uronic acids[3] has made possible the synthesis not only of conjugated uronides but of aldobionic acids as well.

The synthesis of the β-heptacetyl methyl ester of the aldobionic acid, glucose-6-β-glucuronide, has been accomplished by condensing 1, 2, 3, 4-tetracetyl-β-glucose[4] with 1-bromo-triacetyl-glucuronic acid methyl ester in chloroform solution in the presence of silver oxide. The derivative is obtained in yields of 30 per cent. as a crystalline substance melting at 198–199° (uncorrected) and having a specific rotation in chloroform of $[\alpha]_D^{23} = -11.0°$ (C = one per cent.). (Found: C, 48.40; H, 5.48; $OCH_3$, 4.64; $COCH_3$, 46.1).

The β-heptacetyl methyl ester was converted into the α isomer by the action of zinc chloride in acetic anhydride solution. The α-heptacetyl methyl ester melts at 201–202° (uncorrected) and has a specific rotation in chloroform of $[\alpha]_D^{23} = +48.4°$ (C = 0.7 per cent.). (Found: C, 48.78; H, 5.58; $OCH_3$, 4.62; $COCH_3$, 45.4). The difference in molecular rotation of the α and β isomers is equal to 39,500 degrees, a value which is in good agreement with the known differences in molecular rotation of the α and β sugar acetates.

Since the aldobionic acid, glucose-6-β-glucuronide, can be regarded as the uronic acid derived from gentiobiose, the synthetic product described above may be designated as the heptacetyl methyl ester of gentiobiuronic acid. The latter substance is isomeric with the heptacetyl methyl ester of the aldobionic acid derived from the specific polysaccharide of Type III Pneumococcus. The application of analogous synthetic procedures should eventually make possible the preparation in the laboratory of aldobionic acids identical with those elaborated by encapsulated microorganisms in the production of their type-specific polysaccharides.

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[3] W. F. Goebel and F. H. Babers, *Jour. Biol. Chem.*, 111: 347, 1935; S. Morell, L. Baur and K. P. Link, *Jour. Biol. Chem.*, 110: 719, 1935.

[4] B. Helferich and W. Klein, *Ann. Chem.*, 450: 219, 1926.

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## EXOGASTRULATION IN AMPHIBIA AFTER X-RAY EXPOSURE

THE following observations were made in the course of our studies upon regeneration and development as affected by irradiation. These investigations have been in progress for some years assisted by the Committee on Radiation of the National Research Council. In view of their general interest and because we have found no record of exogastrulation produced by x-rays, either in Amphibia or other groups, this note seems justifiable before histological study of the extensive series in hand and before completion of the further experiments now being conducted.

The exogastrulae, which are similar to those described by Holtfreter,[1] have been obtained by exposing blastulae to 1000 r, no filter, and allowing them to develop in tap water. Almost 100 per cent. exogastrulation has been observed after 1000 r, with about 50 per cent. exogastrulation following 500 r. Four series of Amblystoma, two of Rana and one of Bufo have given the same results. These exogastrulae live for only a few days and do not undergo extreme constriction at the blastopore region, as described by Holtfreter. Further experiments with reduced exposures are under way, and it is hoped that viable exogastrulae can be produced.

Control series in tap water included no exogastrulae. Other controls were placed inside the x-ray chamber under lead plates to test the effects of high concentrations of ozone generated by the x-ray machine. No abnormalities have been noted in the subsequent stages of blastulae thus exposed to ozone but protected from x-rays.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A PHOTOKYMOGRAPHIC METHOD WITH CONTINUOUS CATHODE RAY OSCILLOGRAMS[1]

THE method presented was developed during studies of action potentials from the nervous system. It gives photokymographic records of the cathode ray oscillogram, black, and other signals, white, on a gray background with coordinates of time and amplitude.

These have been obtained (4/10 actual size) on bromide paper moving vertically in a recording camera, fitted with a photographic lens of large aperture (F/1.2–5 cm focal length), set (11.5 cm) in front of the screen of the cathode ray oscillograph. On this the fluorescent spot moves horizontally in response to potentials impressed on the corresponding plates.

In the same focal plane tangent to the screen, just below the spot, is mounted a flat, horizontal strip of white, unglazed paper, illuminated by a washlight to

[1] J. Holtfreter, *Biol. Zentralblatt*, 53: 404–431, 1933.

give the gray background and ruled vertically every centimeter to give the amplitude scale. To illuminate this strip, light from a small intense source is focused to a point where it is periodically interrupted by a timing device; then its divergence is reduced by a lens, and the beam reflected by a plane mirror through a cylindrical lens (5 inches long, 4 inches focal length), thus producing a bright band on the upper edge of the strip.

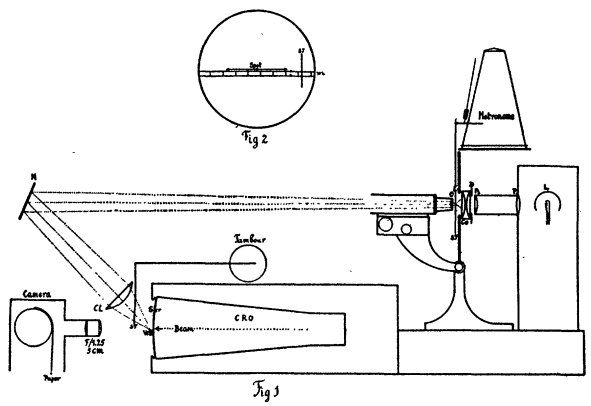


FIG. 1. Diagram of apparatus in side view. CRO = cathode ray oscillograph, Scr = its screen, L = light source, $P_1$ and $P_2$ = projecting lenses, D = diaphragm, Co = Abbe condenser of microscope, ST = time marking arm from metronome, O = low power objective of microscope, M = mirror, CL = cylindrical lens, WL = focal plane of washlight on horizontal strip across Scr, F/1.25, 5 cm = photographic lens of paper-camera. ST = stylet of tambour. FIG. 2. Diagram of front view of fluorescent screen with spot traveling horizontally across screen. WL = horizontal strip illuminated by washlight. ST = stylet of signal marker.

To record stimulus, response and other variables electromagnets, tambours or other devices interrupt the washlight by their vertical stylets, which travel horizontally between the cylindrical lens and the illuminated strip.

As the screen and the strip of paper are photographed simultaneously to produce the records described, the spot, being just above the strip, is recorded, at any speed of paper, a small constant distance (about .55 mm) ahead of the corresponding shadow record.

In practice a small projection lantern has proved a satisfactory source of washlight for records of speeds up to 4 inches per second. Its collimated beam is focused by the condenser of a microscope, in horizontal position, to a point between its stage and low power objective, which reduces the divergence of the beam. The intensity of the light is controlled by the diaphragm of the condenser, and inequalities in illumination of the strip of paper are reduced by a cardboard shield, cut to admit less light in the center and mounted against the cylindrical lens. In order to avoid possible electrical interference from synchronous motors used to drive tooth-wheel interrupters, the time record was obtained from a metronome, with a descending arm so placed that at the center of the swing it interrupts the beam at its focal point between the stage and objective of the microscope.

When necessary, simultaneous tracings (in black) from instruments with mirrors on moving elements can be superimposed upon these records by focusing the beam from the mirror to a spot, which moves horizontally on the strip.

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## PREPARATION OF TRANSPARENT SPECIMENS OF LEAVES, WORMS, BEES, BUTTERFLIES, ETC.

DURING the course of investigation of formic acid on plant and animal material[1] it was noted that this acid dissolves most plant pigments, starches, sugars, gums, dextrines, proteins, etc., but does not dissolve such framework material as cellulose, lignin or the chitin of animal tissues. These observations have been utilized recently for the preparation of transparent sections of vegetables, leaves, grasshoppers, worms, butterflies, etc. These specimens, in addition to being attractive in their transparent forms, which look somewhat like Cellophane wrappers covering the frames of grasshoppers, bees and worms, make it possible to study their structures without the interference of opaque substances.

### METHOD OF PROCEDURE

The usual procedure has been to place the object or material in 95 per cent. ethyl or methyl alcohol, which dehydrates the tissues to a considerable extent and dissolves tannins, sugars, etc., and also prevents distortion through extensive swelling of tissues when formic acid is added. The samples are left in the alcohol for storage, 48 hours or more, until it is convenient to proceed with the preparation of transparent specimens. The alcohol solution is then decanted off and replaced with a 90 per cent. (1.2 sp gr) formic acid. This acid will dissolve about all that the alcohol will and much more; in fact, nearly the only parts about these specimens that do not dissolve are fat, carotin, lignin, cellulose and the chitinous material making up the framework of bugs, grasshoppers, etc.

The time the specimen needs to remain in the formic acid depends somewhat on the material. A week was necessary to dissolve all soluble material in some cases, but no harm seems to result when it is left longer, and in cases hard to clear the liquid may be drained off in a few days and replaced with fresh formic acid. In the case of grasshoppers, bees, etc., which are

[1] R. H. Carr, SCIENCE, 69: 1789, 1929.