and the subsequent condensation of chloral with chlorobenzene followed the work of Mosher *et al.* (*7*).

Starting with 50 mM of barium carbonate containing 20 mc of activity, 15 g of crude DDT (42% yield based on EtOH) was obtained. Two crystallizations from ethyl alcohol yielded 6.11 g of *p,p'* DDT (17% yield) having a melting point of 107–107.5° C. The specific activity of the product was approximately ½ mc/g.

The residue is currently being treated to recover additional amounts of the *p,p'*-isomer as well as the *o,p*-isomer.

It is planned to submit a detailed report of this work elsewhere.

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Protamine, Chylomicrons, and Clot Formation[1]

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The main intestinal lymph channels of 14 rats fed 1 ml of corn oil were cannulated by the technique of Bollman *et al.* (*1*). Addition of 0.2 mg of protamine (clupein, Vitrum, Stockholm) to 1 ml of chyle caused immediate flocculation of the chylomicrons. The floccules rose to the surface during the following 30 min leaving a clear subnatant fluid. Five samples of chyle were adjusted to pH 7.4 with *N* HCl and 0.1 ml aliquot parts from each sample were mixed with quantities of protamine ranging in weight from 2.5 to 20 μg in multiples of 2.5 μg. The minimum quantity of protamine causing flocculation varied from 0.05 to 0.20 mg per ml of chyle. The pH of aliquots of normal chyle (7 samples) and chyle containing flocculated chylomicrons (5 samples) were gradually changed from 7.4 to 2 or 12, and the isoelectric point of normal chylomicrons in intestinal lymph was determined by the technique of Ludlum *et al.* (*2*). This was found to lie between pH 4.6 and 4.9. Lymph chylomicrons in the human subject are apparently stabilized with albumin (*3*) (isoelectric point pH 4.7), and it is clear that the same is true of the rat. The protamine-flocculated chylomicrons dispersed to form a stable emulsion over the pH range less than 4.7 and flocculated again if the pH was readjusted to a value greater than 4.7. Evidently protamine is adsorbed to the interface of lymph chylomicrons in the interisoelectric region of albumin and protamine (pH 4.7–12) causing loss of surface potential followed by flocculation. The reaction appears to be similar to that discussed by Elkes *et al.* (*4*) in the course of examination of effects of proteins on detergent-stabilized emulsions.

Protamine was added to 7 chyle samples (1 mg/ml) and allowed to stand for 30 min. The samples were then centrifuged at 3000 rpm for 2 min when a crystal-clear subnatant fluid was obtained. It is possible that lymph clarified in this manner contains all the non-chylomicron lipid and that the procedure can be used to facilitate analysis of the composition of lymph chylomicrons, or the fluid in which they are suspended. The mean total fatty acid content determined by a modified Schmidt-Nielsen technique (*5, 6*) of the whole chyle samples and clear subnatants was, respectively, 3000 (range 5000–1500) mg%, 34 (60–12) mg%. These observations suggest that the fat in intestinal lymph is almost entirely in chylomicron form during fat absorption, but it remains to be shown that only the chylomicrons are separated from lymph by this treatment. However, no differences in the distribution of proteins in thrombin-treated chyle before and after clarification by protamine were detected (*7*) by the Tiselius electrophoretic technique.

The samples of normal chyle which were collected during the present investigation usually slowly clotted on standing. The clots did not retract and they bound large quantities of lymph which were difficult to expel by compaction. The chylomicrons remained suspended in the lymph fluid recovered by compaction and imparted to it the characteristic milkiness of freshly drawn chyle. In several of the chyle samples treated with protamine, formation of a light clot and flocculation of the chylomicrons occurred within several seconds of treatment. The clot bound chylomicrons so that when it was lifted out of the lymph, the chylomicrons were lifted out with it leaving partially or completely clarified lymph behind. It did not bind lymph fluid and, in this respect, on removal from the lymph it resembled a compacted clot from normal lymph. The reaction was evidently associated with surface changes on the chylomicrons, and it was not a clotting reaction in the usual sense.

Blood was drawn 3 hr after feeding 8 rats 4 ml of olive oil/kg body wt. The effect of protamine on the serum chylomicrons visible in the darkfield microscope (1200 ×) was ascertained. In all 8 samples the chylomicrons were flocculated into immobile clumps by 2 to 3 mg of protamine/ml of serum; 1 mg/ml had little or no visible effect. Thus the minimum dose of protamine causing flocculation of chylomicrons in serum is considerably higher than that required to flocculate chylomicrons in intestinal lymph. Addition of 3.3 ml of a protamine sample/ml of serum induced changes in the electrophoretic pattern of serum owing to association of albumin with protamine (*8*), and it may well be that the larger dose required to flocculate the serum chylomicrons is due to competition between

[1] Many samples of chyle used in this investigation were supplied by B. Borgström and R. Blomstrand.

the stabilizing protein film about the serum chylomicrons and albumin in the disperse phase, for the protamine.

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A Syringe Carrier and Egg Clamp for Intravenous Inoculation of Chick Embryo[1]

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The great range of potentialities of the chick embryo for the study and utilization of the relationships between viruses and susceptible cells has been exploited, in part, by various methods of inoculation: allantoic, amniotic, chorio-allantoic, intracerebral, intravenous, and yolk-sac. Inoculation by the intravenous route was first used by Polk, Buddingh, and Goodpasture in 1938 (*1*), and the current improved technic was described by Eichhorn in 1940 (*2*). It has been stated, however, that widespread application for the intravenous method has not been found (*3, 4*).

A study, undertaken by one of us (R.G.), required a collection of serial blood samples taken at 1–2-hr intervals, from the same, 10–16-day-old chick embryo. To overcome the prohibitively high casualty rate resulting from "free-hand" injection and bleedings, an apparatus was designed and built which consists of a rigid movable syringe carrier and an adjustable egg-clamp (Fig. 1).

The syringe carrier consists of two rigid rods. One, 30 cm × 2.5 cm and one 40 cm × 2.5 cm; the larger, a vertical one (1) fixed to the work table, and the smaller, a horizontal one (2) attached to the upright rod with a bar-clamp (3) allowing for coarse vertical and horizontal movement. Attached to one end of the vertical rod is a revolving stage (4 and 5) consisting of 2 circular plates (10 cm diam.), a fixed one (4) carrying a movable disc (5), actuated by a small spur gear (6) meshed into the geared edge of the moving plate. The center of the stage revolves about the long axis of the horizontal rod. A machine slide with bedded strip (7) is fixed to the revolving stage along the diameter. At 90° to the slide, which is fixed to the revolving stage, and bisecting it, is a second machine slide (8) and bedded strip. This slide and bedded strip is actuated up and down along the first slide and bedded strip by a worm-drive screw (9). The second (the outer machine slide), also actuated by a worm-drive screw (10) has a carriage (11), for holding various sizes of hypodermic syringes.

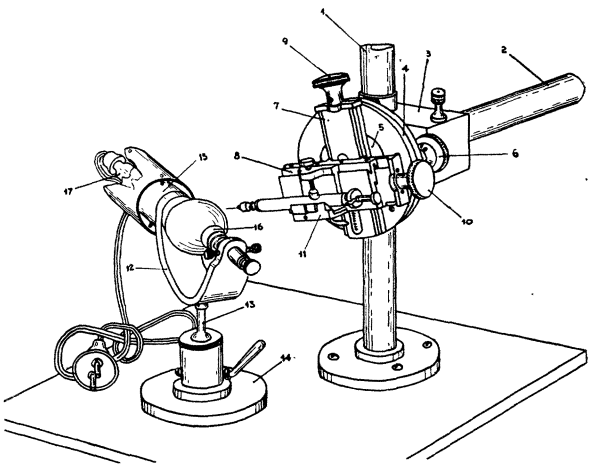

FIG. 1.

The carrier thus allows for the forward and backward motion of the entire syringe, by actuating the outer slide (10); the raising and lowering of the syringe by moving the inner slide (9); and adjustment of the angle of attack of the needle attached to the syringe by rotation (6) of the circular stage. Fine movement along the cylindrical axis of (2) can be achieved by incorporation of a rack and pinion in clamp (3).

The device permits the accurate and precise placement of the hypodermic needle at the proper angle, and entry into, and removal from the blood vessel, without erratic motion.

The other unit, the egg-clamp, consists of a U-shaped cradle, (12), mounted from below to a ball-and-joint swivel, (13), attached to a heavy but movable base (14). The egg is held at the air chamber and opposite ends. The air-chamber end fits into a fixed tube (2.0 cm diam.) (15), while the opposite holding point is an adjustable, spring-loaded rod with a small (0.75 cm diam.) soft rubber cup (16). The air-sac-holding tube extends beyond the U-cradle and contains a 25-w lamp for transillumination (17) of the embryo.

Selected eggs are transilluminated, the position of one of the large fixed veins lying in the chorio-allantoic membrane is marked on the shell, and the direction of the blood flow (which is toward the point where the fixed veins join the free allantoic vein) is indicated. A triangular segment of eggshell over the selected vein, of about 1–1.5 cm on each side, is re-

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