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Observations on the Role of Lipids in Serum-Induced Intravascular Thrombosis.* (26095)

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(Introduced by F. J. Stare)
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Thrombus formation in diseased coronary arteries is the most common clinically important complication of atherosclerosis. Moreover, it has been suggested that the atherosclerotic process itself may result from endothelialization of intravascular thrombi(1). Dietary and serum lipids have been shown to be associated with the incidence and prevalence of coronary heart disease in human populations. Whether lipid alterations can affect coagulability of blood leading to thrombosis, as well as possibly contributing to the development of the underlying atherosclerosis, is not known. Alterations of serum lipids can produce marked atherosclerosis in experimental animals, yet there are few reports of induced thrombosis in such experiments(2). To investigate the possible role of lipids in coagulability of blood, there have been many studies utilizing various *in vitro* technics. The controversial results have been well summarized(3,4).

Wessler(5) reported on a method of inducing intravascular thrombi in dogs in a venous segment isolated after systemic injection of homologous serum. A later communication(6) presented an extension of this *in vivo* technic to production of thrombi in rabbits following infusion of heterologous (human) serum. This communication also presented evidence to show that the active serum factors necessary in this system are Factor IX (Plasma Thromboplastic Component), Hageman Factor, and possibly Plasma Thromboplastin Antecedent(7).

In an effort to quantitate the dose-response-time interrelationships we have adapted Wessler's method to the use of chickens as the assay animals. Chickens are cheap, grow rapidly, and are easily maintained. Some preliminary findings of the effects of lipids on this system are presented.

Materials and methods. White Leghorn cockerels approximately 3 to 4 weeks of age (200 to 300 g) were used. Some of the birds were fed a commercial Chick Starter mash, others received purified diets containing 0.8% cholesterol in which the source of fat was varied. The composition of these has been described(8).

Pooled human serum lots were stored at 4°C and used within one week of collection. Pooled chicken serum from birds on the mash diets was similarly stored for use.

Cholesterol determinations on the assay birds' sera and on the infused test sera were performed by the method of Carpenter *et al.* (9).

Chickens were anesthetized with intraperitoneal sodium pentobarbital (Nembutal) and a 5 cm segment of non-branching jugular vein was exposed. The test serum was infused into the contra-lateral jugular vein and al-

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TABLE I. Quantitative Assay of 2 Samples of Pooled Human Serum.

ml	No. of assays	10 min. Segments clotted No.	%	15 min. Segments clotted No.	%
Serum I					
1.0	13	1	7.7	1	7.7
1.5	13	1	7.7	2	15.4
2.0	13	0	0.0	6	46.1
2.5	13	4	30.8	10	76.9
3.0	13	8	61.5	12	92.3
Serum II					
1.0	12	0	0.0	0	0.0
1.5	12	1	8.3	2	16.7
2.0	12	0	0.0	3	25.0
2.5	12	2	16.7	6	50.0
3.0	12	3	25.0	7	58.3
3.5	12	5	41.7	10	83.3

50% endpoint: Serum I —2.067 ml ± .145 ml.
Serum II—2.579 ml ± .176 ml.
$p < .05$ and $> .02$

lowed to mix for one and one-half minutes at which time the exposed segment was isolated with rubber-tipped clamps so as to create 2 contiguous segments. At appropriate times the segments were opened *in situ* and the contents collected on filter paper. Results were reported as either the presence or absence of gross clot.

Results. Blood in isolated venous segments remained fluid for at least one hour when no serum infusion was given. Neither normal saline nor homologous (chicken) serum produced intravascular clots.

Using 10 and 15 minutes as the endpoints for opening the 2 venous segments, human serum was active in the dose range 1.5 to 3.5 ml per bird. Table I presents the findings in 2 lots of pooled human serum tested at different times on 2 groups of chickens raised on commercial mash diets. These dose response curves are plotted in Fig. 1. When compared by the Reed-Muench method(10) of establishing 50% endpoints (standard error determined by the method of Pizzi(11)), the difference between the 2 sera at 15 minutes is significant at the .05 level.

TABLE II. Clots Induced by 3.0 ml of a Pooled Human Serum Lot at a 15 Minute Endpoint.

Diet	No. of assays	No. of segments clotted	Mean serum cholesterol, mg %
Mash	24	9	140
16% corn oil + 0.8% cholesterol	12	5	492
16% coconut oil + 0.8% cholesterol	6	2	781
8% olive–8% coconut + 0.8% cholesterol	6	3	639
Total—Purified diet	24	10	601

Table II shows the results when a single lot of pooled human serum was infused into birds raised on commercial mash as well as on various types of purified diets. The latter produced considerable differences in degree of hypercholesterolemia. None of the differences is significant (Chi Test).

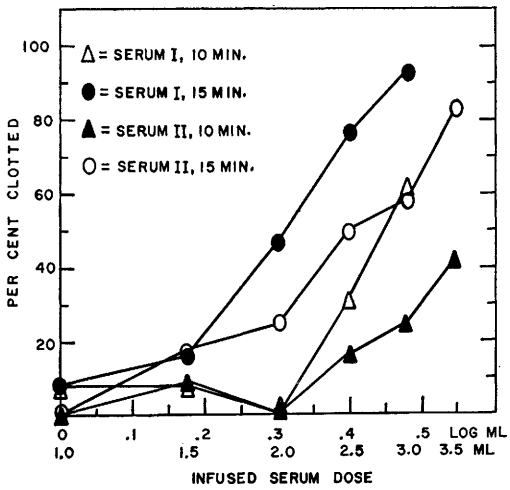

FIG. 1. Assay of 2 lots of pooled human sera with 10 and 15 min. endpoints.

Discussion. That chicken serum is inactive in this system confirms Wessler's finding that Hageman Factor and Plasma Thromboplastic Component are necessary for inducing intravascular thrombosis. Didisheim *et al.* (12) have previously shown that chickens have no detectable Hageman Factor and levels of Plasma Thromboplastic Component equivalent to less than 5% of normal human serum values.

Levels of serum necessary to produce thrombosis are higher in our experiments with chickens (10 ml/kg) than reported by Wessler for rabbits (1.32 ml/kg); duration of segment isolation in the rabbits was 10 minutes. In a small series of experiments on adult rats, we have found that human serum

infusions of 1.25 mg/kg will produce thrombosis within 2 minutes. Chicken serum is inactive in the rat.

Whether the difference between the 2 human serum lots was due to real differences between the lots of serum or to variation between the two groups of assay birds is not known. Until the sources of variation are known, this sort of assay should be done on a single group of birds within a short period of time.

Gross alterations in the plasma lipids of assay birds failed to cause any differences in the clotting rates. Until infused sera from patients with hyperlipidemias or recent thrombotic episodes can be assayed, one cannot exclude a role for lipids in this system. The system described is suitable for quantitation and use of this approach may facilitate the evaluation of the role of lipids in intravascular thrombosis.

Summary. (1) A method of producing serum-induced intravascular thrombi has been adapted to use in a convenient laboratory animal, the chicken. (2) Dose-response curves can be constructed and statistically compared. (3) The factors influencing the clotting rates are not all known, but alterations in serum lipids in the assay animals did not influence the results.

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Survival of Calves Treated with Autologous Bone Marrow After Exposure To Lethal Dose of Whole-Body Irradiation.* (26096)

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In conjunction with earlier experiments on induction of aplastic anemia in calves by trichloroethylene-extracted soybean oil meal (1) or by S-(dichlorovinyl)-L-cysteine(2), we attempted, without success(3), to prevent development of the blood dyscrasia by administration of D,L-batyl alcohol. Inasmuch as the toxic agent, if given in sufficient amounts and without therapeutic treatment, needs to be administered for only a short period of time(2,3) to damage the blood forming organs irreversibly, it appeared possible that hematopoiesis might be restored by intravenous injection of homologous bone marrow or other hematopoietic tissues. Bone marrow transplants have been effective in several species for recovery of hematopoietic function after the bone marrow had been

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