"Get me the brush on the north side of the dresser," "Go and sit in the chair on the east side of the porch." She did not do this to train his sense of direction but merely because it was more convenient and less confusing for her. Out of this training the boy has apparently developed the unusual ability to move about a complicated path for relatively long periods of time and retain his orientation without paying attention to the process.

From a theoretical angle the fact that this boy has learned to orient himself for long periods of time over a devious path while occupying his mind with many other things furnishes a clue to help us to understand how certain birds and animals can wander for long distances and find their way home over unfamiliar territory. If this boy can orient himself to the compass directions without voluntary attention to the task for long periods of time, then it is not difficult to conceive why certain animals, to whom such an ability would have an important survival value in hunting or being hunted, can likewise maintain this sense of continuous automatic orientation, if not to points of the compass, at least to their homes.

It is obvious that other factors need to be discovered to explain the case of an animal being blindfolded, confused and carried for a considerable distance and then finding his way home, but then many animals can not carry out this stunt. Perhaps in the few cases reported where animals have been successful in this performance, certain cues that might have enabled them to retain this continuous automatic orientation were not eliminated.

HARRY R. DESILVA

PSYCHOLOGICAL LABORATORY,
UNIVERSITY OF KANSAS

MENDELIAN DIFFERENCES

MAY I be permitted to correct an error of attribution before it becomes more widely spread in the biological literature. Dr. E. S. Russell, in his recent very interesting book, "The Interpretation of Development and Heredity" (p. 64), quotes Johannsen[1] as responsible for the conception that Mendelian inheritance deals with *differences* rather than similarities. It is necessary to point out that this conception originated in a paper of mine[2] in 1915, where the matter is fully developed. It was also referred to again in my book "The Mutation Factor in Evolution" (1915, p. 313).

It is worth pointing out that in the same paper (p. 141) Johannsen uses the conception of a particular constitution as characteristic of every cell in each genotype. This conception is clearly stated in "The Mutation Factor" (p. 297) and, as is well known, was based originally upon the Oenothera mutations which have an extra chromosome in every nucleus. It was further considered in various aspects in "Mutations and Evolution."[3]

R. RUGGLES GATES

UNIVERSITY OF LONDON,
KING'S COLLEGE, STRAND

[1] "Some Remarks about Units in Heredity," *Hereditas*, 4: 133–141, 1923.

[2] "Heredity and Mutation as Cell Phenomena," *Amer. J. Bot.*, 2: 519–528.

[3] New Phytologist Reprint No. 12, pp. 118, 1921, now published by the Cambridge Press.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE REMOVAL OF TRACES OF OXYGEN FROM NITROGEN

THREE general methods have been used to remove traces of oxygen from nitrogen: (1) To pass nitrogen through a solution which will remove the oxygen; some of the solutions used have been alkaline pyrogallol, ammonium cuprous chloride and alkaline hydrosulfite, with or without a catalyst as anthraquinone sulfonate suggested by Fieser.[1] (2) To remove oxygen with a solution such as ammonium cuprous chloride, which was placed in a large cylinder containing the gas under pressure; nitrogen, free from oxygen, but containing traces of ammonia, is delivered from the cylinder.[2] (3) To pass the nitrogen over a hot metal which will remove the oxygen and form the oxide of the metal; for this, copper has been shown to be much the most efficient, and has been used by the majority of investigators.

Variations in the potentials of solutions of cysteine which were observed in my laboratory when this type of purification was used indicated that under some conditions all traces of oxygen were not removed by the copper and suggested a reinvestigation of the problem concerned with the removal of oxygen from nitrogen.

Previous work had shown that a flow of between 200 and 500 cc of nitrogen a minute was desirable. For the removal of the traces of oxygen from this volume of nitrogen many different forms of tubes and furnaces were prepared and investigated. These need not be described in detail; the results eventually have led to a tube which has been thoroughly tested and has been proved to be satisfactory. It possesses several advantages when compared with the old type of copper furnace.

[1] L. F. Fieser, *J. Am. Chem. Soc.*, 46, 2638 (1924).

[2] H. Wartenberg, *Zeitschr. f. Elektrochemie*, p. 295 (1930).

Popoff[3] has suggested the use of the heated filament of a tungsten incandescent light for the purification of hydrogen. It was hoped that copper wire could be substituted for the tungsten and that it could be electrically heated to such a temperature that the traces of oxygen in nitrogen would combine with the copper oxide thus formed and would not be decomposed. With a 110 volt heating current, either an extremely long wire of large cross-section may be used or a shorter wire of small cross-section. No. 28 copper wire was chosen. A sufficient length to give 10 ohms resistance was wound on a triangular glass frame of pyrex rod. The frame was placed inside of a pyrex tube and suitable arrangements were made for the passage of the electric current and for the flow of nitrogen. It was then found that the wire expanded, short circuited and soon burned out.

This suggested a separation of the furnace into two parts, an inside heating element, and copper in the form of gauze to take up the oxygen. A cylinder of 40 mesh copper wire gauze 60 cm in length was wrapped around the outside of a 26 mm pyrex glass tube. Between the 26 mm glass tube and the copper gauze was placed a strip of mica which covered about a fourth of the surface of the inside of the copper gauze. Two thicknesses of copper gauze were wound around the tube and the strip of mica. The copper gauze was securely fastened with copper wire. The glass tube was then withdrawn, leaving a tube of copper gauze. This was placed inside of a pyrex glass tube 34 mm outside diameter and a coil of nichrome wire 0.5 cm in diameter, which contained 7.5 meters of No. 18 nichrome wire, was placed inside of the tube of copper gauze. A rod of silica 6.5 mm in diameter was placed inside the coil of nichrome wire to prevent distortion of the coil. The nichrome heating element rested on the mica. Copper wire for the connections to the heating element was sealed through the glass tube (Fig. 1).

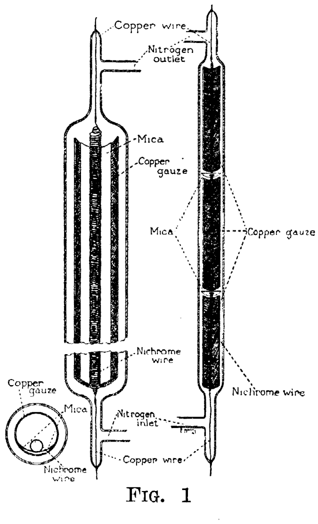

FIG. 1

[3] S. Popoff and A. H. Kunz, *J. Am. Chem. Soc.*, 51, 382 (1929).

Such a furnace has the following six advantages: (1) It is open for the free passage of the gas, the heating element and the copper occupy only a small part of the cross-section of the tube, and the velocity of the flow of nitrogen through the tube is, therefore, not increased. (2) The temperature to which the pyrex glass is heated (not above 300°) is too low to soften the glass. The pyrex tube therefore is not subjected to severe thermal changes and will last indefinitely. (3) The temperature to which the nitrogen is heated may be made maximal. The nichrome coil may be heated to any temperature desired up to decomposition of the nichrome. From 400 to 600° is sufficient to insure a high degree of reactivity of the traces of oxygen so that the oxygen rapidly combines either with the nichrome or the copper gauze. (4) The temperature of the copper gauze is high enough to insure the rapid reaction with all traces of oxygen, but it is not sufficiently high to drive off the copper oxide which has been formed. The position of the gauze in contact with the pyrex glass tube insures the maximal conduction of heat from the copper through the glass. (5) The tube is made of light construction and is not bulky. This fact permits a convenient arrangement of the tube in connection with electrode cells. (6) All parts of the tube are visible and the condition of the tube can be checked up without difficulty.

The first four points which have been considered are in strong contrast to the conditions present in a pyrex glass tube filled with copper wire and heated by an outside furnace. In such a furnace the velocity of the gas through the tube is high because of the bulk of copper. The pyrex glass tubing is subjected to great thermal changes. It may be ballooned out by pressure of the gas or collapsed due to a vacuum which may be produced in the system. The oxygen in the nitrogen is heated to a sufficiently high degree to react with the copper, but copper oxide is readily decomposed into copper and oxygen at a temperature which is sometimes used in this type of furnace.

With the new type of furnace the volume flow can be raised to 500 cc a minute and the copper oxide does not form for a distance of more than 4 cm from the entrance end. Even after 24 hours, not more than 15 cm of the gauze will be tarnished with the oxide. The reduction of the copper oxide with hydrogen requires but a few minutes.

Continued use of the furnace brings about changes in the properties of the copper. It looses ductility and becomes highly reactive to oxygen. If air at laboratory temperature is passed through the tube, the copper becomes tarnished. When first prepared and at occasional intervals it is best to oxidize the copper with a rapid current of air through the tube while it is heated. This appears to burn off oil and other substances which cover the surface of the copper. The copper oxide formed is then reduced with hydrogen. This results in an enormous increase in the surface of the copper because of the cracked and seamed surface of the copper oxide. Reduction with hydrogen does not greatly change the physical appearance of this surface.

The removal of traces of oxygen from nitrogen in such a tube is complete. In addition the tube provides the solution of another important problem. It is possible to pass water vapor through the tube without the formation of even traces of hydrogen. Even if the water vapor is decomposed on the surface of the nichrome wire, the presence of the copper oxide completely removes all traces of the hydrogen so formed. All traces of hydrogen originally present in the nitrogen are removed as completely as is the oxygen.

In connection with studies of the oxidation reduction potentials of cysteine, an apparatus was prepared which consisted of eight electrode cells and two movable burettes, essentially similar to those described by Clark and coworkers.[4] Experience with such an apparatus over a course of several years has shown that deKhotinsky cement seals are frequently the source of contamination of the nitrogen with oxygen. Copper tubing, for the distribution of the nitrogen to the cells and burettes, also has been shown to be unsatisfactory. It therefore became necessary to modify the apparatus in order to make available a continuous source of nitrogen free from oxygen.

As it is desirable to see the contents of the electrode cells, the eight cells and the new type of copper gauze furnace which has been described were mounted on a framework which was counterbalanced and could be raised and lowered into the oil thermostat. Pyrex glass tubing connected the copper furnace to a distributor with eight pyrex stopcocks which terminated in pyrex tubing 8 mm in diameter. The eight delivery tubes from the distributing tubes fitted into eight mercury seal cups which were attached to the eight tubes that delivered the nitrogen to the electrode cells. A "Y" tube was placed in the distributing line and the nitrogen was connected to the two movable burettes by means of flexible copper tubing, 6 mm in diameter. Since copper tubing may be the source of contamination with oxygen, the nitrogen was passed through another small copper gauze purification furnace which was placed at the entrance of each burette.

[4] Hygienic Laboratory Bulletin No. 151, p. 35 (1928).

A diagram of this furnace is shown in Fig. 1. Continuous pyrex tubing connected this furnace with the movable burette.

In addition to these precautions for the removal of oxygen, the construction of the electrode cell was also modified. The tops of ten ounce nursing bottles were ground off on a steel plate with emery powder so that the top of the bottle was in one plane, parallel with the bottom, and presented a broad ground glass surface. Circular Bakelite caps, one cm in thickness, with flat-bottomed circular grooves which fitted the tops of the ten ounce bottles were held in place with a suitable frame which extended around two sides and across the bottom of the cell (Fig. 2). The bottle was

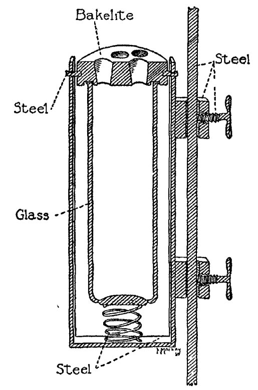

FIG. 2

securely held in the frame by a spring which pressed the cell against the Bakelite top. When vaseline was placed in the groove of the Bakelite top and the nitrogen outlet was closed, the vaseline seal between the ground glass top of the cell and the Bakelite top would withstand a pressure of 2 kilos. Through the Bakelite top, the electrode, the nitrogen inlet, the agar salt bridge and the nitrogen outlet were all arranged by means of tapering aluminum collars. The taper of the aluminum collar precisely fitted the taper in the hole through the Bakelite. The aluminum collars were fastened to the respective glass tubes with deKhotinsky seal and vaseline was used between the aluminum collar and the Bakelite top. The nitrogen outlet was also arranged to provide the inlet for addition of solutions from the movable burettes. This arrangement has two great advantages. First, it removes an opening in the Bakelite top, and, second, nitrogen continually passes around the tip of the burette and rapidly removes all traces of oxygen which may possibly have gained entrance to the outlet tube when the rubber stopper to this opening was removed. If the tip of the burette is placed in the nitrogen outlet and allowed to remain for one half minute or so before addition of the solution to the cell, the nitrogen will rapidly sweep out all traces of

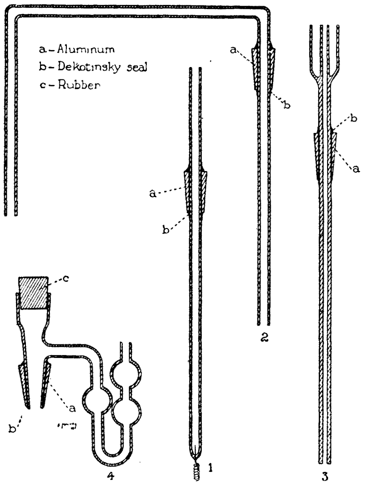

Fig. 3

oxygen. DeKhotinsky seals made as described are much more secure than if made without the collar of metal. Any other suitable material could be used in place of aluminum; this metal is easy to work on the lathe and has been found to be satisfactory in every way (Fig. 3).

Evidence that the copper furnace completely removes all traces of oxygen and that the construction of the electrode cells and movable burettes satisfactorily meets all requirements will not be included in this paper, but will be given in the papers dealing with the reaction of cobalt salts with cysteine, and the oxidation reduction potential of cysteine and glutathione.

E. C. Kendall

Mayo Foundation,
Rochester, Minn.

SPECIAL ARTICLES

THE CHEMICAL CHANGES THAT OCCUR DURING THE CURING OF TOBACCO LEAVES[1]

It has long been assumed that the metabolism of excised leaves follows a relatively normal course for quite appreciable periods of time, and that differences that arise between the chemical composition of such leaves and the intact leaf are due to the cutting off of the food supply from the stem and to the interference with the outlet for the products of metabolism; storage of these products therefore occurs. Subsequently, however, the changes become more patently katabolic; extensive dehydration of the tissue takes place if water is not supplied and this brings in its train a whole series of complex changes, many of which can be recognized as the results of the activity of well-known types of enzymes.

The curing of tobacco under commercial conditions affords an excellent opportunity for the study of these katabolic changes, since the soluble substances in the large thin leaf of this plant can be readily extracted by means of hot water and adequate quantities of leaves of uniform size and development are easily secured.

In order that definite comparisons might be established between leaf tissue which had reached different stages of the curing process five 50 kg lots of leaves (8th to 11th leaf) were picked from the plants the same day (August 1, 1929). One of these lots was immediately extracted with boiling water, the other four lots were strung on cords in the customary manner and suspended in a curing shed. The second lot was removed and extracted at the expiration of 12 days when all the leaves had become yellow, the third lot at the expiration of 18 days when the leaves had first become a uniform brown color, the fourth at the end of 51 days when the leaves were pronounced "fully cured," and the last lot was subsequently fermented along with the main crop of tobacco from the same field. Each lot was extracted with hot water in the same way. The leaves were dropped slowly into a large volume of boiling water to which sufficient sulphuric acid was added from time to time to maintain the reaction at or near pH 4.0; loss of nicotine was thereby avoided. After boiling until the mid-ribs were soft the leaves were pressed at the hydraulic press. The residues were then ground in a meat grinder and re-extracted with boiling water. This process was repeated once more. The three successive extracts were collected quantitatively, were filtered and concentrated *in vacuo* to 8 l. Analyses of extracts and extracted residues were then carried out, the methods for total, ammonia, amide and nitrate nitrogen employed being those recently developed in this laboratory,[2] amino nitrogen was determined by Van Slyke's method and the total solids, ash, crude fiber, soluble carbohydrate and ether soluble solids were determined by standard methods.

Inasmuch as each lot of leaves was initially of the same weight and the leaves were individually of the same size[3] and age, comparison between the different lots could be established by calculation of the absolute

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] H. B. Vickery and G. W. Pucher, *J. Biol. Chem.*, 83, 1 (1929); *Ind. Eng. Chem., Anal. Ed.*, 1, 121 (1929); G. W. Pucher, C. S. Leavenworth and H. B. Vickery, *Ind. Eng. Chem., Anal. Ed.*, 2, 191 (1930); H. B. Vickery and G. W. Pucher, *J. Biol. Chem.*, 90, 179 (1931).

[3] The uniformity of the material is evident from the fact that the 12-day lot contained 2,014 leaves and the 18-day lot contained 2,011.