There seems to be no doubt, therefore, that for experiments such as described testosterone propionate can be more potent for some tissues by the intraperitoneal route than when given subcutaneously. Interestingly enough, when similar doses are used for longer periods of time (*e.g.* from 26 to 80 days) the seminal vesicle may respond better to subcutaneous than to intraperitoneal injections.[8]

Summary. A comparative study of the potency of testosterone propionate administered intraperitoneally and subcutaneously in 45 respective litter mate male albino rats has been made. Injections of 50 γ daily dosage were given for 10 days from 22 to 32 days of age. Body weights before and after the experiment, final seminal vesicular- and testicular-weights were statistically compared. It was found that the intraperitoneally-injected animals gained 11% less weight, and showed a 274% *increase* in seminal vesicular growth, and a 41.9% *decrease* in testicular size over their controls. The subcutaneously injected animals showed practically no difference in gain in weight, a 185% *increase* in seminal vesicular weight, and a 52.8% *decrease* in testicular size compared to their controls. The differences observed for seminal vesicular and testicular weights were statistically "probably significant." The diminished weight increase observed for the intraperitoneally-injected animals while not statistically significant was sufficiently large to indicate a trend.

It is concluded that under the conditions of the experiments cited, testosterone propionate may vary in potency in accordance with its method of administration. But generalizations are as yet invalid since the greater testicular effect (depression) was observed by subcutaneous injection, and the greatest seminal vesicular response (stimulation) resulted from intraperitoneal administration.

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[8] Rubinstein, H. S., unpublished data.

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A Study of Clot Firmness in Viscometer Tubes.

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Determinations of clot firmness have been made by studying the tensile strength of clots. Fonio[1] prepared plasma clots with magnesium sulfate solution and ether. He pressed the clots into discs, suspended them, and determined their tensile strength. Kristenson[2] who criticized this method used clots from plasma which was prepared by centrifugation in ice jackets. Following incubation at 37° C. for 1 hour he wrapped both ends of the coagulum in linen cloth and tied a string around each end. He then measured the tensile strength by adding weights until the clot tore. Schnedorf[3] permitted blood to coagulate around two wire meshes which served as points of attachment. Five to 30 minutes after coagulation at room temperature, weights were added to measure the tensile strength. By employing different amounts of blood and using several modifications of the same principle, he was unable to obtain comparable results. To test clot firm-

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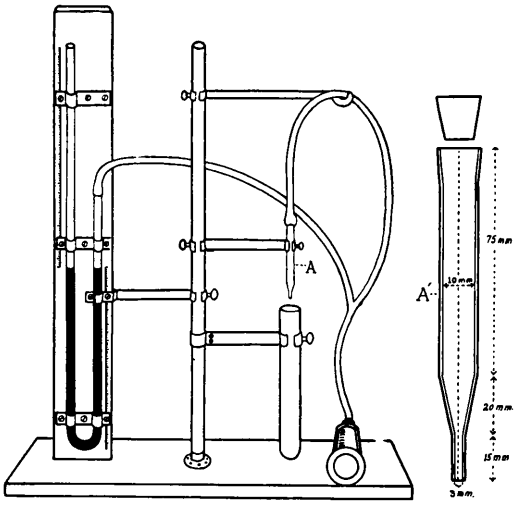
[1] Fonio, A., *Schweiz. med. Wchnschr.*, 1921, **11**, 146.

[2] Kristenson, A., *Acta med. Scandinav.*, 1932, **77**, 351.

[3] Schnedorf, J. G., personal communication, 1942.

ness we constructed a viscometer tube which measures the flow properties of blood clots. Results obtained in this way were sufficiently uniform and exhibited a measurable difference in clot firmness.

CLOT FIRMNESS APPARATUS.



Method. Glass tubes (A'; Fig. 1) are constructed with a wide portion of 10 mm and an outlet 3 mm in internal diameter. The axial length of the tube is 11 cm, its outlet being 1.5 cm, its conus portion 2 cm and the wide part 7.5 cm long. A "00" rubber stopper is fitted into the top, the tube is inverted and filled with 5 cc of blood through the outlet. Admixture of air bubbles with the blood must be prevented. This is very important, since we found that mixing air bubbles with the blood may change the firmness of the clot significantly. Tissue juice which enters the syringe initially can affect the firmness of the clot. For this reason, when 15 cc of blood were drawn, 2 tubes were filled and the remaining 5 cc were discarded. The coagulation time is determined by slightly tipping the tube at intervals of 30 sec. After the blood has coagulated, the tube is incubated at 37°C for 30 min. The viscometer tube is inverted while the stopper is removed, and the retracted clot and the serum are allowed to settle against the outlet. The tube is placed into a clamp with the outlet directly above a vessel that catches the tested material (Fig. 1). The viscometer tube is then connected to a vasolined 50 cc syringe and to a U tube mercury manometer capable of measuring 450 mm of Hg pressure. Pressure is slowly increased with the syringe by increments of 10 mm of Hg until the clot is forced out of the viscometer tube at a rate, if possible, of 5 mm per min. The pressure necessary to produce this rate of flow is taken as the index of firmness of the clot. Blood was drawn from the antebrachial vein of humans, the heart of rabbits, and the jugular and femoral veins of dogs.

Results. Similar tubes of 8 and 10 mm in internal diameter correspondingly required

TABLE I.
Agreement of Clot Firmness of Blood Withdrawn on Different Days from the Same Animal.

No. of blood withdrawals	Clot firmness in mm of Hg pressure						
	Dog I	II	III	IV	Rabbit I	II	III
1	130	170	160	420	450	320	440
	100	170	140	400	440	300	440
2	100	210	160	360	410	300	380
	100	210	150	370	380	320	320
3	100	230	140	350		460	420
	100	200	120	340		260	360
4	140	210	130	250		280	
	120	210	130	400		290	
5	190	240	140	270			
	120	200	160	300			
6	130	210	130				
	140	200	160				

TABLE II.
Coagulation Time and Clot Firmness of Blood from One Hemophiliac.

May 5, 1942			May 11, 1942		
Tube	Coagulation time, min	Clot firmness, mm of Hg	Tube	Coagulation time, min	Clot firmness, mm of Hg
1	48	50	1	91	10
2	16	110	2	85	0
1*	88	15	3	91	0
2*	88	10	4	91	0
3*	78	0	5	94	10
4*	23	90	6	33	30

*Blood from the second withdrawal.

different pressures to force the clots through an outlet, 3 mm in internal diameter. Clots from the same dog were incubated in 8 mm and 10 mm tubes for 30 min. Pressures of 30 to 40 mm of Hg were needed in 12 different 8 mm tubes, while 12 different 10 mm tubes required pressures of 100 to 140 mm of Hg to force the clots out.

The effect of incubation on clot firmness was studied in 3 normal dogs and 1 normal rabbit. Six 10 mm tubes were filled from the same withdrawal in each case. Duplicate determinations of clot firmness were done after 30, 60, and 120 min. The findings showed that a period of 30 min suffices to produce maximal clot firmness, and that incubation of 2 hr did not further alter the clot firmness.

Table I demonstrates that duplicate tests done on clots from the same animal on different days are reproducible. In a few instances the values do not agree closely, however, agreement can be obtained by repeating the test. It may be noted that dog IV whose clots were firmer than those of the 3 normal dogs was operated 3 weeks previously. Blood clots of normal rabbits constantly exhibited greater firmness than those obtained from normal dogs or humans. In a group of 8 normal humans clot firmness varied from 80 to 160 mm of Hg. Results from duplicate tests in this group agreed within 30 mm of Hg.

Table II shows the coagulation time and clot firmness in blood obtained from one hemophiliac. The clots were incubated from 30 to 40 minutes at 37° C. On the first day during the first withdrawal 10 cc of blood were drawn with difficulty. The blood which was emptied last from the syringe had the shortest coagulation time and a firm clot. On the second withdrawal 20 cc of blood were obtained. In this instance blood emptied last from the syringe also had the shortest coagulation time. Clot firmness in the last tube was within the range found with clots from normal subjects, while the clots in the remaining 3 tubes were soft. Six days later 30 cc of blood were drawn without difficulty. At this time the last tube showed the shortest coagulation time and the greatest clot firmness, even though all clots were soft. No pressure was necessary for 3 clots, whereas in 3 other clots only minimal pressure was required.

Comment. We realize that this clot firmness test is empirical. For this reason the viscometer tubes have to be standardized to obtain comparable results. By this means, one determines the applied stress at which the blood clot begins to flow. Beyond this yield value,[4] which determines the firmness of the clot, we did not study the flow properties of blood clots. Clot firmness may vary from the same withdrawal especially when the coagulation time is prolonged and the blood is mixed with tissue juice. When 5 cc of blood which entered the syringe first were discarded, the difference in duplicate tests became minimal. We found that clots from normal animals required 30 min incubation at 37° C to attain their maximal firmness. Incubation from 30 to 120 min did not produce significant changes in the firmness of blood clots from normal animals. After the blood

[4] Copley, A. L., Krchma, L. C., and Whitney, M. E., *J. Gen. Physiol.*, submitted.

gelates completely, incubation for 30 min is necessary, since at temperatures of 20 to 25° C maximal firmness may not develop within 1 hr. It is of interest that in hemophilia both the clot resistance[5] and the clot firmness are decreased. Blood clots from 2 heparinized rabbits exhibited firmness equal to 10 to 40 mm of Hg which was not affected by 2 hr of incubation.

An exploratory study on clot firmness was done on blood clots of a "dicoumarinized" patient with a history of recurrent thrombophlebitis. Following therapy with 3,3′-methylenebis (4-hydroxycoumarin) the coagulation time and prothrombin time were prolonged, whereas the bleeding time and clot resistance were normal. The clot firmness equalled 70 mm of Hg after 40 min incubation and increased to 200 mm of Hg by 80 min. This phenomenon of an increase in clot firmness after 30 min incubation was observed on 3 separate occasions in blood clots from this patient. We believe this could be due to a delayed formation of fibrin, although it is possible that the change might occur subsequently in the fibrin itself. These findings, and those in normal animals, indicate that a clot which forms in a tube continues to increase in firmness for a period of time.

[5] Copley, A. L., and Lalich, J. J., *J. Clin. Invest.*, 1942, **21**, 145.

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Influence of Essential Fatty Acid Deficiency on Transport of Fatty Acids into the Liver.*

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Although it is well recognized that linoleic acid is not synthesized by the animal body and is a dietary essential for several species of laboratory animals there is no evidence as to the metabolic function of this fatty acid. The common observation that linoleic acid is a normal and apparently ever-present constituent of tissue phospholipids led Burr and Burr[1] to consider the possibility that linoleic acid is necessary for the normal metabolic functioning of tissue phospholipids. The nature of the functions of phospholipids is not definitely known, but one well recognized characteristic is the exchange of phosphorus and fatty acids in the phospholipid molecule. Two attempts have been made to study the rate of phospholipid turnover in the tissues of rats suffering from a deficiency of the essential fatty acids, by Hevesy and Smedley-MacLean,[2] using radioactive phosphorus as a tracer, and by Barnes, Miller, and Burr,[3] using labeled (spectroscopically active) fatty acids. In the labeled acid study essential fatty acid deficiency resulted in a slight decrease in the rate of fatty acid incorporation into the phospholipids of the intestinal mucosa, hence it was thought desirable to extend this type of study to other tissues. The present investigation was undertaken to study fatty acid incorporation into the liver phospholipids of rats subjected to a fat deficient regimen.

Details of the various methods employed

* Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration, Official Project No. 265-1-71-236, Subproject 612.

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

[2] Hevesy, G. C., and Smedley-MacLean, I., *Biochem. J.*, 1940, **34**, 903.

[3] Barnes, R. H., Miller, E. S., and Burr, G. O., *J. Biol. Chem.*, 1940, **140**, 773.