of ouabain was paralleled by a lack of antagonism of ouabain-induced changes in contractile and electrical properties of the atrial muscle. Therefore no evidence was found to support the view that d-aldosterone may competitively antagonize the K^+-depleting actions of ouabain on isolated atrial myocardium.

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Influence of Dietary Saturated and Unsaturated Fats on Blood Coagulation In Miniature Pigs.*† (28653)

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Following the observations of Keys and coworkers(1,2) that the fat content of a diet bears a direct relationship to the plasma cholesterol level of the population and to the incidence of coronary heart disease, it was shown by other investigators(3,4) that unsaturated fats decreased the plasma cholesterol level and that saturated fats increased it. Whether saturated and unsaturated types of dietary lipids can affect coagulability of blood, as well as possibly contributing to the development of atherosclerosis, is not known. Numerous studies have attempted to ascertain whether, and to what extent, dietary fats bring about changes in blood coagulation and fibrinolytic systems. Both animal and vegetable fats produced the same shortening of the recalcification time of plasma of patients, 3 and 4 hours after ingestion of fat(5). Keys *et al*(6) found that the silicone clotting time of blood was much shorter on a butterfat or coconut oil diet than on a corn oil diet. Using pigs as experimental animals, Rowsell *et al*(7) suggested that feeding butterfat increases both the incidence and severity of aortic lesions, perhaps due to alterations in the blood clotting mechanisms.

At The Hormel Institute, miniature pigs are being used in studies of the fundamental causes of atherosclerosis, including the influence of various dietary fats on blood coagulation. This paper presents some of the differences observed in the coagulability of blood on miniature pigs maintained for a

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† These results are part of an experiment designed and supervised by Dr. Eldon G. Hill, the results of which will be reported later. Our thanks are due to M. C. L. Silbernick, for technical assistance in this work.

TABLE I. Composition of Diets.

Ingredient	Group I (%)	Group II (%)
Corn	33	33
Oat groats	30	30
Soybean oil meal (solvent ext'd)	10	10
Dry skim milk	5	5
Meat and bone scraps	5	5
Minerals*	1.05	1.05
Vitamin-mix†	.85	.85
Cholesterol	.5	.5
Beef tallow	15.0	—
Safflower oil	—	15.0
	100.4	100.4

* Minerals added to ration: 0.5% dicalcium phosphate, 0.5% iodized salt, 0.05% Hi-Zinc trace-mineral mix for swine (Calcium Carbonate Co.).

† Vitamins added to ration: 0.2% Vit. B mix (2-4-9-10), 0.05% dry stabilized Vit. A palmitate and Vit. D_2 mix (as Quaknex IV, Nopco, 10,000 units of A and 1250 units of D_2 per g); 0.1% dry stabilized Vit. E acetate (20,000 units/lb); 0.5% Vit. B_{12} and antibiotic mix (Aurofac).

year on diets containing beef tallow and safflower seed oil.

Materials and methods. Twelve weanling miniature pigs were fed a low fat basal diet for 3 weeks and then were divided into 2 groups. One group was supplemented with 0.5% cholesterol and 15% beef tallow and the other with the same amount of cholesterol and 15% safflower oil. The composition of the diets is shown in Table I. Diets were fed on an equal basis of calories/kg of body weight. The animals were equilibrated with these diets for one year. The average weight of the animals of the tallow group was 310 lb. and that of the safflower group, 260 lb. The general appearance of all the animals was very good. Fasting blood samples were taken in the morning. Lipemic blood was taken 3 hours after feeding following an overnight fast. Samples of blood were obtained from the anterior vena cava with siliconized needles and syringes by the 2-syringe technique.

Whole-blood clotting time was measured by a modified 2-tube Lee-White method (2 ml whole blood per tube). A stop watch was started at the instant the needle was withdrawn from the animal. The first tube was tilted at one-minute intervals until a firm clot was obtained. The second tube which was kept undisturbed was thereafter tilted at 30-second intervals until clotting occurred. The clotting time of the second tube was taken as the whole-blood clotting time.

The silicone clotting time was measured in a single siliconized tube containing 2 ml of blood. The tube was tilted at 30-second intervals after clotting had occurred in unsiliconized tubes, and the clotting time recorded.

Plasma was prepared by mixing blood with a 20% sodium citrate solution (0.2 ml/10 ml blood) and centrifuging at 2500 RPM for 15 minutes. Recalcified plasma clotting time (R. P. C. T)(8), prothrombin time (one-stage, using simplastin‡)(9), stypven§ time (10), and plasma optical density at 640 mμ were determined.

Results. Table II shows the mean whole-blood clotting times in glass and silicone coated tubes. The optical density of the fasting plasma obtained from both groups of pigs was almost identical and indicative of the absence of hyperlipemia. Yet clotting time of the tallow group was shorter than that of the safflower group both in the silicone tube ($P < 0.05$) and in the glass tube ($P < 0.025$). In the lipemic state, *i.e.*, 3 hours after feeding, the shortening of the clotting time in the tallow group was much more pronounced ($P < 0.001$). However, the plasma turbidity of the tallow group was much higher than that of the safflower group. Consequently, the shortening of the clotting time of the tallow group might be related in part to the degree of lipemia.

The results of the recalcified plasma clotting times (R. P. C. T.), prothrombin times and stypven times of both groups in the fasting and lipemic states are shown in Table II. In the fasting state, the R. P. C. T. of the tallow group was much shorter than that of the safflower group, whereas there was no significant difference in prothrombin and stypven times between the two groups. In the lipemic state the R. P. C. T. of the tallow group was much shorter than that of the safflower group. Although there was no difference in prothrombin time between the two groups, the stypven time was shortened

‡ Warner-Chilcott Div., Morris Plains, N. J.

§ Burroughs Wellcome & Co., Tuckahoe, N. Y.

TABLE II. Blood Coagulation of Miniature Pigs Equilibrated for One Year on Diets Containing Beef-Tallow or Safflower Oil.

Group	Fasting state A Safflower oil	Fasting state B Tallow	Significance of differences: A – B	Lipemic state† C Safflower oil	Lipemic state† D Tallow	Significance of differences: C – D
Whole-blood clotting time (min):						
Silicone tube	20.1 ± 2.44*	14.9 ± 4.9	P <.05	13.6 ± 2.1	4.6 ± 2.6	P <.001
Glass tube	11.5 ± .75	9.3 ± 1.6	P <.025	10.25 ± 1.3	4.2 ± 2.4	P <.001
Plasma O.D.	.12± .02	.122± .03		.218± .09	.406± .19	
R.P.C.T. (sec)	78.4 ±14.8	53.8 ± 5.6	P <.005	60.8 ±11.2	35.8 ± 1.9	P <.001
Prothrombin time (quick) (sec)	14.9 ± 1.3	15.2 ± .9	N.S.‡	14.2 ± 1.2	13.5 ± 1.6	N.S.
Stypven time (sec)	21.8 ± 2.9	20.5 ± 2.1	"	19.3 ± 2.2	15.2 ± 1.9	P <.01

* In all columns, refers to mean ± standard deviation.
† 3 hr after feeding following overnight fast.
‡ Not significant.

in the animals receiving tallow. Again, it should be pointed out that both fats were capable of shortening the R. P. C. T. and stypven time 3 hours after ingestion of the respective fatty meals, and the reduction of these values brought about by tallow was much more than that induced by safflower.

Discussion. The influence of various fat-containing diets on blood coagulation has been studied by several investigators to determine the possible relationship of diet to thrombosis. Most of these studies have been concerned with the effects on coagulation of a single fatty meal or very short term feeding. The present work is concerned with measurements of coagulation times of blood of miniature pigs maintained for one year on diets containing 2 different fats: 1) beef tallow, a saturated type of fat, and 2) safflower oil, an unsaturated type of fat. The experimental diets were designed to approximate the normal human diet in that about 40% of the calories were fat calories. The animals were equilibrated on these diets for one year so that lipid distribution in the blood of these animals would reflect, to some extent, the nature of the dietary lipids. Lipid analyses will be reported later.

Even though no hyperlipemic condition was observed in the fasting state, the mean whole-blood clotting time of the tallow group was shorter than that of the safflower group, in both glass and silicone tubes. In the lipemic state, both fats shortened the whole-blood clotting times in glass and silicone tubes; the effect produced by tallow was greater than that produced by safflower oil. This could be ascribed to the higher degree of lipemia observed in the plasma of the tallow group. However, it is doubtful whether the same degree of lipemia would produce identical clotting times in both groups. Correlation of clotting time with degree of lipemia in both groups needs further study.

The mean R. P. C. T. of the tallow group is also shorter in fasting and lipemic states. There is no difference in the stypven time of both groups in the fasting state, whereas it is shortened in both groups 3 hours after feeding their respective diets. In the lipemic state, the stypven time of the tallow group was much lower than that of the safflower oil group ($P < 0.01$). Perhaps this difference is the result of the difference in the plasma turbidity levels of the two groups. If so, this is consistent with the view that the stypven time test is a rough index of lipemia(11). There was no difference in prothrombin time between the two groups either in the fasting state or 3 hours after ingestion of their fatty meal.

Prothrombin time is not affected by lipemia, nor is it useful to detect differences in coagulability brought about by saturated and unsaturated fats. Although the stypven time is affected by lipemia, its usefulness for the latter purpose still remains to be demonstrated. The R. P. C. T. on the other hand is

significantly different in the two groups, that of the tallow group being much shorter than the safflower oil group. The mechanism by which dietary fats influence clotting times including stypven and recalcification times is not known. Studies on the influence of dietary fats on various clotting factors and on rate of thromboplastin generation in miniature pigs are in progress.

Summary. Miniature pigs maintained for one year on diets containing 15% of either beef tallow or safflower oil did not exhibit lipemia in the fasting state, yet the clotting time was much shorter in the tallow group than in the safflower group. The clotting time was shortened 3 hours after ingestion of either fat, but the effect produced by the saturated fat was much more pronounced than that produced by the unsaturated fat. The recalcified plasma clotting time was also a satisfactory measure of the increase in coagulability brought about by dietary fats, and served to detect differences in coagulability brought about by saturated and unsaturated fats.

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Partial Explosion of Erythrocytes, Induced by Hyaluronic Acid.* (28654)

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Inorganic acids and alkalis hemolyse different erythrocytes. Acids may elicit at certain pH a transitory "fixation-effect" without causing any unusual morphological changes; alkalis do not induce any fixation. We assumed a transitory "fixation-effect" with some of the inorganic acids, because the hemolysis observed under phase-contrast was not instantaneous as with alkalis. In studying the action of inorganic and organic acids, we observed a surprising but consistent phenomenon on various erythrocytes, when hyaluronic acid was added. Instead of hemolysis, which might be expected at an acid reaction, the hemoglobin exploded in microscopically well visible granules. In a few seconds several hundred granules were extruded through one sector of membrane. The liberated granules showed a vigorous brownian movement. The membranes retained some of the hemoglobin. The membranes were sharply visible in phase-contrast revealing single or multiple empty splits, or, occasionally, buds filled with hemoglobin.

Materials and methods. Sheep, beef and horse erythrocytes were obtained from defibrinated blood and washed 3 times with 0.85% NaCl solution. One per cent suspension of washed erythrocytes was used for phase-contrast examinations. Human erythrocytes were also examined unwashed in a similar concentration.

Hyaluronic acid (highly polymerized from human umbilical cord) and lyophilized hya-

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