A Blood Sampling Technique for Sea Turtles

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This booklet describes a safe procedure for taking venous blood from adult and juvenile loggerhead turtles. The same method is applicable to green turtles and presumably for other marine turtle species. It also details separating plasma from the cellular components of the blood for subsequent analysis. As with any procedure involving endangered species, the animals should be handled with care to insure their comfort and survival. Federal law states that no experiment shall be conducted that requires undue injury, death or suffering to the specimen. This procedure is in full compliance with this requirement as it does not injure or cause suffering to the animal. The blood sampling shown in this booklet was done under agent designation from the Southeast Fisheries Center, National Marine Fisheries Service, under permit FWS PRT 2-4481.

LIST OF EQUIPMENT

Centrifuge
Centrifuge tubes
10 ml syringe and needles
5 ml syringe with needle
Heparin
Vial
Biomailer
Labels and pen
Data notebook

The equipment listed above and explained below is required for taking turtle blood and isolating the plasma for analysis. The equipment is illustrated in Figure 1.

1. The 10 ml syringes and 20 guage 1-1/2" needles are used to take the blood from the neck sinus. 18-20 guage needles are recommended for the adult and subadult turtles; 20-24 guage 1" needles are suitable for juvenile turtles.

2. Sodium heparin is an adequate anticoagulant for blood taken for most analyses. For plasma sodium analyses another heparin salt would be necessary e.g. calcium or ammonium heparin.

3. Centrifuge tubes of 10-15 ml capacity provide 6-9 mls of plasma. These tubes can be glass or disposable plastic. If glass is used they should be cleaned with an acid wash before use.

4. A sturdy, portable centrifuge, should be used. Most of the centrifuges have 3 pronged plugs, therefore an adaptor to two prongs is often needed when used in the field, on boats, etc.

5. A 5 ml syringe with needle is used to remove the plasma from the centrifuge tube. Pipettes are generally inadequate outside the lab (i.e. on board small boats), as they are difficult to control.

6. The vials should have secure caps and be freezer-proof. These can also be either glass or disposable plastic; if glass they should be cleaned thoroughly.

7. Pencil or waterproof pen and labels should be used to mark each vial. Use labels with waterproof glue. The notebook should be of good quality paper with sewn bindings.

8. Styrofoam boxes sturdy enough to be mailed and with sufficient volume to hold the samples and dry ice should be used.

PROCEDURE

A 10 ml syringe with a 20 guage needle should be heparinized before the sample is taken. This is done by taking up 1/2 ml of heparin and swirling it in the barrel of the syringe. With the needle point up the air and excess heparin can be expelled (Fig. 2). This will leave a small but sufficient quantity of heparin in the needle and barrel of syringe.

The turtle to be sampled is carefully placed plastron down (i.e. right side up) on a box, stool or cement block. The box should be under the central anterior plastron (chest) so that the front flippers are partially off the deck (Fig. 3). It is generally necessary for one person to hold the turtle while another samples it. The

head is held with one or two hands and pressed firmly but gently out and down until the neck is fully stretched.

The needle should be inserted approximately half way between the anterior border of the shell and posterior border of the head. A visible muscle band runs from the shell to the neck (Fig. 4). In a 30-50 kg turtle the point of entry is approximately 1 - 1-1/2" from the midline of the neck, just lateral to the muscle band.

The needle should enter at a 30-45° angle to a (mid) sagittal plane i.e. so that the needle enters toward the center of the neck (Fig. 5 and 6).

A relatively quick entry of the needle through the skin and neck disturbs the turtle less and seems to be more successful in entering the vessel than a slow needle entry. The needle should go in 1" to 1.5" depending on the size of the turtle. Care should be taken not to strike the vertebrae with the needle by an overly deep insertion. After the needle is in place a slight suction should be sufficient to cause blood flow. Excessive suction can collapse the vessel temporarily and cause the needle to be occluded. If blood does not flow into the syringe the needle should be withdrawn slowly while maintaining a slight suction. If still unsuccessful, release the suction, withdraw the needle completely, and try again up or down from the original point of entry or at a slightly different angle. If after three attempts no blood is obtained, the other side of the neck should be tried. Locating the vessel can be difficult and this process requires practice.

Once you have located the vessel and blood flow has started, continue to withdraw the blood slowly until a sufficient sample is taken (Fig. 7). If at anytime when the needle is in the turtle's neck, the animal succeeds in withdrawing its head, the needle should be removed immediately. A 10 ml sample can be safely taken from a 20 kg or larger animal. A total of 30 ml of blood have been taken from 35-

40 kg captive animals during 3 hours repetitive sampling causing no decrease in hematocrit or other ill effects. If the turtle is to be sampled more than once, it should be taken off the block. As with all turtles taken out of the water, it should be kept wet and cool. After sampling allow the turtle a short recuperation time before returning the animal to the water.

Gently squirt the blood sample into the centrifuge tube and place it in the centrifuge (Fig. 8). As the centrifuge must always be balanced, the tube opposite the sample tube must be filled with another blood sample or water of equal weight. Spin the tubes at top speed (4000 rpm) for 5-10 min. This will pack the cells into a discrete lump at the bottom of the tube. The supernatant will be clear plasma.

The 5 ml syringe with attached needle is then used to withdraw the supernatant. Be careful not to draw up any of the cells as this contaminates the sample (Fig. 9).

The plasma should be put into a container for storage (Fig. 10). For hormone analysis, three separate 1-2 ml samples should be taken. All storage vials should be tightly capped. Each vial should be labelled clearly with the turtle's identification number. The label must be waterproof and able to withstand handling and mailing. All relevant data on the turtle, the date, the sample and any other pertinent information should be recorded immediately in the data notebook. The samples should be stored in the freezer or on ice as soon as they are placed in vials and labelled. They should be kept frozen until analyzed.

Figure 1. The equipment necessary for blood sampling.

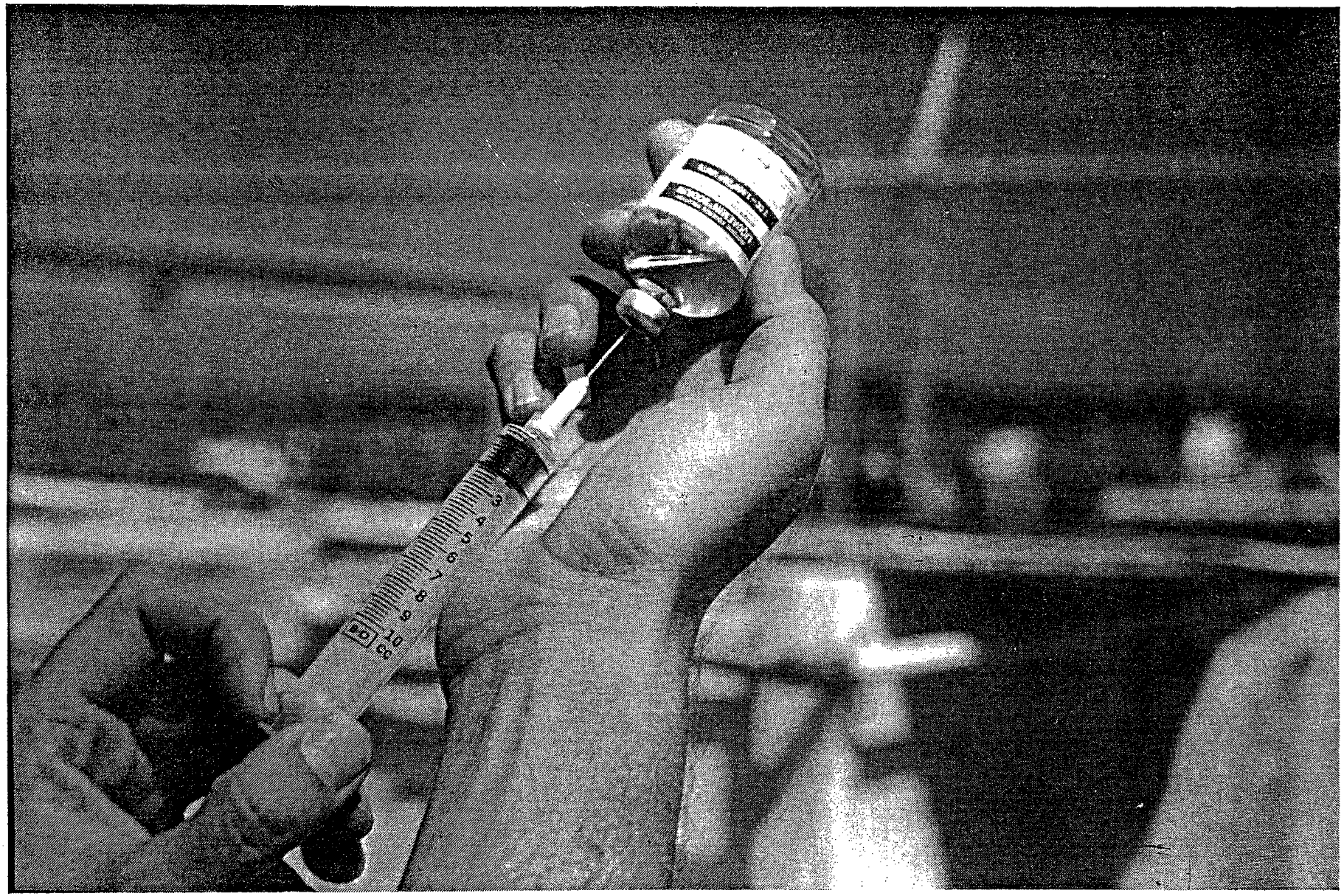

Figure 2. Heparinizing the 10 ml syringe.

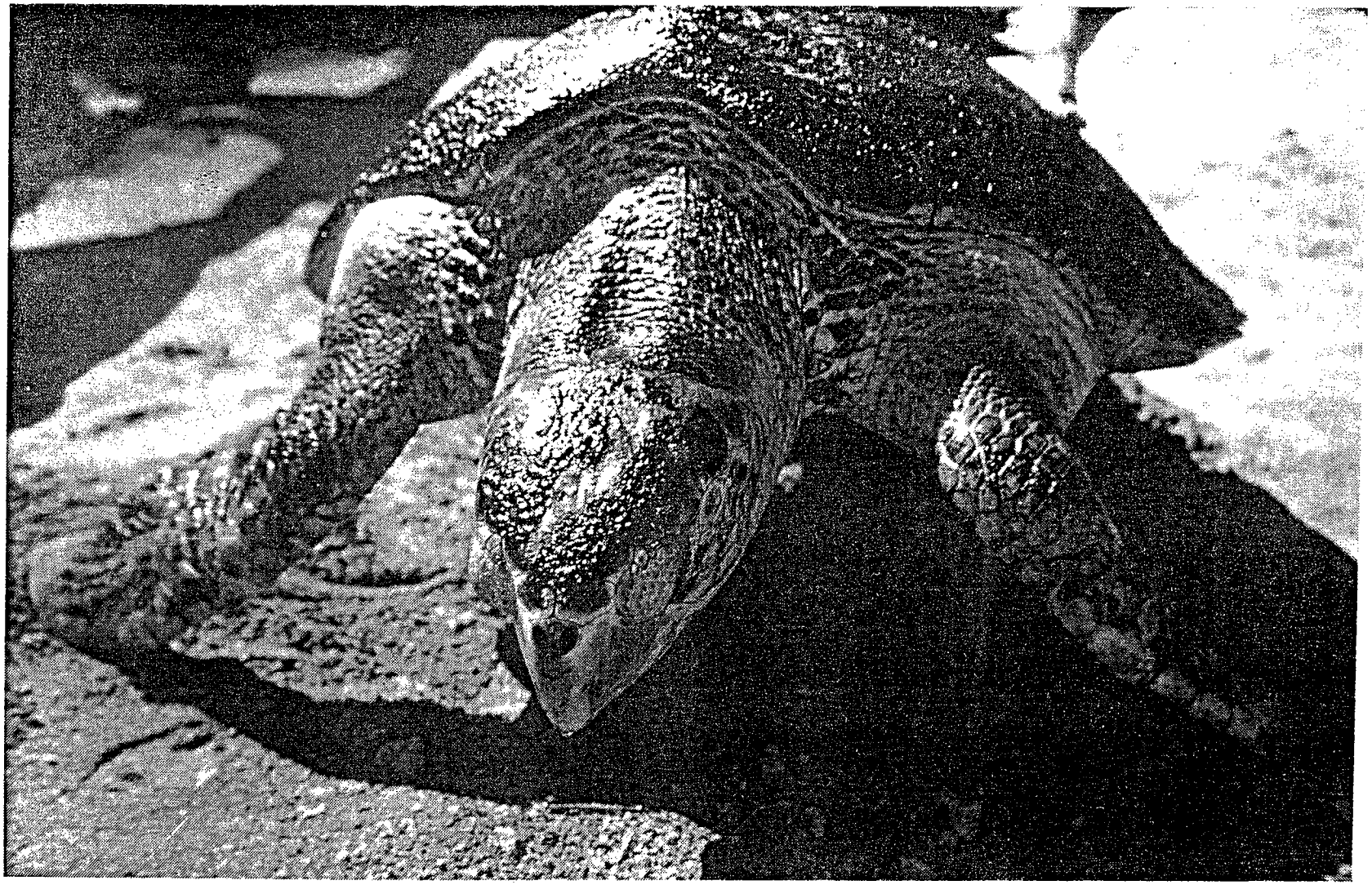

Figure 3. The turtle is raised off the ground and resting on a block.

Figure 4. The turtles' head is held down and out. Note the centrally located muscle band.

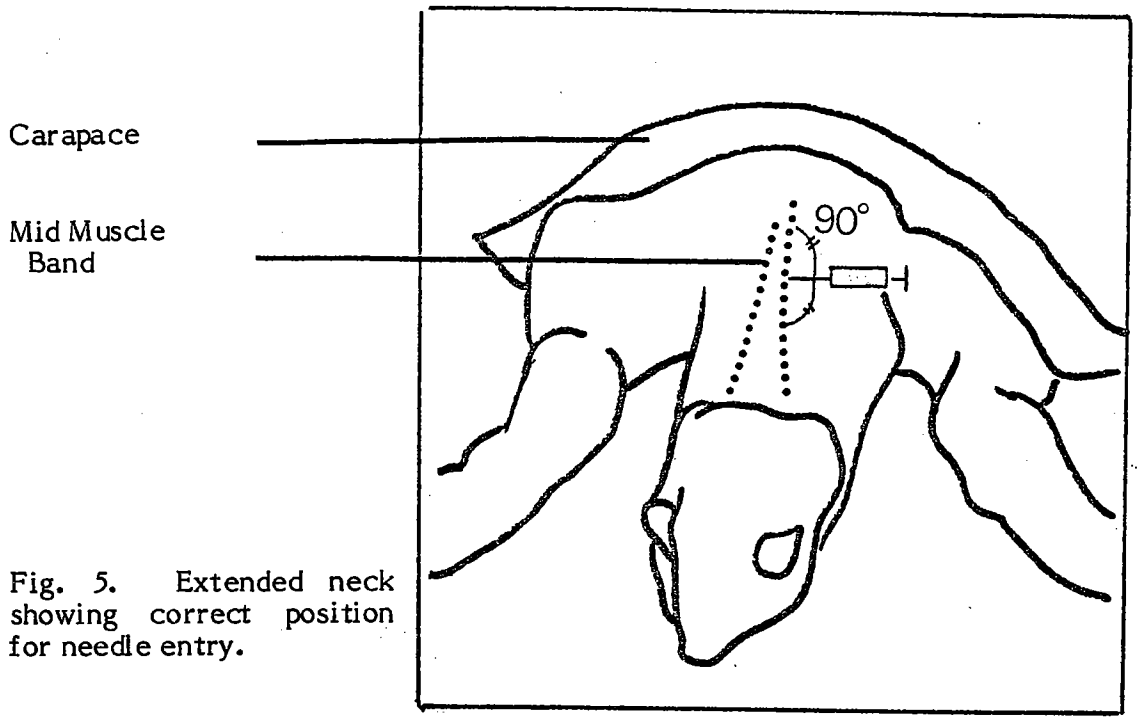

Fig. 5. Extended neck showing correct position for needle entry.

Fig. 6. Cross-section of neck showing position of neck showing position of correct needle entry.

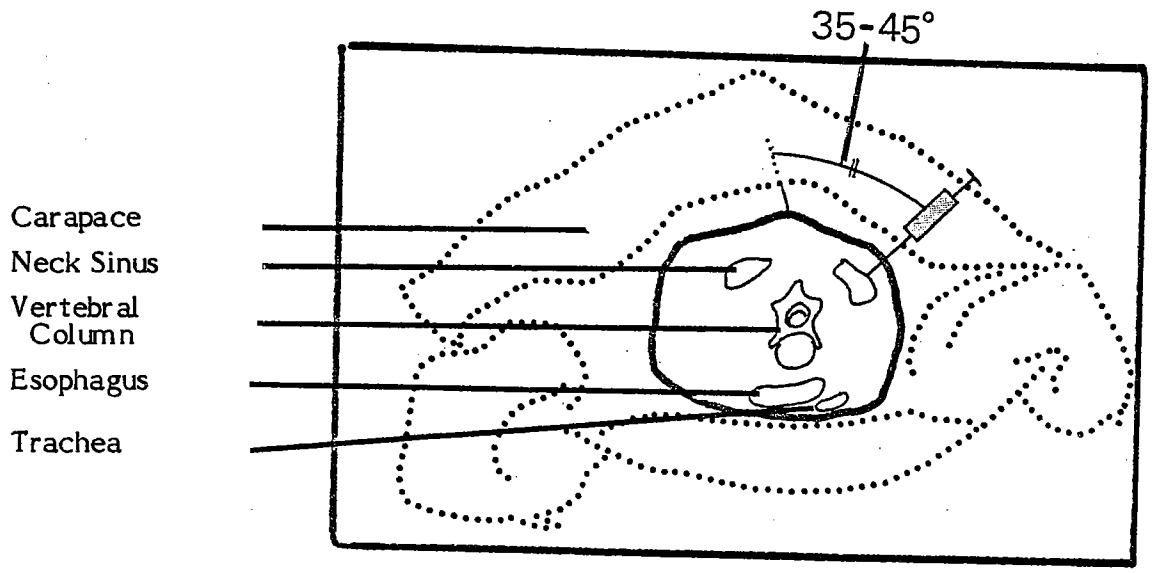

Figure 7. The needle is in the correct position and blood is being withdrawn.

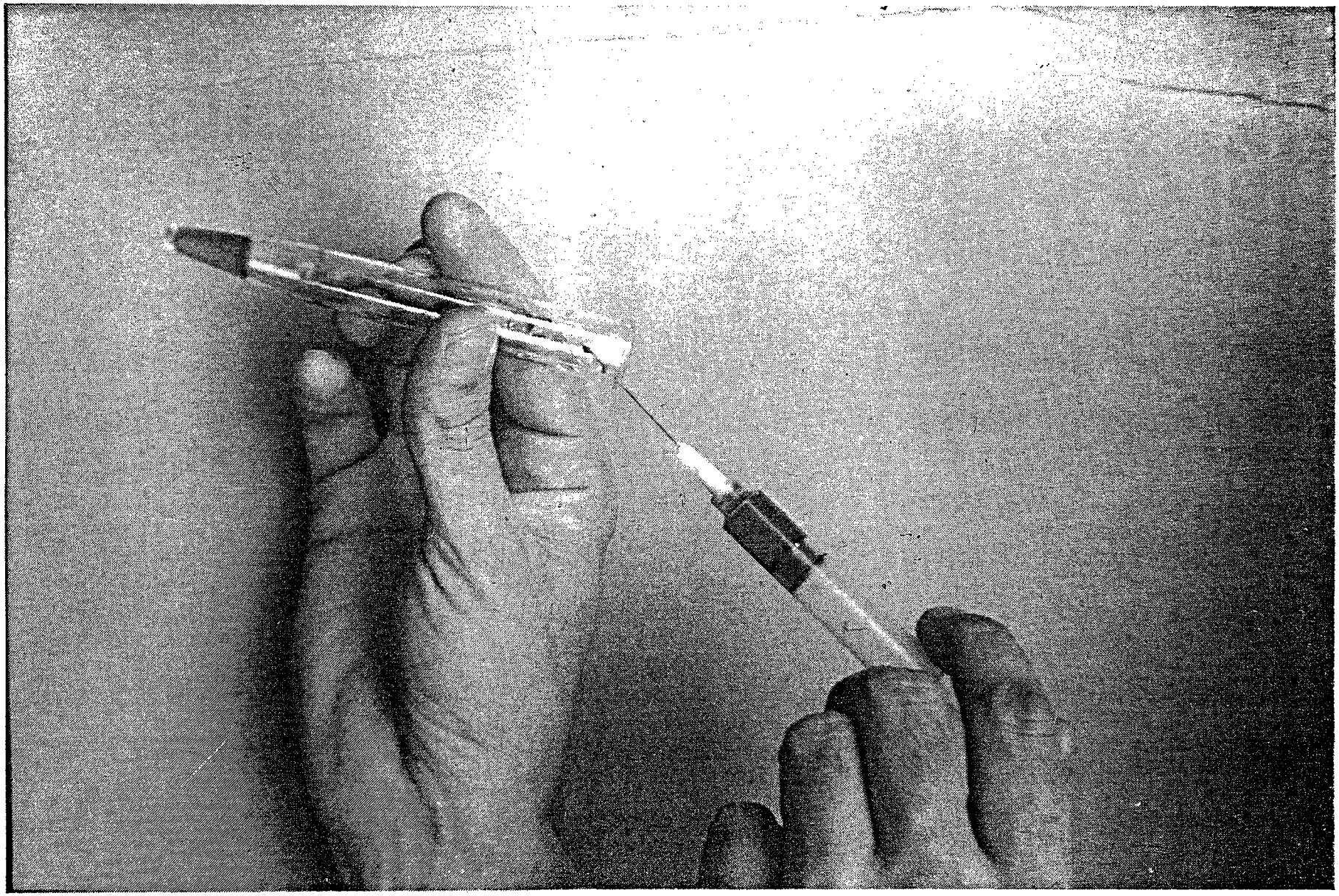

Figure 8. The syringe is emptied into the centrifuge tube.

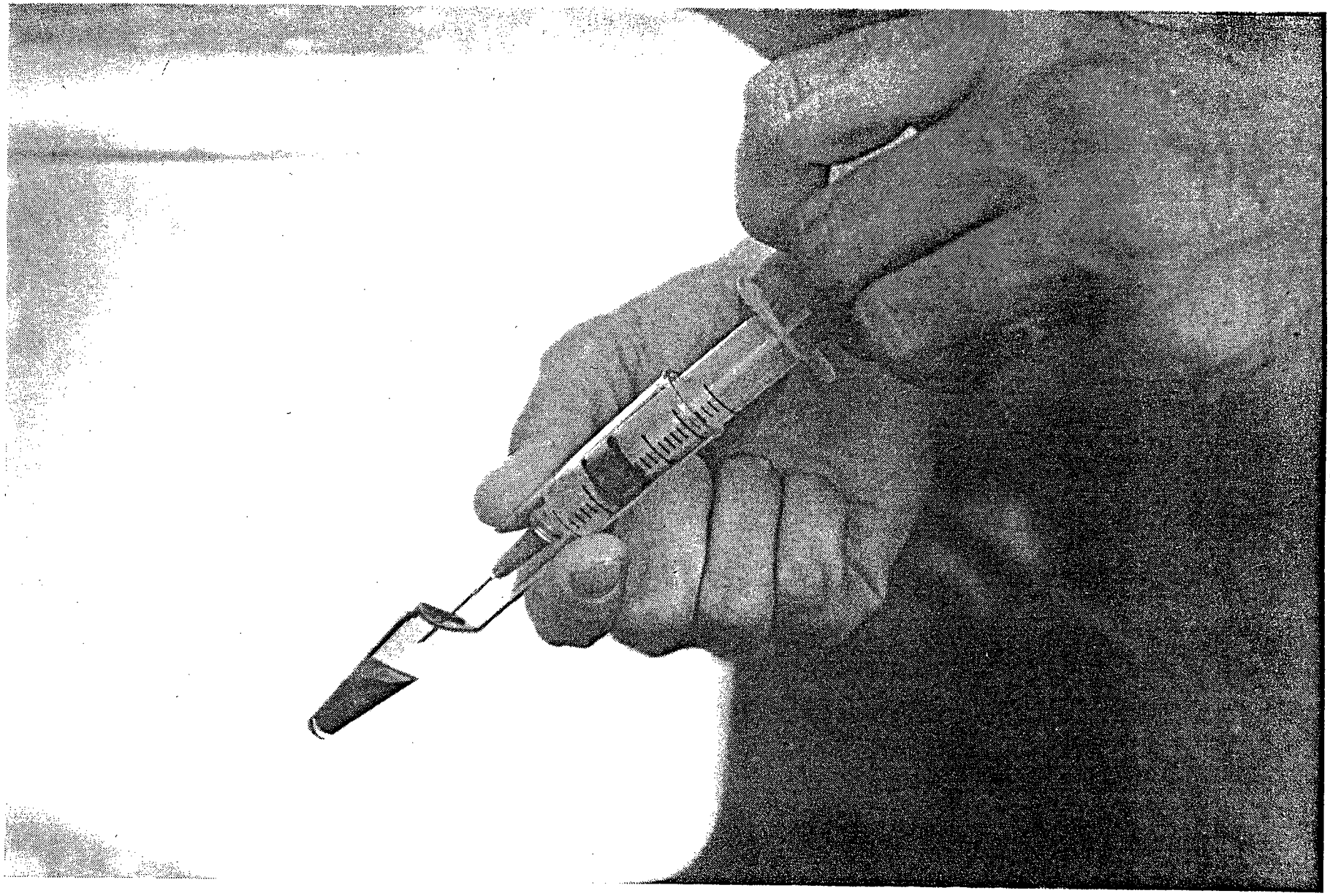

Figure 9. Plasma is withdrawn from the centrifuge tube.

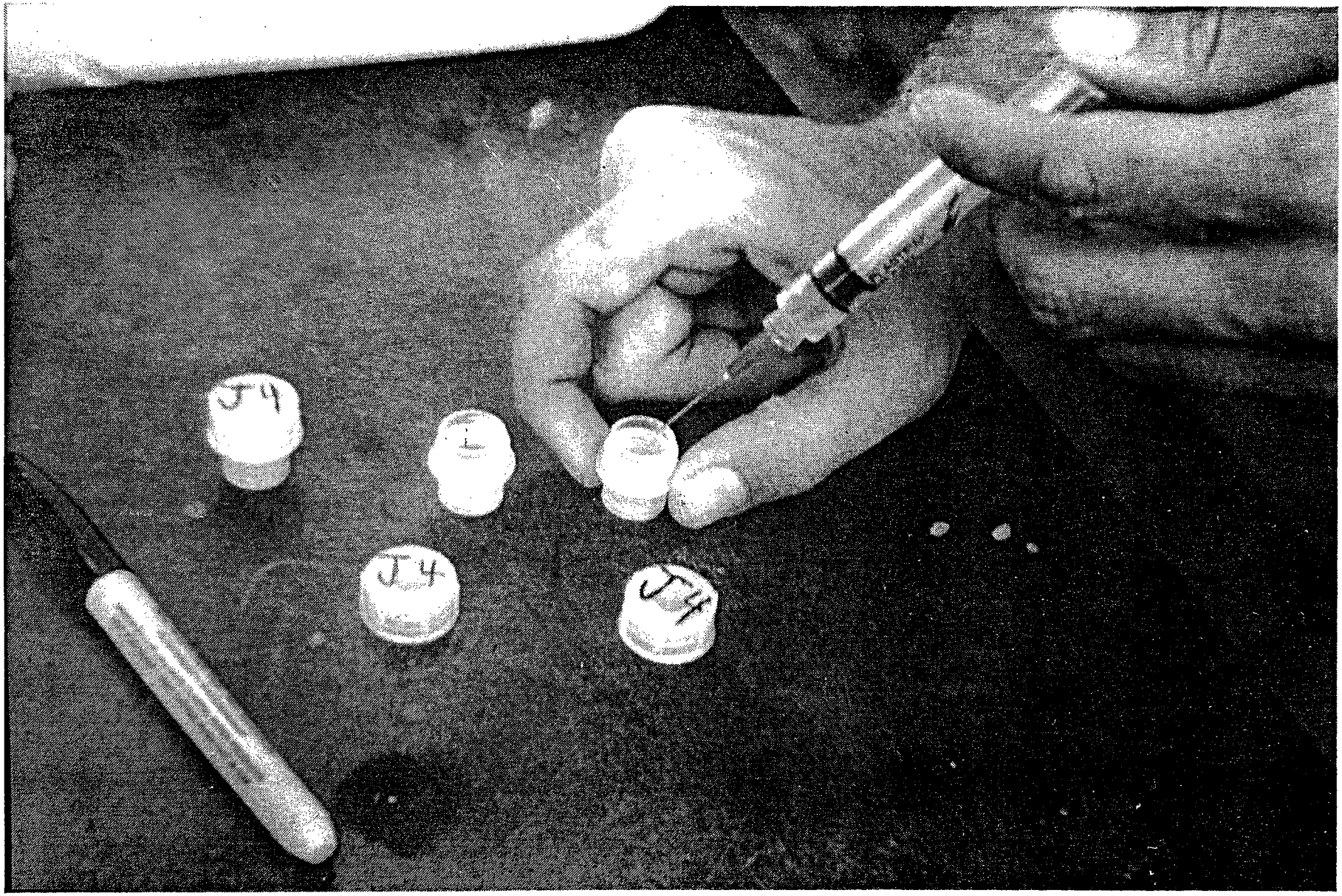

Figure 10. The labelled sample containers are filled with plasma.