have been used preceding the employment of either or both dioxane and tertiary butyl alcohol and many surprising results have been noted. Much of the obloquy heaped upon certain killing and fixing fluids appears to be wholly gratuitous and should be laid instead against absolute ethyl alcohol, xylol, chloroform, benzene and similar fluids employed for "clearing" purposes.

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IMMUNOLOGICAL REACTIONS AND VISCOSITY

IN 1923, while at the Rockefeller Institute in New York, we devised a microviscosimeter of high sensitivity[1] which enabled us to follow continuously the changes of viscosity occurring in a sample of solution, as a function of time, temperature or as a consequence of a reaction. It occurred to us that this instrument was well adapted to the study of immunological reactions—immune serum plus antigen—and that it might be interesting to find out whether flocculation and precipitation were not preceded or accompanied by some variations in the viscosity of the mixture. We found that such was the case, and that the addition of one drop of specific antigen to 1 cc of immune serum determined a considerable but *momentary* increase in the viscosity of the mixture. The amplitude of the phenomenon reached, in certain cases, 300 per cent. of the original viscosity. In a few minutes, the viscosity comes back almost to its former value. This reaction is strictly specific. Although we did not publish it at the time, we described the phenomenon at the Pasteur Exhibition in Strasbourg, 1923. In 1933 we reported it before the Congress of Immunology (Rome, Convegno Volta) and in 1934 in the Ergebnisse der Hygiene.[2]

Late in 1934, we took it up again with Miss V. Hamon and applied it to the study of the diphtheria toxin-antitoxin reaction which had been shown by Ramon[3] to yield quantitative results, making it possible to titrate the antitoxic activity of a serum *in vitro*. We found that, under the conditions specified by Ramon, who kindly supplied us with toxin and antitoxin, the same increase in viscosity could be observed, and that, for a given ratio of concentrations of the two substances, a quicker and more important phenomenon took place. It was thus possible to titrate the antitoxin content of a serum by a new and entirely different method, which may prove to be more accurate than the flocculation method.

The same method was then applied to precipitins by our associate, Dr. M. Coppo,[4] in our laboratory. It was found that the increase in viscosity in this case was proportional to the concentration in precipitins of the serum (rabbits injected with horse serum). The reaction is extremely sharp. A few interesting results were obtained: The higher the titer in antibodies of the serum, the smaller the quantity of antigen required to obtain the maximal viscosity. For instance, if 1.5 cc of immune serum precipitating at 1/1000 is mixed with antigen, the maximum of viscosity will be attained when .7 cc of antigen is added. If the serum precipitates at a dilution of 1/15,000 it will only be necessary to add .01 cc of antigen to 1.5 cc of serum in order to obtain the maximal viscosity. And in that case the absolute value of the maximum will be much higher than in the first case. In addition, it was observed that the rapidity at which the viscosity increases, then decreases, is a function of the concentration in antibody of the serum. It was also found that, for a certain and different ratio antigen antibody, corresponding to an excess of antigen, a minimum of viscosity occurred, the amplitude of which was much smaller than that of the maximum.

Similar maxima and minima, when the serum is mixed with tannin in definite proportions, were observed in our laboratory by another of our associates, Dr. F. Seelich. A detailed account of the experiments will appear shortly.

This new reaction seems, therefore, to raise questions of interest, not only from the practical, but also from the theoretical standpoint.

LECOMTE DU NOÜY

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF THE PHOTOELECTRIC CELL IN PHYSIOLOGICAL EXPERIMENTS

THE photoelectric cell, as ordinarily used, measures the density of light to which its total active surface is exposed.

In searching for a method of conveniently obtaining a record of the motion of a beam of light, it occurred to us that the photoelectric cell could be made to record a continuous curve of the arc traversed by the beam, rather than simply the fact that it impinged or did not upon the surface of the cell. It was found that this could be done by interposing between the

[1] Lecomte du Noüy, *Jour. Gen. Physiol.*, 5: 329, 1923.
[2] Lecomte du Noüy, *Ergebn. Hygiene, Bakter., Immunit., u. Exp. Therap.*, 15: 304–334, 1934.
[3] G. Ramon, *C. R. Soc. Biol.*, 86: 661 and 711, 1922.
[4] M. Coppo, *C. R. Soc. Biol.*, 118: 1307, 1935; 119: 165, 1935.

beam of light and the cell a neutral wedge of glass, possessing a suitable, graded range of total light transmission from its thin to its thick end. Such a wedge was obtained through the cooperation of Dr. A. T. Williams, head of the Photometric Division of the Weston Electrical Instrument Corporation, who aided us in determining the proper values, and of Dr. H. P. Gage, chief of the Optical Division of the Corning Glass Works.

The light, sweeping across the surface of the wedge, reaches the cell with an intensity determined at each point by the graded thickness of the wedge. Thus, corresponding continuously with this modulated stimulus there is generated a progressively varying current, which can be led off to a reflecting galvanometer. A second beam of light, reflected from the galvanometer mirror to a camera, will trace the desired curve.

As the cell can be placed where it would ordinarily be impractical to place a camera, this constituted just the advantage which was desired in securing, simultaneously with the action potential and recorded on the same paper, a mechanical record of the contraction of a muscular organ situated in a somewhat inaccessible portion of the abdominal cavity.

Finding any modification of the usual lever or tambour recorder unsatisfactory, both because of the difficulty of attachment to the delicate, thin muscle without doing damage, and because of the lightness of the moving system demanded by the frailty of the muscle, optical registration was resorted to as meeting these requirements, and, moreover, possessing the necessary short period essential to an accurate tracing. Fig. 1 illustrates the set-up. The small supporting yoke, Y, made of heavy wire, has stretched between its extremities a phosphor-bronze or gold wire (such as used for galvanometer coil suspensions) to which is attached at the center, with rubber cement, a small fragment of a silvered cover glass. By means of the yoke this mirror is placed over the muscle and, after gentle torsion of the suspension wire, is allowed to rest almost flat upon the surface. A contraction wave, passing over the muscle, will rotate the mirror, causing a vertical excursion of the beam of light, MW. A system of mirrors necessary to bring the reflected beam from the preparation to the camera, and to convert the vertical motion into a horizontal one, adapted to the horizontal slit of the camera, K, proved to be tedious, necessitating readjustment with each experiment. It also resulted in a beam of light so long that the ensuing amplification made it difficult to confine the excursion to the camera slit, as well as requiring a very powerful source of illumination. The photoelectric cell as here described was, therefore, used. The cell, P, with the wedge, W, attached is placed about 6 inches from the mirror, M, which receives its light from a 50 candle power automobile bulb, L, through a condensing lens, C, also about 6 inches away. The galvanometer has its own source of light, L_2. The

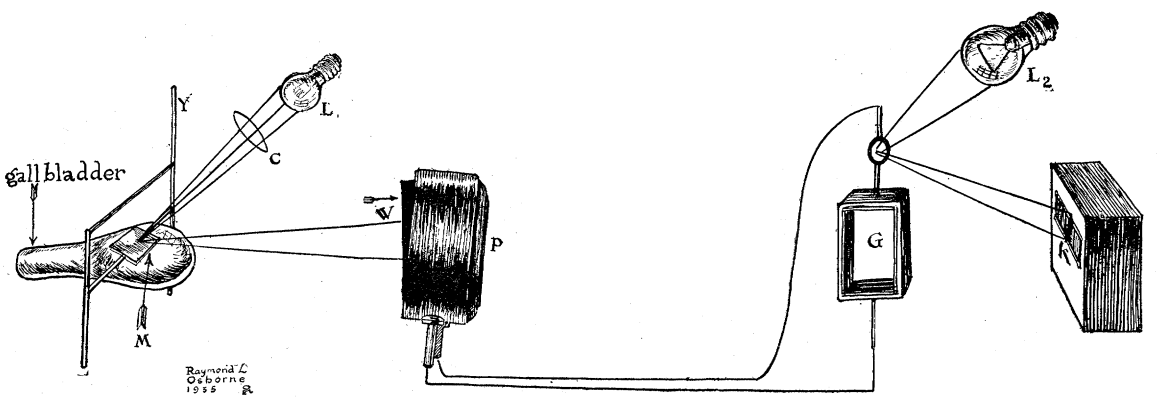

FIG. 1

cell used was the Weston Photronic. Fig. 2 shows a test curve made by manually rotating the mirror, M.

We feel that this arrangement renders optical registration for physiologic experiments a more flexible technique. The photoelectric cell, with interposed

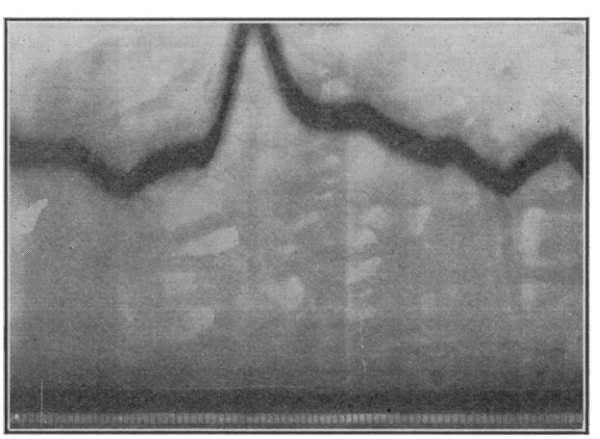

FIG. 2

wedge, can also be used to record the position or the motion of an opaque object in an illuminated field.

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SODIUM AMYTAL FOR ANESTHESIA IN STUDIES ON MITOCHONDRIA[1]

DURING the course of some studies on the mitochondria of the hepatic cell it became necessary to employ an anesthetic. As chloroform and ether are known to modify these cells[2] sodium amytal was tried. Eleven rabbits (New Zealand Whites), selected according to weight to five and three fourths to six pounds, received three grains of sodium amytal intramuscularly. Complete anesthesia usually occurs in fifteen minutes. After one half hour the abdominal cavity was opened and a portion of the liver excised and fixed. One other rabbit required six grains of amytal for anesthesia. The mitochondria of all twelve of the livers were compared to four controls killed at the same time and fourteen used in earlier work. There was no difference between control and anesthetized material. Accordingly, it is concluded that sodium amytal is a safe anesthetic for use in experimental work on the mitochondria of the liver, and when one considers the alteration of the osmotic qualities of the blood effected by ether it seems probable that amytal would be less likely to introduce error in investigations of mitochondria in any tissue.

In addition to the quality of not affecting the morphology of the mitochondria a single injection of amytal has the advantage of keeping the animal anesthetized for hours without harmful results, enabling the performance of prolonged surgical procedures without further dosage.

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A MODIFIED CULTURE JAR

AN experiment was conducted, in which 500 cc capacity inverted bell glasses were used as culture receptacles for soybean, Black Wilson variety, grown in Shive's[1] three salt R2S1 solution. Five hundred cc of fresh solution were supplied to each culture daily through a thistle tube.[2] Minute but equal quantities of iron[3,4] in the form of ferric citrate were added to the culture solutions of each series.

In series A, excess solution was siphoned from near the upper surface of the solution in the jar. In series B, excess solution was carried away from the bottom of the jar by means of a piece of bent glass tubing. Fig. 1 shows the arrangement of the jars in the two series.

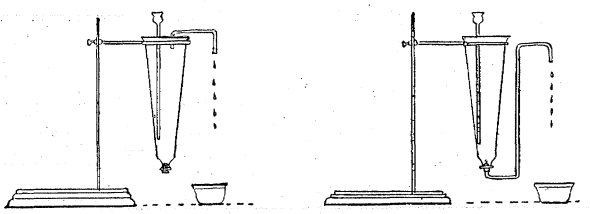

FIG. 1

The main difference in the technique employed in series A and series B was the method of draining away the excess solution. By draining it from the bottom of the jar, as in series B, the solution remained perfectly clear throughout the growth period of the plants, while the solution in series A became definitely clouded. The highest pH value recorded in the solution from series B was 5.4, while the highest pH value recorded in the solution from series A was 5.8. The total green weight of tops and roots from series A, per culture, was 4 grams, while the total green weight of tops and roots from series B, per culture, was 5.2 grams.

The results of this experiment show that more nearly uniform conditions are maintained in the culture solutions when the excess is drained from the bottom of the culture jar rather than being siphoned from near the top of the jar. Better plant growth also occurred when the solution was drained away from the bottom of the jar. This is due, probably, to the elimination of precipitate and plant wastes that could collect in type A jar, largely near the bottom, but were carried away from type B jar.

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[1] This work was assisted by the Grants-in-Aid Committee of the National Research Council.

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