

for one acetyl group. Repeated observations over a period of two months always gave identical results for each rabbit.

By substituting monoacetylmorphine for the diacetylmorphine it was found that the animals that were able to remove both acetyls from heroin were able to hydrolyze the 6-carbon acetyl in monoacetylmorphine. Sera from the other three rabbits did not liberate acetic acid from the monoacetyl compound. Therefore, all the rabbits were able to remove the 3-carbon acetyl group, but the sera of only three of the animals hydrolyzed both acetyl groups present in diacetylmorphine.

Further work using diacetyldihydromorphine as the substrate showed that all six rabbits were able to remove acetic acid from this compound, equivalent to approximately 100 per cent. of the theoretical for one acetyl group. Again, however, there was a distinct separation of the rabbits into two groups of three animals on the basis of the rate at which hydrolysis took place. The sera from the three rabbits that were unable to deacetylate monoacetylmorphine hydrolyzed the dihydrodiacetylmorphine at a much slower rate.

At this point it was predicted that the diacetyldihydromorphine was hydrolyzed at the 3-carbon, and subsequent determinations with monoacetyldihydromorphine as substrate proved this to be true, since none of the animals were able to deacetylate this compound.

Preliminary experiments have shown that human blood serum is able to deacetylate heroin, but at a distinctly slower rate than any of the sera from the rabbits so far investigated.

Physostigmine inhibits the activity of the enzyme

responsible for the deacetylation of the acetylated morphine derivatives. This indicates the possibility that the enzyme might be choline esterase. However, all six rabbit sera have almost identical capacity for hydrolyzing acetylcholine. Also, the human sera so far investigated have much higher concentration of choline esterase than the rabbit sera and at the same time a lesser capacity for the hydrolysis of heroin.

From these results it seems probable that, in the rabbit at least, the difference in potency of heroin and morphine might be fundamentally due to physical factors such as solubility rather than chemical structure, since it appears likely that the animal, in the final analysis, is reacting to morphine, whichever of the two alkaloids is injected. The same applies to monoacetylmorphine.

It would be of considerable interest to determine whether the esterase attacking the acetylated morphines is present in the tissues, especially the central nervous system and to extend the investigation to include other species of animals. It is also possible that certain other morphine derivatives, after entering the body, are converted into morphine. If so, this would be of considerable aid in clarifying some of the similarities and differences in physiological activity that have been found among the chemical compounds related to morphine. The investigation of these possibilities and others not so obvious is now planned.

This is a preliminary report and will be published in detail elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A TECHNIQUE FOR THE INTRAVENOUS INOCULATION OF CHICK EMBRYOS

In the course of experimentation on the growth of various viruses in chick embryos, the need of a simple technique for the inoculation of embryos directly into the blood stream became very evident.

The method of Goodpasture, *et al.*,¹ consisting in removal of that portion of the shell over a vein or the air sac, application of mineral oil to the membrane to render it transparent and then picking up the veins for injection and subsequent searing of these veins, was tried. While some success by this method may be noted, it has several disadvantages, notably difficulty encountered in injection due to the mobility of the vein during inoculation.

Secondly, there was considerable hemorrhage on withdrawal of the needle even after cauterization of the vein at two points before withdrawing the needle.

A third difficulty is that the removal of the shell cap over the air sac exposes a large area and subsequent maintenance of sterile conditions is difficult even after sealing with Scotch tape.

Accordingly, the following procedure was developed at the laboratories of the Pathological Division of the Bureau of Animal Industry.

Ten- to eleven-day-old embryos were found to be the youngest which could be easily injected routinely. The eggs are candled to locate a vein of the terminal sinus which is fairly straight and which lies embedded in the chorio-allantoic membrane.

This section of vein is then marked on the shell for about 1-1.5 cm, and an arrow indicating direction of blood flow is marked nearby.

¹ Polk, Buddingh and Goodpasture, *Am. Jour. Path.*, 14: 1, January, 1938.

Injection of the material in the direction of the blood flow is important, as hemorrhage usually results if the needle is inserted against the flow.

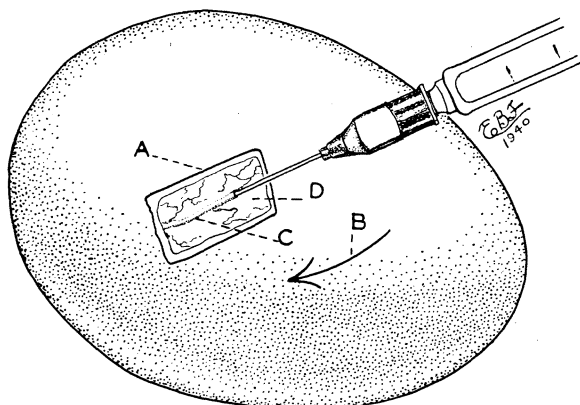


FIG. 1. A. Section of shell removed leaving shell membranes intact. B. Arrow showing direction of blood flow. C. Vein. D. Shell membranes made transparent by oil and adhering chorio-allantoic membrane.

A square of approximately 1 cm is then cut through the shell to the inner shell membrane around the portion of the vein marked on the shell. This is done by means of a high-speed grinder such as the Handee or Moto-tool. If the grinder is clamped to a stand and the egg manipulated, the cutting of the shell is facilitated as more accurate pressure may be applied. Care must be taken not to cut through the outer and inner shell membranes, as the chorio-allantoic membrane will then usually pull away from the outer shell membranes and it will be impossible to expose the vein. The cut section of shell is then lifted away by inserting any fine-pointed instrument in the cut and lifting.

A drop of light, sterile mineral oil is then placed on the membrane, which immediately becomes transparent, exposing the vein, and the egg is ready for injection.

It is advisable to use a $\frac{1}{4}$ cc tuberculin syringe with a 25-27 gauge needle, and the angle of insertion should be as acute as possible using the posterior edge of the cut as a support, while the bevel of the needle is held uppermost. Withdrawal should be extremely slow and caution exercised to prevent possibility of hemorrhage. In this connection it was found that the probability of hemorrhage with veins larger than the diameter of the needle is greater than with those slightly smaller, hence it is preferable to pick out, if possible, a portion of a vein of medium size.

After the needle is withdrawn, the egg is sealed with a 6 per cent. solution of paralodion in ether.

In bleeding the embryo, the same procedure is followed, with the exception that the needle is inserted against the direction of flow.

Quarter cc amounts have been bled from embryos without apparent damage and quantities of blood up to 0.1 cc injected with survival. Allantoic fluid, however, has proved fatal in a few minutes when injected intravenously in 0.01 cc quantities, producing tremendous hemorrhage in the embryo proper.

In conclusion, there have been 32 passages to date from egg to egg in series by this method, while mortality rate due to hemorrhage in faulty manipulation has been approximately 30 per cent., but there is no reason why this can not be considerably reduced with improved technique.

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MOUNTING EMBRYOLOGICAL MUSEUM SPECIMENS WITH GLASS WOOL

By using the following technique a practically transparent, as well as artistic, suspension of specimens may be obtained.

First, pour mounting medium in jar (10 per cent. formalin, or glycerin, etc.). Second, place specimen in jar of medium and encircle it with the least possible amount of loosely meshed, fine, glass wool, enough to hold the object suspended and freely oriented to present the most satisfactory picture.

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BOOKS RECEIVED

- ADDICKS, LAWRENCE, Editor. *Silver in Industry*. Pp. vii + 636. Illustrated. Reinhold. \$10.00.
- CLAUSEN, JENS, DAVID D. KECK and WILLIAM M. HIESEY. *Experimental Studies on the Nature of Species: I, Effect of Varied Environments on Western North American Plants*. Pp. vi + 452. 155 figures. Carnegie Institution of Washington. \$3.50.
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- DE VRIES, LOUIS. *French-English Science Dictionary; For Students in Agricultural, Biological and Physical Sciences*. Pp. viii + 546. McGraw-Hill. \$3.50.
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- WHITEBECK, R. H., FRANK E. WILLIAMS and WILLIAM F. CHRISTIANS. *Economic Geography of South America*. Third edition. Pp. xi + 469. 203 figures. McGraw-Hill. \$3.50.
- WINDLE, WILLIAM F. *Physiology of the Fetus; Origin and Extent of Function in Prenatal Life*. Pp. xiii + 249. 70 figures. Saunders. \$4.50.