by some competent authorities as being, at least in part, of lower Mississippian age. This would place the Indiana wood in the Mississippian, and thus extend the range of *Callixylon* from the Devonian up into the Carboniferous. However, there is no record of its occurrence any higher than this basal member.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN AUTOMATIC MICROSPIROMETER

IN metabolic studies on the incubating hen's egg the writer has carried out continuous determinations of oxygen absorption and carbon dioxide production. At the beginning of incubation the egg is placed in a specially constructed metabolism apparatus in which it remains excluded from the outside air until hatched. The rate and total oxygen consumption and carbon dioxide elimination may be calculated at any time during the development of the embryo. A complete report will be published at a later date.

The oxygen spirometer developed to meet the requirements of the experiments has proved adaptable to a wide range of respiratory experiments. It is employed with satisfactory results by Dr. J. O. Ralls, of the biochemistry department of the University of Buffalo, in metabolic studies on rats.

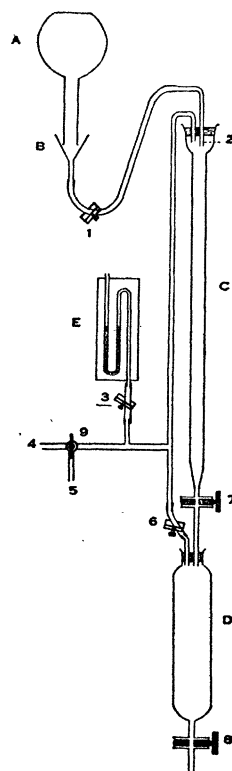
The spirometer used in my experiments is illustrated in the accompanying figure. It is simple in construction, being made up of ordinary standard laboratory equipment. The neck of a 1,000 cc volumetric flask *A* rests snugly in funnel *B*. *C* is a 50 cc burette and *D* a 500 cc separatory funnel. *E* is an ordinary manometer constructed of 4 mm glass tubing and filled with red kerosene. These parts are connected as shown in the illustration.

In principle the apparatus depends upon the replacement of consumed oxygen by fluid without abnormal variations in pressure. In order to control humidity I have employed a saturated solution of sodium chloride.

The procedure for a continuous experiment is as follows: The manometer *E* being empty the volumetric flask *A* is filled with the sodium chloride solution and inverted in funnel *B*. By manipulation of cocks 7

and 6, the solution is allowed to displace the air from the apparatus through the manometer. The solution is allowed to pass up in the manometer tubing until it reaches manometer fluid level at *e*. By closing pinch cock 1, the fluid is drawn from the manometer through stop cock 8, then by closing pinch cock 3, the fluid is displaced through 8 by oxygen coming in at 5, taking care to leave enough fluid to fill the lower end of burette *C* to the 50 cc mark.

By measuring the displaced sodium chloride solution the volume of the spirometer above and below cocks 6 and 7 is determined. The sum gives the total volume of the spirometer. The two volumes are determined separately so that the burette part may be used independent of the separatory funnel especially during the first eight days of incubation when the chick will not use over 50 cc of oxygen in twenty-four hours. The air volume of the egg chamber having been previously determined, the total volume of the apparatus is known. These data are necessary for barometric and temperature corrections. An ideal place of operation is a constant temperature room.



After releasing cock 3, kerosene is placed in the manometer to level *e*. Then *B* is adjusted level with 2 which is a glass tube drawn to a suitable capillary point. Cock 6 is closed, 7 being already closed with fluid in the burette to the 50 cc mark. Cock 1 is released and the spirometer connected to the egg chamber by the three-way cock 9. By opening cock 8 the manometer is adjusted to zero, at which time barometric and temperature readings are taken.

The CO₂ is absorbed in the egg chamber and as oxygen is consumed a negative pressure results, as will be indicated by the manometer. But if *B* is properly adjusted on a level with 2 the oxygen used will be immediately replaced with fluid from the volumetric flask flowing into the burette. Thus the oxygen consumption may be read at any time by the fluid level in the burette.

The readings are made at some selected age hour interval. About thirty seconds before the hour the manometer is adjusted to zero by either letting in or drawing out fluid as the case needs be. The zero reading is maintained as the hour strikes, at which time the fluid level in the burette is noted, then immediately the barometric and temperature readings are taken. With the data in hand the oxygen consumed may be reduced to standard readings.

The separatory funnel serves as a reservoir. When the burette is filled near its limits, cocks 6 and 7 are opened and the surplus fluid drained in to *D*, the oxygen being automatically displaced from *D* into *C*. When *D* is filled the fluid is displaced with oxygen from the supply tank as at the beginning of the ex-

periment. The fluid is measured and replaced in the volumetric flask, the same fluid being used repeatedly.

During the first five days of incubation the 50 cc burette will take care of the entire oxygen consumption. In the last half of incubation the rate of oxygen consumption of the egg rapidly approaches 40 to 50 cc per hour, which it reaches just before hatching. When the oxygen consumption is likely to be more than the measuring capacity of the burette during the absence of the observer, the cocks 6 and 7 are left open, allowing the fluid to flow into *D*. On the observer's return the fluid may be displaced with oxygen and measured. Care must always be taken to first adjust the manometer reading to zero and to note barometric and temperature readings at the same time any fluid measurement is made. The egg chamber is of sufficient size to permit the spirometer to be shut off for a short period without disturbing the physiological control.

The advantage of this spirometer is that it permits fairly accurate measurements of rapidly varying rates of oxygen consumption. The measurements may be made over an entire biological period. This is very advantageous in studies of ontogenetic energy. I feel that the principle may be used for studies of oxygen consumption in tissue cultures, insects and small mammals or vertebrates.

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SPECIAL ARTICLES

COW'S MILK AS A SOURCE OF VITAMIN B FOR LACTATION

In 1924 I produced experimental evidence¹ that a ration containing 50 per cent. skimmed milk powder as the only source of vitamin B was inadequate for rearing of young when lactating rats were allowed litters of six young to nurse. When such diets, however, were fortified with a brewer's yeast concentrate entire failure was changed to complete success in lactation.² The conclusion was then made that the vitamin B requirements for lactation are much greater than those for growth, and that cow's milk is deficient in vitamin B for milk secretion. Since no method is available for collecting the milk secreted by a rat, the

¹ B. Sure, *J. Biol. Chem.*, 1924, 62: 371-396.

² Since these milk diets were fortified with ferric citrate, iron was ruled out as a limiting factor in failure of lactation. That copper, another mineral element deficient in milk, is not a complicating factor in the vitamin B lactation problem has also been recently demonstrated. See *J. Biol. Chem.*, 1928, 80: 289-295.

criterion for successful lactation used by all nutritional investigators is the character of growth of the nursing young when a specific dietary essential is the only limiting factor in a ration. In 1927, using diets (provided with a satisfactory salt mixture containing an abundance of ferric citrate) composed of purified food substances, I demonstrated that the vitamin B requirements for lactation are at least three times those necessary for normal growth.³ Recently I have developed quantitative biological methods for studies of lactation and vitamin requirements of nursing young of the albino rat⁴ which have disclosed the fact that the great requirements of vitamin B for lactation are due to the lactating mother's dissipating over 60 per cent. of the vitamin in the metabolism of transfer to the milk. My methods involve transfer experiments from stock diets to purified synthetic

³ *J. Biol. Chem.*, 1927, 74: 55-69.

⁴ *Ibid.*, 1928, 76: 685-700.