IN THE LABORATORY

An Interval-Timing, Automatically Resetting Switch to Operate After Any Given Number of Counts

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Two commercially available pieces of apparatus were inserted into a circuit that automatically rewinds a camera every time a given number of frames is exposed, thus preventing the tension of the camera's spring-drive mechanism from becoming so low as to result in erratic performance of the shutter. The frames are counted by causing the solenoid operating the single-frame plunger on the camera to close a switch at each stroke, thereby actuating an electrically operated counter[1]. The counter is modified[2] by removing all but the first two wheels and sliding onto its shaft a brass wheel arranged to turn in unison with the second digit wheel. Two electrical contacts are made to the circumference of the brass wheel. One is a permanently closed wiping contact, and the other is interrupted by insulating segments so spaced that contact is made every 180°, or twice in each revolution of the second digit wheel, *i.e.* once every 50 counts.

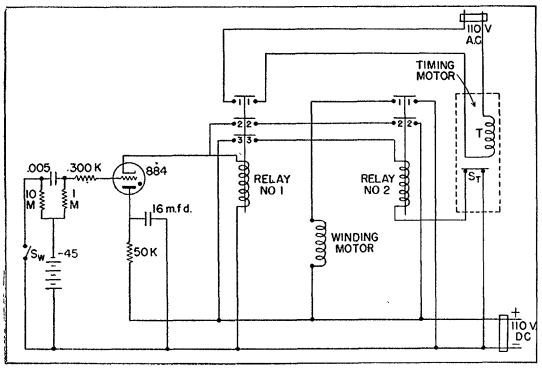

FIG. 1

On every 50th count, therefore, a second switching circuit is closed (at the point shown as S_w, Fig. 1). This simultaneously energizes the camera winding motor and a timing motor (synchronous)[3] set to cut both itself and the winding motor off after a predetermined interval (15 seconds in this case) sufficient to rewind the camera mechanism enough to restore the original spring tension in the camera drive. The synchronous timing motor has a magnetic clutch bringing the gear train of the timer into place when the motor is energized. When the clutch is demagnetized upon opening the motor circuit, a spring disengages the gear train, allowing another spring to reset the time-delay mechanism. To make the timing motor circuit independent of the duration of closure of the counter contact, which would vary with exposure rate and would remain closed if the counter were stopped on the 50th or 100th frame, the circuit from the counter is arranged so that, when closed, it produces only a short triggering pulse firing a thyratron. The output of the thyratron closes relay #1, and relay #2 is closed through contact 3 of relay #1, switch S_t in the timing motor being normally closed. Closure of contacts 2 on both relays completes a holding circuit to keep the relays closed after the pulse from the thyratron has ended. Contact 1, relay #1, starts the a-c timing motor. Contact 1, relay #2, starts the camera winding motor. After 15 seconds the timing motor opens switch S_t, which breaks the holding circuit and allows both relays to open, removing the voltages from timing and winding motors. The timing motor then resets itself, and conditions are returned to the original state.

The application described prevents the spring drive on our camera from running down enough to produce erratic performance of the shutter, which has to be maintained in synchronization with recurring single sweeps of the cathode-ray beam being photographed. Other related timing problems could be solved in this fashion by suitable small modifications of the counter and the choice of a timing motor of appropriate range.

[1] Manufactured by Gorrell & Gorrell, New Jersey.

[2] We wish to thank Gracient Eidt, school machinist, for his cooperation in constructing the modified counter.

[3] Manufactured by Haydon Manufacturing Company, Connecticut.

Simplified Techniques for Inoculating Chick Embryos and a Means of Avoiding Egg White in Vaccines[1]

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Most of the current techniques for inoculating chick embryos, as, for instance, those outlined in the report by Beveridge and Burnet (*1*), involve the use of a dental drill, an instrument seldom available except in specially equipped laboratories. The techniques described below render the drill unnecessary and also have the advantage of avoiding nonspecific lesions which drilling often causes on the chorioallantois.

Chorioallantoic inoculation. The eggs are candled, and the margin of the air space is marked on the shell in pencil on the side where the chorioallantois is best developed. The shell around this area is disinfected with 1/100 Zephiran or other suitable disinfectant. Using a pair of round, pointed forceps, a hole is made in the shell over the air space, a few millimeters from its margin. The shell is jabbed with the points of the

[1] Work carried out while the author was working under a grant by the Australian National Health and Medical Research Council as a guest worker at the Pasteur Institute.

forceps until it and the attached membrane are breached, and then the hole is carefully enlarged until it is roughly 1 cm. long and 3–4 mm. wide, running parallel with the margin of the air space. Next, the shell without the attached shell membrane is broken away for 3–4 mm. beyond the margin of the air space—that is, over the chorioallantois. In doing this, care must be taken to lift the fragments of shell slowly outwards with the forceps and not to allow them to hinge back and pierce the chorioallantois. The exposed outer layer of shell membrane is then torn away, and the inner layer of shell membrane is found to have a fold about 1 mm. wide marking the margin of the air space. A fine pair of forceps is used to tear the shell membrane along this fold. Up to this stage of the operation the egg is held in the left hand in an approximately horizontal position; it is now tilted with the air space downward. With 11-day embryos the chorioallantois usually falls readily, giving an artificial air space in the same position as with the standard Burnet technique. With 12-day embryos it is usually necessary to tap the egg with the fingers to commence the separation of the chorioallantois from the shell membrane. When the separation commences, the egg is returned to a horizontal position. The inoculum is placed on the chorioallantois with a Pasteur pipette, the hole in the shell sealed with cellophane adhesive tape, and the egg incubated in a horizontal position.

Amniotic inoculation. After preliminary incubation with the air-space end uppermost, in most of the eggs the embryo is found close to the margin of the air space, which is essential with this technique. The eggs are candled and the hole commenced as for chorioallantois inoculation, but it is not extended beyond the margin of the air space. A sharp, pointed pair of forceps is thrust through shell membrane and chorioallantois, close to the margin of the air space, and the hole thus made extended by blunt dissection to about 1 cm. As the air enters, the embryo enclosed in the amnion is presented at the hole. It may be necessary to remove some of the shell membrane adhering to the chorioallantois. Sometimes an air bubble blocks the hole; this may be disposed of by touching it with a needle heated in the flame. The amnion is grasped with forceps and inoculated with a Pasteur pipette drawn to a fine point and beveled, or with a syringe and needle. The egg is sealed with cellophane adhesive tape and returned to an upright position for incubation.

Allantoic and yolk sac inoculations. The current technique in many laboratories is to pierce the shell with a needle, and it is mentioned here merely to complete the series of techniques not requiring a dental drill. The hole is made in the shell with a surgical needle mounted by driving it through a rubber bung, after which the standard techniques are followed.

Position of the egg during incubation. Although it is well known among embryologists that the position of the egg during incubation determines the position of the embryo within the egg, this fact does not appear to be generally known by virus workers.

After 4 days incubation the embryo often moves freely within the shell, rising immediately to the uppermost point as the egg is rotated. Between the 5th and 9th days movement is slower: if an egg lying horizontal is rotated 90°, the embryo and membranes rise to the top after several hours. Between the 10th and 12th days the position of the membranes becomes fixed, but the position of the embryo may still change to some extent if the egg is turned.

If eggs incubated horizontally for 6–9 days are turned 180° so that the embryo and membranes are on the lower side, about 25 per cent die overnight. On the other hand, among eggs that are not turned at all during incubation up to the 12th day I have observed very few deaths—no more than occurred in eggs turned slightly once or twice a day.

If eggs have been incubated in the horizontal position, it is often not easy to harvest the allantoic fluid because, on reflecting the membrane under the air space, one encounters yolk sac and egg white as well as the allantoic cavity. If an egg has been incubated with the air space down, it is impossible to harvest either allantoic fluid or the yolk sac via the air space, because under the membrane in the air space there is only egg white. The embryo will be located in the "sharp" end of the egg, and the egg white is always in the lowermost point.

When cultivating influenza virus for vaccine production by red cell concentration technique, it is especially desirable to have the embryo centered under the air space. Furthermore, unless it is in this position, inoculation via a hole in the air-space end of the egg will often fail to infect the allantoic cavity.

It is of considerable importance that vaccines produced from the developing egg should be free of egg white; therefore, for the production of vaccines from allantoic fluid, yolk sac, or embryos, the eggs should be incubated before and after inoculation in a nearly vertical position with the air space upwards. They may be inclined 30°–40° from the vertical and turned by tipping back and forth once or twice a day. The only advantage in turning in this way is that it results in a more symmetrical development of the chorioallantois and allantoic cavity. On the other hand, for inoculation on the chorioallantois it is somewhat of an advantage to have a large area of chorioallantois on one side of the egg, so the eggs are better not turned before this type of inoculation. For intracerebral inoculation or amniotic inoculation by the standard Burnet technique it is better to incubate eggs in a horizontal position before inoculation.

Reference

1. Beveridge, W. I. B., and Burnet, F. M. *Med. Res. Coun., Lond., Spec. Rep. Ser. No. 256*, 1946.

Use of Trisodium Phosphate With Herbarium Material and Microfossils in Peat

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A brief article recommending the use of dilute aqueous solutions of trisodium phosphate for reclaiming dried zoological specimens (*1*) suggested the possibility of using the same technique for restoring herbarium specimens to a condition for sectioning or clearing. In one test, for example, two leaves and a twig of dried *Viburnum acerifolium* L. were placed in 25 cc. of the solution and kept in an oven at 60° C. for 2 hours. The leaves assumed the external appearance of fresh material (except for a slightly less brilliant green color) and approximately the natural flexibility, toughness, and turgidity of the fresh state. Free-hand sections of the leaves showed the par-