tion procedure (step 8). Metabolic heterogeneity of the histones was indicated by the differences in the specific activities of the histone fractions in both liver and hepatoma. Each of the protein fractions of the nuclei of the liver cells had higher specific activities than corresponding protein fractions of the tumor with the notable exception of the histones which were in the reverse relationship. This difference is most readily seen in the ratios of the specific activities of protein-glycine in various protein fractions of the tumor to those of corresponding fractions of liver. These ratios are: total cytoplasmic proteins, 0.54; nuclear fraction I, 0.53; nuclear fraction II, 0.22; nuclear fraction III, 0.52; nuclear histone II, 1.8; nuclear histone III, 1.6. It is not surprising that labelled glycine was incorporated to a greater degree into most of the protein fractions of liver than into corresponding fractions of the hepatoma since the transport of the intraperitoneally injected amino acid to the liver probably would be more favorable than the transport to the subcutaneously transplanted hepatoma. However, the fact that the histones were in the opposite relationship to the other proteins and had higher specific activities in the tumor than in the liver was unexpected, and this observation is of considerable interest. These data suggest that the histones may play a particularly important role in the nuclei of the hepatoma cells. However, further work will be required to determine whether this relationship is characteristic of tumors or whether it is fundamentally characteristic of rapidly dividing cells as contrasted with non-dividing or slowly dividing cells.

Summary. A procedure is described for the separation of the proteins of cell nuclei into several fractions which differ in solubilities and in glycine content. Twenty hours after intraperitoneal injection of glycine-l-C^{14} into adult rats bearing subcutaneously transplanted hepatomas the specific activities of the protein fractions of the nuclei of liver and tumor were in the descending order: residual lipoprotein > globulins > histone fraction III > histone fraction II. Each of the protein fractions of the nuclei of liver cells had higher specific activities than corresponding protein fractions of the hepatoma with the notable exception of the histones which were in the reverse relationship.

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Methods for Comparing Effects of Various Fats on Fibrinolysis.* (23385)

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Recently, much attention has been directed toward differing biologic effects of various common dietary fats. Ahrens(1,2), Beveridge(3,4), Bronte-Stewart(5), and others have shown in man that fatty meals of substances such as butter (a highly saturated fat

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containing only small amounts of essential fatty acids) will elevate cholesterol levels of blood whereas meals of substances such as corn oil (a relatively unsaturated fat containing a large amount of essential fatty acids) will lower them. The effect of these alterations in cholesterol levels of blood on tissues is not yet known. Maclagan and Billimoria (6), following an entirely different line of inquiry have found in *in vitro* experiments (using human plasma and various fats in test tubes and adding Russel-viper venom) that butter has a profound effect on clotting; that lard, oleomargarine, and several other fatty substances have a lesser effect; and that olive oil and several other fats have none. Keys, Buzina, Grande and Anderson found that fatty meals of butter, coconut oil and corn oil decreased siliconized tube clotting time approximately equally but that a meal of marine fish oil had a lesser effect(7).

We have produced myocardial or renal infarcts by dietary means in 10 of 17 rats given a diet containing 40% butter or lard by weight plus cholesterol, sodium cholate and thiouracil(8). None of 9 animals fed 20% corn oil with 20% Crisco (plus the same quantities of cholesterol, sodium cholate and thiouracil given to the rats receiving butter or lard) developed myocardial or renal infarcts. We are now attempting to extend these results with more animals. It is important to utilize many approaches in evaluating the biologic effect of various fatty substances. Therefore we have followed other lines of investigation and have studied *in vivo* and *in vitro* the effect of various types of fatty meals on the fibrinolytic mechanism in rabbits. The results of studies *in vivo* have been presented (9). We demonstrated in rabbits that intermittent fatty meals of butter or oleomargarine greatly increased (as compared with controls) number of thrombi that organize instead of lysing after repeated intravenous injections of standard amounts of clot. Similar fatty meals of corn oil had no effect.

In experiments reported herein 2 new approaches have been utilized. In Exp. 1, using a partially *in vitro* method, the effect in rabbits of single meals of butter, corn oil, coconut oil or water on fibrinolytic activity of blood was measured by a technic utilizing streptokinase to activate conversion of profibrinolysin (10) to fibrinolysin. In Exp. 2, we have modified this technic to study the effect by a completely *in vitro* method of various fats on streptokinase-lysis times of clotted human plasma. Results of both experiments indicate that butter significantly lengthens streptokinase-lysis time (under conditions specified) but that corn oil and coconut oil have little or no effect.

Materials and methods. Exp. 1. Ninety-two young adult rabbits (New Zealand White) of both sexes, weighing 2.5 kg or more were used. They were offered Purina rabbit pellets daily and water *ad libitum*. A tube was passed transorally into the stomach of each rabbit and 25 ml of either butter, corn oil, coconut oil or water introduced. Three hours later, 25 to 30 ml of blood were obtained from each rabbit by cardiac puncture and placed in a tube containing 2 mg of potassium oxalate per ml of blood to prevent clotting.

Briefly stated, the test‡ used for lytic activity consisted of adding to bovine thrombin and streptokinase a portion of oxalated blood and mixing thoroughly. The thrombin rapidly produced a clot which the streptokinase lysed presumably by activating profibrinolysin. Time required for lysis was recorded for each sample and average lysis times in various groups (butter-fed, water-fed, etc.) were compared.

Details of the procedure are: 5.5 units of bovine thrombin were used for each ml of oxalated blood; under our conditions, this amount clotted the blood in less than 30 seconds. Quantities of streptokinase varied because vials differed in activity making it necessary to alter the amount when new ones were required. However, animals were so arranged that streptokinase from each vial was used for all groups (butter-fed, water-fed, corn oil-fed). Thus variations in vials were

‡ This test was suggested to us by Dr. Sol Sherry, Professor of Medicine at Washington University. Dr. Sherry also provided us with a purified streptokinase preparation made by Lederle Laboratories. Bovine thrombin used was a commercial preparation sold by Parke Davis.

distributed and not concentrated in a single group. The amount of streptokinase to be used was determined by preliminary testing and was that quantity necessary to produce lysis within 10 to 15 minutes in random blood samples from the water-fed group. In most tests 325 units were employed but in one vial only one-tenth of this amount was required. Nine ml of blood were used in most tests although in some only half this amount, and thrombin and streptokinase were reduced accordingly. All samples were numbered so that the examiner did not know the groups from which each came until after lysis times had been recorded. After the blood had been mixed with streptokinase and thrombin in 19 mm tubes, test tubes containing the clots were placed in a water bath at 37° and examined every 2 or 3 minutes by holding the tube up to a light. Each time the clot was examined, the tube was rotated and after lysis began it was also shaken to insure thorough mixing. The clot slowly divided into small clumps which eventually became fluid. "Lysis time" in this study was the time that elapsed between clotting and the return of blood to a fluid state containing particles not larger than one millimeter. A few samples from all groups failed to clot (or lyse) at all and were discarded.

Exp. 2. For the second experiment 5 ml of blood were obtained by venipuncture from 46 healthy individuals. Their average age was 33 years (range 23 to 51), and most (88%) were males. Blood was placed immediately into tubes containing 2 mg of potassium oxalate for each ml to prevent clotting. Erythrocytes were allowed to settle or were centrifuged down and the plasma removed. Subsequent procedures were carried out with plasma rather than with whole blood (which was used in Exp. 1). Plasma from each individual was divided into 4 samples of 0.45 ml each (for studies with butter, corn oil, coconut oil, and saline). Amount of bovine thrombin and streptokinase to be used was determined by preliminary testing of each vial as described in Exp. 1. In general, amount of thrombin used was that required to produce clotting in 30 to 40 seconds, and enough streptokinase was added to cause lysis of the clot within 30 to 50 seconds in samples of plasma to which only saline was added. Plasma from each individual was tested with all 4 substances (butter, etc.) concurrently, using identical amounts of thrombin and streptokinase.

Fats to be tested were commercial products (unsalted butter,§ corn oil‖ and coconut oil¶) obtained from retail firms. A small sample of each was placed in individual tubes in a 37°C water bath for one-half hour before being tested along with a tube containing saline. Butter began to melt at approximately 29°C, while other substances were, of course, in a liquid state even at room temperature. The melted butter separated into a clear supernatant layer and a turbid layer which were mixed by shaking before using. Equal amounts of the various fats and saline were used for each test. In half, this amount was 0.2 but in the other half only 0.05 ml was used.

Briefly stated, the test for the effect of the various substances on fibrinolysis was: appropriate quantities (as described above) of bovine thrombin and streptokinase were placed in 4 test tubes. Butter, corn oil, coconut oil, or saline in equal amounts were added. Plasma (0.45 ml) was added to each tube using a blow-out pipette, the mixture shaken vigorously and placed in the water bath at 37°C. As soon as the mixture appeared to be in a gel state, clotting was considered complete. After the clot had formed, the tube was removed from the water bath every 5 or 6 seconds for shaking and naked-eye inspection with a light behind the tube. Part of the clot rapidly liquified and the remaining solid portion gradually disintegrated. "Lysis time" in this study was that number of seconds measured by a stop-watch for the clot to become completely liquid except for residual threads or minute particles which persisted indefinitely.

Results. Exp. 1. Average lysis times are given in Table I for all 92 rabbits. Although individual variations occurred, average time

§ Sealtest

‖ Mazola Oil.

¶ Magnus, Maybee & Reynard.

TABLE I. Average Clot-Lysis Times (Streptokinase Method) for Several Groups of Rabbits Given Single Meals of Butter, Corn Oil, Coconut Oil or Water.

Type of meal	No. of animals in group	Avg lysis times (min.)	Stand. error of mean
Butter	31	22.0	1.47
Water	23	15.0	1.53
Corn oil	16	15.5	2.63
Coconut oil	22	13.0	1.62

Avg lysis time for the group fed butter is significantly longer than that for any other group. Differences in lysis time among the other groups are not statistically significant.

for the group that received butter was 22 minutes, significantly higher than that for any other group (using the t test $p = 0.01$ for comparisons between butter and water and between butter and coconut oil; $p = 0.05$ for comparison between butter and corn oil). Average lysis times for the groups receiving corn oil (15.5 min) and coconut oil (13 min) do not differ significantly from that of the group receiving water (15 min). Plasma from rabbits given fatty meals did not appear fatty. Plasma cholesterol levels of many samples were never above 116 mg%.

Exp. 2. Results are presented in Tables II and III. Average lysis time in seconds for samples of human plasma containing 0.2 ml of the various substances were: butter 158, corn oil 68, coconut oil 60, saline 43. Corresponding times when 0.05 ml of lipids or saline were added were: butter 176, corn oil 68, coconut oil 63, saline 37. With either 0.05 or 0.2 ml of fat added, butter greatly lengthens lysis time as compared with all other substances tested ($p = 0.01$ as determined by the t test). The minimum amount of any fat tested that would be required to produce a significant lengthening of the lysis time was not determined; in each case, however, it was found that 0.05 ml of lipid produced as great an effect as 0.2 ml. Lysis time of human plasma with corn oil and coconut oil was significantly longer than that with saline with either 0.2 or 0.05 ml of lipid added (using the t test $p = 0.01$ for comparisons with saline and corn oil; for comparison with saline and coconut oil $p = 0.01$ with 0.05 ml added and $p = 0.05$ with 0.2 ml added). Difference in lysis times using corn oil and coconut oil was not significant ($p = 0.1$ with both 0.2 ml and 0.05 ml added).

TABLE II. Average Streptokinase-Lysis Times of Clotted Human Plasma after Addition *In Vitro* of 0.2 ml of Various Fats to 0.45 ml of Plasma.

Substances tested	No. of samples	Avg lysis time (sec.)	Stand. error
Saline	23	43	5.6
Butter	23	158	12.1
Corn oil	23	58	4.9
Coconut oil	23	60	6.2

Lysis times for butter, corn oil and coconut oil are significantly longer than that for saline. Difference in lysis times between corn oil and coconut oil is not significant but differences between butter and corn oil and between butter and coconut oil are.

TABLE III. Average Streptokinase-Lysis Times of Clotted Human Plasma after Addition *In Vitro* of 0.05 ml of Various Fats to 0.45 ml of Plasma.

Substances tested	No. of samples	Avg lysis time (sec.)	Stand. error
Saline	23	37	2.1
Butter	23	176	16.7
Corn oil	23	68	6.8
Coconut oil	23	63	4.5

Lysis times for butter, corn oil and coconut oil are significantly longer than that for saline. Difference in lysis times between corn oil and coconut oil is not significant but differences between butter and corn oil and between butter and coconut oil are.

Discussion. Our experiments provide further evidence that various fats (butter, corn oil, coconut oil) differ in their biologic effects. The significance of differences reported by others and herein in relation to development of arteriosclerosis and thrombosis in man is not yet clear. Broad conclusions and recommendations are not warranted. However, in view of possible implications for man, some tentative conclusions are suggested.

It must be emphasized that procedures so far used to evaluate the biologic effects of various fats are highly artificial. Also, although butter and corn oil have been studied most extensively, other fatty substances may have similar biologic effects or at least differ only in degree (for example, the coconut oil used here). Further, butter, corn oil and other commonly used fats are actually complex mixtures of many fatty substances. Additional investigation may disclose that highly specific

constituents (that can be added or removed at will) are responsible for the differing biologic effects. At least in the experiments reported herein differences cannot be simply a reflection of degree of saturation since coconut oil (highly saturated) had no demonstrable effect whereas butter, (another highly saturated fat) had a significant effect.

Definitive conclusions cannot now be drawn but facts thus far discovered cannot be ignored. At least 5 different approaches have been utilized in comparing biologic effects of various fats: (1) on cholesterol levels in man (1-5); (2) on clotting of human blood *in vitro* (6); (3) on production of myocardial infarcts in rats by dietary means(8); (4) on thromboembolic-induced pulmonary arterial lesions in rabbits(9); (5) on fibrinolytic activity of blood of rabbits and man (reported herein). In every instance butter and similar substances have had an "undesirable" effect whereas in most instances corn oil and similar substances have had either a negative or a "desirable" effect. We believe available information regarding differences in biologic effects of fats such as butter and corn oil is impressive enough to make imperative an immediate, concentrated effort to obtain definitive data regarding the importance of the differences.

Our interest in the effect of a substance on the fibrinolytic system stems from the fact that the precipitating cause of death or disability in individuals with arteriosclerosis is usually thrombosis. If it could be prevented or if thrombi once formed could be lysed rapidly, victims of arteriosclerosis might live quite satisfactorily with their lesions. Thrombosis seldom occurs in normal vessels with smooth linings but frequently occurs in roughened arteriosclerotic vessels. The body has a defense mechanism in the form of a fibrinolytic system for rapidly lysing thrombi. It is obvious that factors influencing this system are exceedingly important, particularly to individuals with arteriosclerotic plaques.

Summary. In experiments reported herein, 2 new methods have been utilized to compare the biologic effect of 3 dietary fats. (1) In Exp. 1 a study has been made in rabbits of the effect of single meals of butter, corn oil, coconut oil and water on fibrinolytic activity of blood (drawn 3 hours after feeding and measured by a technic utilizing streptokinase to activate profibrinolysin) *in vitro* under controlled conditions. Lysis time so measured was significantly prolonged in rabbits fed butter as compared with those fed either corn oil, coconut oil or water. Meals of corn oil and coconut oil had no more effect on lysis time than did water. (2) In Exp. 2 the effect of butter, corn oil, coconut oil and saline added *in vitro* on fibrinolytic activity of clotted *human plasma* was measured by a technic utilizing streptokinase to activate profibrinolysin under controlled conditions. Under these conditions butter markedly and significantly ($p = 0.01$) prolonged lysis time of human plasma, whereas corn oil and coconut oil had a lesser effect. (3) Other substances and approaches must be studied before definitive conclusions can be drawn regarding relationships of various fats to arteriosclerosis and thrombosis in man. Our experiments are significant in that an additional approach (which may be particularly useful for rapid screening) has been added to our armamentarium for study of biologic effects of various fats.

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Metabolic Effects of Phenethyldiguanide, A New Hypoglycemic Compound.* (23386)

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With the introduction of the sulfonylureas for treatment of diabetes mellitus, renewed attention has been paid to the use of orally effective compounds. The sulfonylureas have proven to be useful in selected diabetic patients, but in a significant number of subjects they have failed to control the metabolic imbalance. Therefore, a considerable effort has been made to find other compounds which might be more effective than the sulfonylureas. In a search for such compounds, Ungar (1) recently discovered that phenethyldiguanide (PEDG), administered orally or parenterally, caused profound hypoglycemia in certain animals. This interesting observation prompted investigation of the mechanism by which hypoglycemia is produced. An understanding of the mode of action of PEDG would be of great help in its clinical applications. In an attempt to elucidate this problem, we have made studies of the effect of PEDG on glucose, glycogen, lactic acid, urea and non-protein-nitrogen *in vivo* and *in vitro*.

Materials and methods. Guinea pigs weighing between 450 and 750 g were used in these studies because they have been shown to be more sensitive to PEDG† than other animal species(1,2). In each type of experiment at least 24 animals were sacrificed, 12 serving as experimentals and 12 as controls. The drug was injected subcutaneously because responses after administration by this route were more uniform than after oral or intraperitoneal intake. The dose used was 20 mg/kg.

The following determinations were made 4 hours after PEDG administration as well as at longer and shorter intervals: blood glucose by the method of Somogyi(3), liver and muscle glycogen, using KOH for tissue digestion by the anthrone reagent of Carroll *et al.*(4), blood urea nitrogen by the method of Barker (5) and blood lactic acid by the method of Barker and Summerson(6). Non-protein-nitrogen in the total amount of urine produced during the 4 hours following PEDG injection was determined by the method of Fee *et al.*(7). In order to collect all of the urine, a small abdominal incision was made, the bladder emptied and the urethra ligated just before PEDG was given. Four hours later the urine in the bladder was collected with a needle and syringe.

The first part of the study *in vitro* was carried out on guinea pig liver slices. Slices weighing approximately 100 mg were incubated for 45 minutes at 37° in 2 ml of a solution containing 4 parts of 0.9% NaCl and one part of 0.1 M potassium phosphate buffer, pH 7.5. The beakers were placed in a Dubnoff apparatus and shaken at a rate of 110 oscillations per minute. Three different concentrations of PEDG were tested, at 50, 250 and 500 μg per ml of medium. At the end of the incubation, glucose and lactic acid concentration in the medium and glycogen concentration in the tissue were measured, using the same chemical methods mentioned in the *in vivo* studies.

The second part of the *in vitro* study was conducted with isolated rat diaphragms.

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