served cobalt protection effect remains to be investigated. It may be that not one mechanism alone but all three together, working in a functional correlation, produce the effect. The parallelism is so close, and the possibilities of obtaining more information on certain phases of the protection mechanics are so obvious, that further investigations are indicated.

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Manuscript received July 25, 1952.

Collection and Storage of Chicken Plasma for Tissue Culture

V. Armaghan, Helen M. Pavlech, and Norman O. Olson

Department of Pathology, School of Medicine,
and Division of Animal Pathology,
Animal Husbandry Department,
West Virginia University, Morgantown

Since Carrel and Burrows introduced the use of chicken plasma in tissue cultures, many attempts have been made to replace it by something more readily available, such as agar (*1*), cellophane (*2*), or commercial thrombin and fibrinogen (*3*). These agents have proved fairly successful in the hands of their sponsors, but generally speaking the chicken plasma clot is still the most satisfactory substrate for tissue culture.

It has been said that experienced operators seem to draw blood without difficulty by intuition rather than by method. We hope that the method here described will be of help to the inexperienced and simplify the procedure for the experienced. Steps eliminated in the collection of plasma are based on our observations and are supported by information obtained from a survey of nine tissue culture laboratories.

Bleeding methods. Carrel and Burrows (*4*) in 1911 recommended collecting blood from the carotid artery by "the method similar to one used by Delezenne and by Gengou" (no reference given). The bird is etherized, and the carotid artery is exposed. The vessel is rubbed with dry gauze and covered with olive oil. A glass cannula sterilized in olive oil is used to run the blood into chilled paraffin-coated tubes, and after centrifugation plasma is removed with paraffin-coated pipettes. This is a delicate and time-consuming operation and is used in only three of the nine laboratories that we surveyed.

In 1928 M. R. Lewis (*5*) introduced the cardiac puncture method, using a lateral approach. This simplified the procedure but introduced the need for an anticoagulant. No toxic effects are observed when heparin is used, and six of the nine laboratories surveyed use this method.

The survey confirmed our observations on the following points:

1) Fasting is not essential to collection of clear plasma and does not affect clotting quality of blood. The fat content of chicken feed is not high enough to result in chylous plasma. Overnight fasting, however, is advantageous in the interclavicular approach to the heart. A full crop might get in the way. Fasting also eliminates unassimilated digestion products from the circulation. (One laboratory bleeds without fasting.)

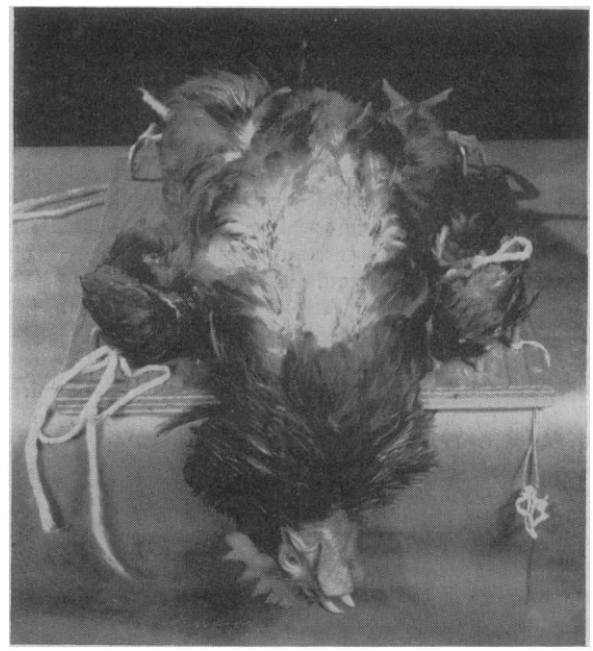

FIG. 1. Bird in position for bleeding.

2) Paraffin coating of tubes and pipettes is unnecessary; the important thing is to keep them well chilled. Three laboratories use plain tubes; one collects in plain tubes but stores in paraffin-coated tubes; five use paraffin-coated tubes.

Modified MacArthur (6) technique of collecting blood. Briefly, this is a cardiac puncture method of reaching the heart through the interclavicular space. The Pavlech modification uses the same approach, but the blood is collected in tubes instead of in a syringe.

Birds selected for bleeding should be 10–12 months old, as management of the bird is easier and the

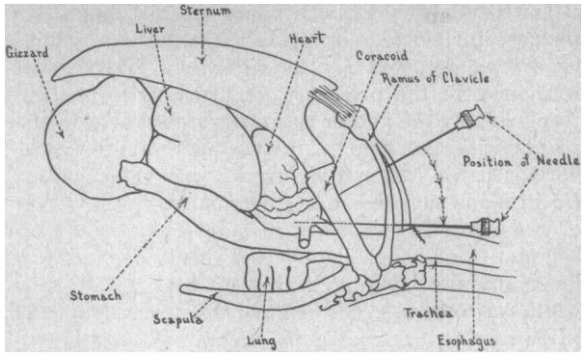

FIG. 2. Diagram showing location of heart and position of needle.

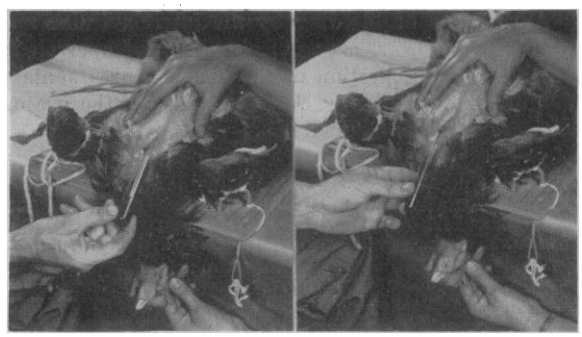

FIG. 4. Undraped bird: collecting the blood.

plasma yield is more adequate. Plasma from older birds is supposed to contain growth-inhibiting factors.

In preparing the bird for bleeding the feathers in the clavicular area are plucked and trimmed to present a clean field of operation. The bird is then placed on its back on a board $\frac{3}{4}'' \times 14'' \times 18''$ with U-shaped bolts or hooks at each corner. Two-inch bandage or muslin ties are used to immobilize the bird. The head hangs over the edge of the board and the operating table (Fig. 1). The bird remains remarkably quiet in this position throughout the operation, without anesthesia. The amount of jerking toward the end of bleeding is no more than that observed under anesthesia.

After proper disinfection of the skin the bird is draped for aseptic operation. The operator uses a 15- or 16-gauge 3- or 4-in. needle (depending on the size of the bird) attached to a 1-cc tuberculin syringe. The syringe aids in keeping the hub of the needle sterile while the heart is being located. There is no need to rinse either the needle or the syringe with olive oil (Figs. 2, 3).

Before starting the operation, the triangular opening to the thoracic cavity formed by the coracoid bones and the neck is palpated. The needle is inserted in the median plane, approximately ½ in. below the angle formed by the clavicle and is directed downward at approximately a 45° angle until the point of the needle is in the dorsal angle (ventral when the bird is standing) formed by the coracoid bones. Then the syringe is lowered until it lies approximately ½ in. above the neck, and the needle is pushed inward and upward 2°–5° through the interclavicular space until the heartbeat is felt. The heart is penetrated, and at the same time slight negative pressure on the syringe is maintained. As a rule, blood will appear with sufficient force to push back the plunger. The syringe is then removed from the needle and the first milliliter collected is discarded along with the syringe to avoid contamination with tissue juice. In a successful operation blood flows rapidly through the needle and is collected in 10–12 ml amounts in chilled tubes (Fig. 4). Should a clot form in the needle before bleeding is completed it can be pulled out with a pair of fine forceps and bleeding continued. Each tube must be corked and placed immediately in a bowl of ice chips. The amount of blood collected depends on the size of the bird.

Collection and storage of plasma. After the blood has been completely chilled the tubes are placed in chilled carriers, carefully balanced, and centrifuged at about 2500 rpm for 8–10 min. After centrifugation the plasma is pipetted into chilled tubes and stored at 5° C. The crucial point in collecting chicken blood for plasma seems to be the immediate chilling of the blood. It is possible that chilling blocks the clotting mechanism, which is released upon the addition of tissue extract. Once "blocking" has taken place the plasma remains fluid under ordinary refrigeration.

Tightly corked tubes of plasma will keep at 5° C for 4–6 months. For longer storage it is best to lyophilize it. Sometimes stored plasma will gel. This is different from a plasma clot, in that the plasma can be thinned out with an equal amount of Tyrode solution; a satisfactory clot forms when tissue extract is added to it.

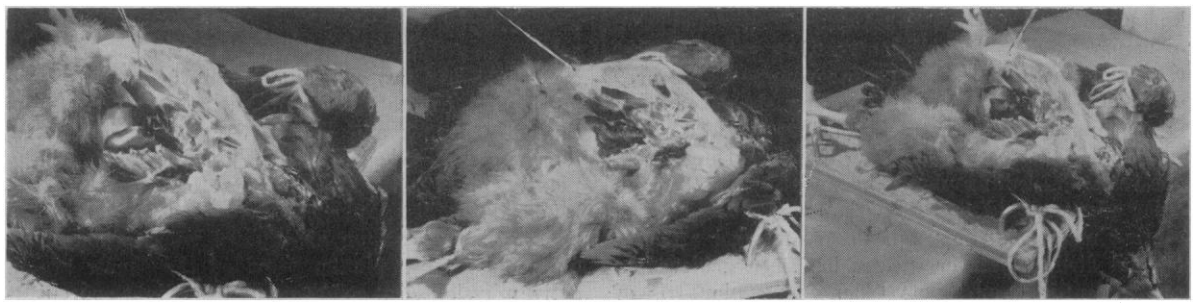

FIG. 3. Lateral views; dissected bird shows position of the heart and needles.

Advantages of bleeding by the interclavicular approach to the heart are: (1) simplicity of operation; (2) fractional collection of the blood insures against loss of all the blood by clotting; and (3) the use of anticoagulants is eliminated.

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Manuscript received May 21, 1952.

Vegetative Propagation of Mango (*Mangifera indica* L.) by Air-Layering (*Gootee*)

L. B. Singh

Government Fruit Research Station, Saharanpur, U.P., India

Mango is one of the most popular fruits of the tropics and subtropics; and it is definitely the best fruit of India, where it commands the largest acreage of any single fruit crop. At present it is propagated commercially by inarching, or "approach grafting," on seedling stocks of unknown heritage. There has been a long-felt need for the exploitation of clonal root stocks in the cultivation of mango, because it will not only help in standardizing the innumerable mango varieties, but will also enable the multiplication of desired fruit plants. The situation was similar in apple orchards in temperate countries until Hatton (*1*) selected a few types of root stocks of known performance, with a view to eliminating variations in apple varieties. These root stocks produced dwarf, semivigorous, or vigorous trees as desired and are now very extensively used in apple orchards practically all over the world.

In the case of mango, however, the exploitation of such known root stocks, even if they were selected, was rendered impossible until now, as no method was available to multiply them vegetatively. Guha-Thakurta and Dutt (*2*) attempted vegetative propagation of mango by cuttings and *gootee*. They got promising results when the trees were quite young—i.e., 2 or 3 years old—but failed in air-layering shoots or rooting cuttings from older trees. Gardner and Piper (*3*) reported 31% success in rooting mango cuttings from one-year-old seedling plants but negative results from older plants. Said and Shoushan (*4*) succeeded in rooting mango cuttings, but these shriveled up later on and died. Whatever success has been achieved in rooting cuttings or shoots from young plants is of very little practical importance, as it provides only a limited number of plants and will hardly serve any purpose for clonal root stocks since the tree will not have been observed for its vigor and fruiting performance. In the present work attempts were therefore made by the author to propagate selected mother plants with the help of well-known root-producing hormones. Six hundred vigorous 2-year-old shoots, 1.5 cm in diameter, were selected from 30-year old trees of two vigorous varieties of mango—Samarbahist Alibag and Gopalbhog. The selected shoots were 18″–24″ long, and for layering a ring of bark about 1″ in width was removed. The exposed wood was scraped off with a knife to remove the cambium cells, if any.

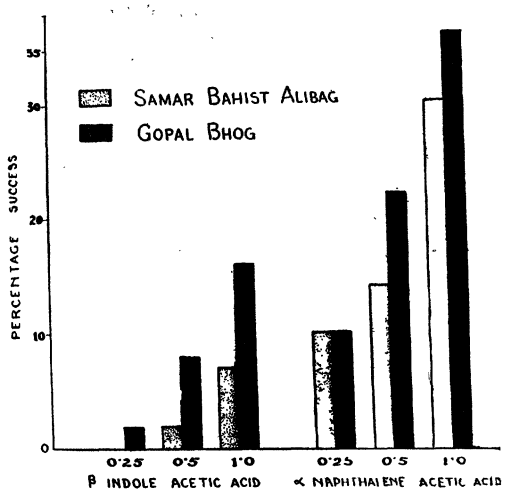

FIG. 1. Effect of hormones on rooting of mango shoots.

Two hormones—α-naphthalene acetic acid and β-indolacetic acid—were then applied to the upper edges of the bark, in three concentrations, 0.25%, 0.5%, and 1.0%, in lanolin paste. The ringed surface was then covered with wet sphagnum moss and wrapped securely with hessian cloth. The shoots were treated in June 1950. Fifty shoots of each variety were thus treated with concentrations of the hormones given above. Roots were formed in 45–60 days, when shoots were detached from the mother plant and potted. Results of these treatments are shown in Fig. 1.

These plants have been established in the field as mother plants for the past 2 years and have taken well. It is evident that shoots from older mango trees can be air-layered quite successfully. Of the two hormones tested, α-naphthalene acetic acid gave better results than β-indolacetic acid. Higher concentrations of both acids proved better than lower ones. The latest view in rooting mango cuttings or shoots was that of Gardner and Piper (*3*), who believed that its success is linked with the juvenile stage of the plant, which varies from species to species. Results reported in this paper clearly prove, however, that mango shoots can be air-layered successfully from quite old mother plants, provided the shoots are in active physiological condition and the right concentration of hormones is applied to them.