The colony is removed from the surface of the agar by a platinum inoculating loop and mixed with several loopfuls of a M/750 phosphate buffer at a pH of 7.3, containing 0.04 per cent. phenol red in a depression slide. By means of the loop (or a smaller one, if several substrates are to be studied) droplets are removed from the depression to the flat surface of the slide. A loopful of distilled water is mixed with one droplet as a control, and a loopful of a 1.0 per cent. distilled water solution of a given carbohydrate is mixed with another droplet. The suspensions of cells prepared in this manner are then taken up in capillary tubes having an inner diameter of 0.35 mm and observed for change in color of the indicator. If the substrate is oxidized the solution will become acid, due either to the liberation of fixed acid or to an excess CO_2 production, while the control will remain unchanged, since the CO_2 production is too low to affect the buffer. In the case of a 2 mm colony of *Escherichia coli* treated in this manner, the change from orange-red to yellow occurred within fifteen minutes, with glucose as substrate, while the control underwent no change over a period of several days in the incubator.

The same method has been fully tested on a slightly larger scale, using capillary tubes of 1 to 2 mm inner diameter, with the washed cells of luminous bacteria (*Vibrio phosphorescens* and *Achromobacter fischeri*) as well as *Escherichia coli* and yeast. Thirty substrates, including pentoses, hexoses, heptoses, polysaccharides and two-, three- and six-carbon polyhydric alcohols, have been tested with each of the above species. The method works equally well in small test-tubes, 10×75 mm, which insure partial anaerobiosis by virtue of the fact that oxygen diffuses in sufficiently rapidly only for the cells in the uppermost layer of the suspension to remain aerobic. Washing the cells with the aid of the centrifuge is desirable in order to get the results of the "resting" metabolism, uncomplicated and unobscured by extensive products and processes of vigorous growth. Except with the most slowly fermented substrates, such as dulcitol, the results are obtained within a few minutes, or at most a few hours. The rate depends, of course, on the concentration of cells, the concentration of buffer, and the rate and products of decomposition of the substrate, but in general, satisfactory results are obtained when washed cells from a heavily inoculated 18-hour nutrient agar Petri plate are resuspended in 15 to 20 cc of M/150 buffer. The suspension is aerated by a stream of air for about fifteen minutes, and 1 cc portions diluted with an equal volume of substrate solution in small test-tubes.

This method has given results entirely consistent with those found in the case of the aerobic oxidation of substrates by the "resting" cells of luminous bacteria in Warburg respirometers, except for glycerine which is easily oxidized aerobically, but apparently not in a limited oxygen supply. While only the oxidations occurring at an initial pH of 7.3 have been studied, it should be possible, with appropriate indicators, to study different pH ranges.

Gas formation could be detected in the case of yeast by large bubbles in the capillaries, but not very well with *Escherichia coli*. With the latter, gas formation could be detected by means of very small glass tubes sealed at one end, and filled by a micro-pipette, and inverted in the small test-tube containing the suspension. In this case, however, gas was not formed from certain substrates apparently because acid accumulated so fast that the cells were precipitated in a few minutes. The accumulation of gas required incubation over night. No further change occurred after several days, and the controls never indicated any decided acid production or gave any indication of gas formation.

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SPECIAL ARTICLES

THE CULTURE OF WHOLE ORGANS

THE method to be described consists of the transplantation of an organ or of any part of the body into a sterile chamber, and of its artificial feeding with a nutrient fluid through the arteries. It is not in any way a substitute for the method of tissue culture. Its techniques, as well as its purposes, are quite different. As is well known, tissues and blood cells grow like bacteria in flasks containing appropriate media. The techniques for the cultivation of tissues are somewhat analogous to bacteriological techniques, although far more delicate. But it is through the employment of complex mechanical and surgical procedures that organs are enabled to live isolated from the body Tissue culture deals with cells as units of bodily structures; the new method, with cellular societies a organic wholes. Its ultimate purposes are the manufacture *in vitro* of the secretions of endocrine glands the isolation of the substances essential to the growth differentiation and functional activity of those glands the discovery of the laws of the association of organs the production *in vitro* and the treatment of organic and arterial diseases, etc.

The idea of maintaining alive a portion of the body in order to study its functions is not new. In 1812, the physiologist Le Gallois[1] wrote that, "if one could sub

stitute for the heart a kind of injection . . . of arterial blood, either natural or artificially made, . . . one would succeed easily in maintaining alive indefinitely any part of the body whatsoever." But Le Gallois did not attempt to supply organs with such artificial circulation. The first perfusion apparatus probably originated from Ludwig's laboratory. In 1866, de Cyon kept the heart of a frog beating for 48 hours.[2] Later, he demonstrated by a similar technique that a perfused liver still manufactures urea. Brown-Séquard observed the reestablishment of certain cerebral functions by circulating blood through the vessels of a head separated from its body.[3] The later history of the perfusion of organs is well known.[4] But the best and more recently constructed apparatus have never been capable of maintaining a gland in a condition of survival for more than a few hours. Even during this short time the organ was being rapidly invaded by bacteria. It is only recently that surgical and mechanical procedures became sufficiently perfected to allow organs to be cultivated *in vitro*. With the study of the transplantation of organs and their blood vessels,[5] techniques were developed for handling the arteries, washing the organ free from blood without injuring the cells, and preventing emboli. During the last war, antiseptic procedures that permit complete protection of tissues from bacteria in the course of surgical operations came into being in the laboratories supported by the Rockefeller Institute in Compiègne. But there was no apparatus capable of playing the rôle of heart and lungs and of keeping an organ free from infection indefinitely. In this laboratory, a long search has been made for the proper apparatus. An ingenious magnetic pump was constructed some years ago.[6] Later, a much simpler apparatus was designed,[7] which maintained an artificial circulation through a segment of artery for a month without infection. Still other kinds of apparatus, based on different principles, were built in the following years. Finally, in 1935, a model was developed that has for the first time permitted an entire organ to live outside of the body.[8] The purpose of the present article is merely to show how, after 123 years, the conception of Le Gallois has been realized.

The organs are removed from an animal that has just been killed by bleeding under anesthesia. Adult fowls or cats are generally used. An ovary, a suprarenal, or a thyroid gland is removed by a procedure similar to that employed in the transplantation in mass of organs.[9] That is, the organ is extirpated, together with its surrounding tissues, arteries, veins, nerves and lymph vessels. In the explantation of the ovary, for instance, the Fallopian tube, the ovary and a flap of peritoneum and connective tissue containing the ovarian artery are dissected as far as the aorta and removed. During the course of the operation, the abdominal cavity and the organ are constantly protected with gauze pads soaked in Dakin solution. In this manner, the operation can be performed successfully in a room that is not dust-proof.

The culture medium varies in quantity and composition. In order that the supply of glucose and bicarbonate may be sufficient for several days, the volume of medium must be about 2,000 times greater than that of the tissues. For instance, a cat's thyroid gland, varying in weight from 85 to 110 mgs, demands about 230 cc of nutrient fluid. The apparatus may be operated with from 200 to 900 cc of medium. This medium consists of blood serum or of more or less growth-activating solutions prepared by L. E. Baker. Such solutions contain protein split-products, hemin, cysteine, insulin, thyroxine, glutathione, vitamin A, ascorbic acid, blood serum, etc. A small amount of phenol red is indispensable as an indicator of the metabolic activity of the organ, as well as of the occurrence of bacterial infection. In some experiments, hemoglobin is added to the fluid. The gaseous medium consists of 40 per cent. oxygen, and from 3 to 4 per cent. carbon dioxide, the remaining part being nitrogen.

The organ is introduced into the culture chamber while being protected by a sheet of Cellophane, and the artery is connected with the cannula of the apparatus. Then the chamber is closed by a rubber stopper and sealed with a cellulose acetate cement. The apparatus maintains a sterile pulsating circulation through the organ for a length of time limited only by the condition of the organ and the perfusion fluid. The pulsation rate and both maximum and minimum pulsation pressures are adjustable. The perfusion fluid is kept well aerated and in contact with gas of controlled composition. Pulsation pressures are practically unaffected by changes in the rate of flow through the organ. Filming and evaporation are prevented. Both the organ and perfusion fluid may be observed at all times. The apparatus is kept in an incubator at a temperature of 37–38° C. The circulation is started about 1 hour after the death of the animal. The number of pulsations, in most of the experiments, has been about 60 per minute, the systolic pressure, 120

[1] C. J. J. Le Gallois, "Expériences sur le principe de la vie," Paris, 1812.

[2] E. De Cyon, *Arch. ges. Physiol.* (Pflüger's), 77: 215, 1899; *Compt. rend. Soc. Biol.*, 52: 372, 1900.

[3] E. Brown-Séquard, *Jour. Physiol. de l'Homme et des Animaux*, 1: 95, 353, 1858.

[4] A. E. Belt, H. P. Smith and G. H. Whipple, *Am. Jour. Physiol.*, 52: 101, 1920.

[5] A. Carrel, *Jour. Exp. Med.*, 10: 98, 1908.

[6] H. Rosenberger, SCIENCE, 71: 463, 1930.

[7] C. A. Lindbergh, SCIENCE, 73: 566, 1931.

[8] C. A. Lindbergh, *Jour. Exp. Med.*, 1935, in press.

[9] A. Carrel, *loc. cit.*

mm Hg., and the diastolic pressure, 60 mm Hg. Some fluid may leak and spurt from small blood vessels without markedly lowering the pressure. The progressive decrease of the pH of the medium is detected by comparing the color of the fluid feed tube of the apparatus with that of a set of standard tubes.

Twenty-six experiments have been performed since the last model of the apparatus was made. The organs were thyroid, ovary, suprarenal, spleen, heart and kidney. Some organs were transferred several times from apparatus to apparatus. Infection occurred twice only. This accident took place in spleens that obviously were contaminated before removal from the abdomen. Thyroid glands were kept more than 20 days with pulsating arteries and active circulation. They could have remained in the apparatus much longer. No emboli and no important hemorrhages were observed. The lowering of the pH of the medium occurred more or less rapidly according to its composition. In diluted blood serum, cat's thyroid glands consumed only about 7 mg of glucose per 24 hours. When they were perfused with a growth-promoting fluid, the glucose consumption increased more than three times.

Changes in form and volume took place in the organs from day to day. Thyroid glands perfused with diluted serum were observed to decrease in size progressively. On the contrary, ovaries or thyroids perfused with a growth-promoting medium modified their form and grew rapidly. In 5 days, the weight of an ovary increased from about 90 to 284 mg. Simultaneously, three corpora lutea developed. At the end of the experiments, that is, after from 5 to 21 days, small fragments taken from the organs and cultivated in flasks engendered active colonies of epithelial cells and fibroblasts. The epithelium of an adult thyroid was found to have recuperated its fetal activity after being perfused for a few days with a growth-activating fluid. The sections showed an almost normal structure of fragments of the thyroid, even when the gland had been roughly treated and perfused with dilute serum for a long period of time, when the pH had been lowered to 7.2 for several days or when the circulation had markedly decreased. The presence of colloid was observed in some follicles. If perfused with a growth-promoting fluid, the structure of the gland was altered. The colloid substance disappeared. The epithelial cells proliferated within and also outside the follicles. In ovaries treated in the same manner, growth was also accompanied by disorganization. There was a luxuriant and disordered proliferation of the stroma and of the epithelial cells. Obviously, a large amount of new tissue had been manufactured by the organ. The fluids that had circulated through the apparatus were tested for modifications induced by the organs. In every case, the thyroid glands were found to have set free substances that stimulated the proliferation of leucocytes more or less markedly.

The structural and functional changes undergone by the organs during their life *in vitro* are complex. They obviously depend on the chemical composition and the physicochemical and physical conditions of the perfusing fluid. They will be discussed in subsequent articles. From the present experiments, it must merely be concluded that an entire organ, such as an ovary, has been maintained alive *in vitro*. It not only survived, but increased in size and in weight. This increase was due to the appearance of new cells and tissues. It is, therefore, probable that this method provides important uses in physiological chemistry, physiology and pathology.

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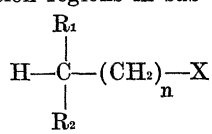
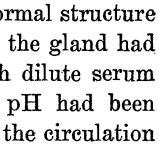
ANALYSIS OF ROTATORY DISPERSION OF CHEMICALLY ANALOGOUS SUBSTANCES

IN view of an article by W. Kuhn and H. Biller announced to appear in *Z. phys. Chem.*, "Drehungsbeitrag chromophorer Gruppen bei analog gebauten Verbindungen," we wish to report briefly the results of similar work which has been in progress in our laboratory in course of several years.

In Table I are given the directions of the partial rotations of the individual absorption regions in substances of the general type of



(R_1 and R_2 being alkyl groups, X a functional group, , N_3, SH, Halogen; n = 0 or an integer). All substances in which X = N_3, NH_2 and  are configurationally correlated; when X = SH or Halogen, all substances in which n = an integer are likewise configurationally correlated by direct chemical methods and those in which n = 0 are not so correlated.

The most significant facts appearing from the data given in Table I are:

(1) The band of group X with the lowest frequency in some instances furnishes the principal partial rotation determining the direction of the optical rotation of the substance in the visible.

In other substances the partial contribution of the absorption region of low frequency depends upon the