species.[12] It appears, therefore, that intravenous carbohydrate tolerance curves obtained on healthy pigs are quite similar to those procured on normal human subjects.

[12] Bell, F. R., and Jones, E. R., *J. Comp. Path.*, 1945, **55**, 117.

Summary. The form of the carbohydrate tolerance curve obtained after the administration of glucose to normal growing pigs by the intravenous route, apparently closely parallels the type of curve procured when the test is made on normal human subjects.

15860

Thromboplastic Activity of the Urine.

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Hemophilics having hematuria often experience severe attacks of renal colic and pass thin clots obviously representing ureteral casts. Since the clotting time of the blood of one such patient observed in January 1947 was in excess of 2 hours, the formation of such clots within the ureter was difficult to explain, unless blood stagnation existed because of obstructive lesions, or the urine itself exerted a clot accelerating action on the blood. The presence of thromboplastic substances in the tissues of the body, in the saliva from normal[1,2] and hemophilic men[3] and in human milk[4] is known. No mention of the presence of these substances in the urine has been found, and there seems to be no adequate explanation of the mode of formation of clots in the renal pelvis of hemophilics with hematuria.[5-10] The evidence to be presented indicates that urine from normal or hemophilic men contains a thromboplastic substance. Contact with such a substance is probably what causes clotting of the blood extravasated in the renal pelvis.[11]

Testing of clot accelerating activity was done in most instances by adding 0.1 cc of the material to be tested to 1 cc of normal or hemophilic venous blood collected with oiled syringes and kept in paraffin-coated tubes. Unless otherwise stated, collodion-coated glass tubes (13 mm internal diameter) were used for the clotting time determinations. The urine was collected, usually from males, over a period of 24 hours in clean jugs containing a crystal of thymol; it was filtered or centrifuged before use. The usual chemical and microscopical studies were carried out on each urine. Those specimens containing sugar, albumin, casts, blood cells or bacteria were not used.

1. The simple addition of intact urine to normal or hemophilic blood accelerates its coagulation in glass or collodion-coated tubes (Table I). Dilution of the urine reduces its clot accelerating ability. Urine obtained by catheterization from the bladder or the renal pelvis does not differ significantly in its ac-

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[2] Glazko, A. J., and Greenberg, D., *Am. J. Physiol.*, 1939, **125**, 108.

[3] Tocantins, L. M., unpublished observations.

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[10] Fonio, A., *Ergeb. der Inner. Med. u. Kinder heil.*, 1936, **51**, 443.

[11] Tocantins, L. M., and Lindquist, J. N., *Fed. Proc.*, 1947, **6**, 215.

TABLE I.

Rate of Coagulation of Normal and Hemophilic Venous Blood to Which Were Added Variously Collected and Filtered Normal or Hemophilic Urine (0.1 cc urine, 1 cc blood in collodion coated tubes at 38°C). Clotting time in seconds.

	Source of urine																	
	Normal Man*						Hemophilic Man*						Normal Woman†				Control	
	Filtration						Filtration											
	None		Paper†		Berkef.‡		None		Paper†		Berkef.‡		Bladder		Right Renal Pelvis	Left Renal Pelvis	0.85% NaCl	
Dilution:	0	1-5	0	1-5	0	1-5	0	1-5	0	1-5	0	1-5	0	1-5				
Normal blood	438	642	477	808	510	705	514	794	600	792	492	696	394	642	368	373	898	
Hemophilic blood	530	812	461	811	1414	2914	506	795	671	786	1493	2878	670	1760	537	562	>7200	

* Urine voided spontaneously. Tests for protein and glucose negative. Microscopic examination negative.
† Urine filtered through double thickness Whatman No. 2 paper.
‡ Urine filtered once through a Berkefeld V candle.

TABLE II.

Physiochemical Changes in Urine After Dialysis; Effect of Addition of Fresh and Dialysed Urine on Rate of Coagulation of Normal and Hemophilic Blood.

Type of urine	Spec. grav.	pH	Total nitrogen (g %)	Chlorides (g %)	Sulfates (as SO_3) (mg %)	Inorganic phosphates (mg %)	Clotting time (sec)‡	
							Normal blood	Hemophilic blood
Fresh								
Undilut.	1020	5.35	3.32	1.0	250	300	530	506
Dilut. 1-5							812	795
Dialysed*								
Undilut.	1000	6.5	0.005	0	117.4	1.8	275	261
Dilut. 1-5							518	720
Controls								
1. 0.85% NaCl							898	>5600
2. Dialysed H_2O alone†							—	>5600

* Salt content adjusted by adding NaCl to 0.85% concentration before mixing with blood.
† Distilled water "dialysed" against running water for 48 hours.
‡ 1 cc blood, 0.1 cc solution in collodion coated tubes at 38°C.

TABLE III.
Effect of Heating (15 min) Undialysed and Dialysed Urine on Its Thromboplastic Activity Tested on Hemophilic Blood.*

	Unheated urine		Urine heated 55°C		70°C		100°C	
Dilution:	0	1-5	0	1-5	0	1-5	0	1-5
1 cc blood, 0.1 cc intact urine (sec)	401	666	887	775	2950	2108	7087	3050
1 cc blood, 0.1 cc dialysed† urine (sec)	337	563	518	712	599	1009	763	1482

* Clotting time in collodion tubes > 7200 sec.
† Dialysis in "Visking" casings for 48 hr against cold running water.

TABLE IV.
Effect of Heating Urine to 70°C (15 min) Before and After Dialysis on Its Thromboplastic Activity Tested on Hemophilic Blood.

	Fresh urine*		Urine heated at 70°C only		Urine dialysed only		Urine heated at 70°C then dialysed		Urine dialysed, then heated at 70°C	
Dilution:	0	1-5	0	1-5	0	1-5	0	1-5	0	1-5
1 cc blood, 0.1 cc urine, clotting time (sec)	401	666	2950	2108	337	567	4400	6035	599	1009

* Filtered through double thickness Whatman No. 2 paper.

tion from urine voided spontaneously. Urine collected from hemophilics behaves essentially like that collected from normal men. The activity is not altered by paper filtration (or centrifugation), but passing the urine through a Berkefeld V candle reduces its clot acceleration power, evident only when the testing is done on hemophilic blood (Table I). The clot accelerating effect is not due to the addition of prothrombin since a mixture of brain thromboplastin, $CaCl_2$, the urine or its dialysed dried residue, and prothrombin-free fibrinogen will not lead to clotting. It is not due to thrombin, since direct addition of intact or dialysed urine to a fibrinogen solution will not change it to fibrin. Moreover, when the urine or its dialysed dried residue are added to unrecalcified citrated plasma no clot forms, after standing 24 hours. The clot accelerating activity of urine and its extracts is apparently due, therefore, to the presence of a thromboplastin-like activity.

2. When the clear protein-free urine is dialysed in cellophane bags for 48 hours against cold running water, most of its electrolytes and nitrogen containing compounds are removed and the clot accelerating power of the urine is somewhat increased (Table II). The effect of heating is more pronounced on intact than on dialysed urine (Table III). The heated undialysed urine has a greater clot accelerating power when diluted, an indication that in fresh urine, inhibitors are present which reduce the effectiveness of the clot accelerators. If the urine, however, is dialysed after being heated, the relatively greater clot accelerating effect of the diluted urine is no longer observed (Table IV). Dialysis may remove a substance which has an anticoagulant action of its own; when the intact urine is only heated, the reduced activity of the urine thromboplastin may allow the action of a heat-resisting anticoagulant to assert itself.

3. Clear, filtered dialysed urine may be frozen and its water content removed by the lyophil method. A fine light brown powder results. The average yield of 10 lots was 43.4 mg of solids per 100 ml of dialysed urine (range 72.-29.5 mg). In concentrations above 100 mg %, the material is poorly soluble.

The addition, *in vitro*, to normal and hemophilic blood, of concentrated solutions of the dried residue has a pronounced clot accelerating action. With the stronger solu-

TABLE V.
Effect of Addition of Dialysed Urine Residue on Rate of Coagulation of Normal and Hemophilic Blood in Collodion and Glass Tubes.

	Mg of lyophilized dialysed urine residue added to each ml blood							
	1.0	0.5	0.1	0.05	0.01	0.005	0.001	Control 0.85% NaCl
Hemoph. blood*								
Collod. tube (sec)	244	282	307	484	1550	2566	4466	>7200
Glass tube (sec)	234	242	340	375	725	998	2600	4300
Normal blood*								
Collod. tube (sec)	230	220	250	289	498	595	878	1652
Glass tube (sec)	229	252	280	332	514	554	781	936

* 1 cc blood, 0.1 cc sol. of residue in 0.85% NaCl.

TABLE VI.
Diminution in Thromboplastic Activity of Urine Standing at 5°C for Several Days.

	Days after collection						
	1	3	4	12	16	27	38
	Clotting time in sec.						
Hemophilic urine*	184	253	265		589	966	903
Normal urine*	234	278	227		210	210	365
" " *	245			795		1180	1167

* 0.1 cc urine, 1 cc hemophilic blood (clot. time >7200") in collodion coated tubes at 38°C.

tion the intensity of the effect is about the same, whether glass or collodion tubes are used; with weaker solutions the thromboplastic effect is less marked on hemophilic blood in collodion tubes (Table V). The coagulation was not reduced below 200 seconds, even when the concentration of the powdered material in the solution added was increased so that 3. mg were added to each ml of blood.

4. Standing at 5°C of intact or dialysed urine leads to slow diminution of its clot accelerating activity (Table VI). The activity of the dried material is likewise reduced on standing, even when kept in evacuated ampuls. Dialysed urine appears to maintain its activity longer than intact urine. Mixing the lyophilized residue with ethyl ether or absolute ethyl alcohol (100 cc per g of residue) for 2 hours does not seem to affect the clot accelerating activity of the residue. Advantage was taken of this fact to render the preparations bacteriologically sterile.

5. A single or repeated intravenous injection of the dialysed lyophilized material (60 mg/kg body weight) into rabbits at a rapid rate (20 mg per second), has a temporary prostating effect lasting about 5 minutes, from which the animal recovers without any subsequent deleterious effects. Intravenous injection into anesthetized dogs of the same material in amounts of 2.5 mg per kg body weight, over a period of 60 seconds, has a moderate transitory depressing effect on blood pressure, from which the animal usually recovers within 10 minutes.

Intravenous infusion of a solution of the lyophilized residue into a hemophilic, in doses indicated on Chart I, leads to a diminution in the rate of blood coagulation in both glass and collodion tubes, the intensity and duration of the response varying according to the amount of material injected. There are no symptoms during the infusion when the rate is maintained between 5-10 mg per minute. When this is exceeded, flushing, pounding behind the eyes and in the abdomen and a slight headache is felt. In 2 instances when the dose of material was 3-4 mg per kg body weight, slight fever, malaise, joint pains and diuresis occurred for a period of 24 hours following the infusion.

Comment. Both intact urine and that free of most of its solutes by prolonged dialysis seem to contain a substance capable of accelerating the coagulation of normal and

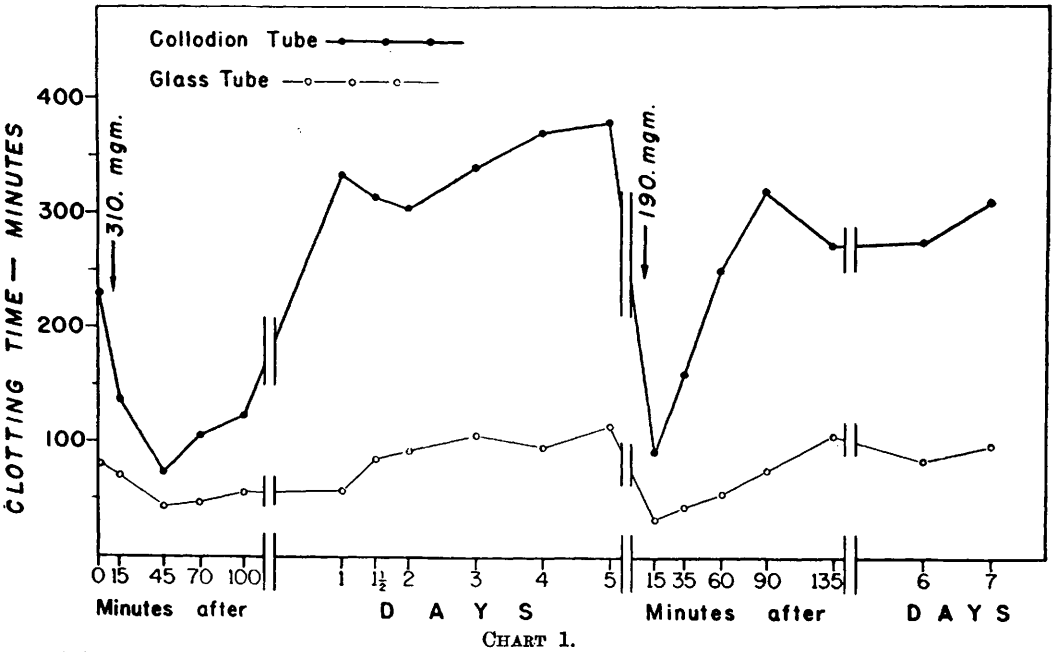

CHART 1.

Effect of 2 intravenous infusions of solutions of the lyophilized dialysed urine residue on the clotting time of the blood of a hemophilic.

hemophilic blood *in vitro* and *in vivo*. The clot accelerating substance in the urine behaves like a thromboplastin and may be derived from one of the cellular or chemical components of the urine, the kidney itself, or represent thromboplastic material separated from the blood and cleared through the kidney in the urine. If the latter proves to be the principal source of the urinary thromboplastin, it is difficult to understand how hemophilic urine often displays as much and occasionally greater activity than normal urine, since hemophilic plasma is said to contain less thromboplastin than normal plasma.

Undialysed and dialysed urine are known to contain blood pressure depressors which apparently differ in action from histamine.[12] A fraction has been separated[12] which has a pronounced blood pressure depressing effect. The substance responsible for this effect is apparently nondialysable, since it may be found in the dialysed urine or a solution of its lyophilized residue. If the blood pressure depressing fraction proves to differ from that having the clot accelerating effect, it may be possible to separate the 2, and to use the thromboplastic fraction for the temporary correction of the coagulation defect in hemophilia. By supplying an excess of thromboplastic activity, the excess of anticephalin activity in hemophilic blood[13] may be temporarily offset, and the coagulation of blood made to approach normal levels.

The urine thromboplastin behaves toward heat somewhat like aqueous solutions of brain thromboplastin. The latter when heated to 100°C lose much of their activity. The potency and heat stability of thromboplastic solutions are known to be influenced by the presence of other substances in the solution. This may explain why the thromboplastin of the dialysed urine is more thermoresistant than that of fresh urine. It is also possible—and the dilution experiments so indicate—that fresh urine contains clot inhibiting substances as well, which are more resistant to heat than the thromboplastin. These substances seem to be lost during dialysis, thus

[12] Frey, E. K., and Kraut, H., *Arch. f. Exp. Path. u. Pharmac.*, 1928, **133**, 1; Westerfeld, W. W., and coworkers, *Am. J. Physiol.*, 1944, **142**, 519.

[13] Tocantins, L. M., *Blood*, 1946, **1**, 156.

allowing the effect of the thromboplastin to become the dominant one in the dialysed urine.

Summary. The clear protein and cell-free urine from normal men and women and from hemophilic men has thromboplastic activity when tested on normal and hemophilic blood. The thromboplastin in dialysed and lyophilized urine seems more resistant to heat than that in fresh urine. The lyophilized dialysed urine residue has a clot accelerating action on hemophilic blood *in vitro* and *in vivo*. The clots which form in the ureter of hemophilics with hematuria are probably due to contact of the extravasated blood with the thromboplastin of the urine.

15861 P

"Control" Erythrocytes for Hemolysis Studies.*

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In several previous experiments the permeability of chicken erythrocytes at 37.5°C was studied over periods of many hours' duration. It was noted that the time for hemolysis in glycerol of untreated cells became less after several hours (Hunter).[1] Since no attempt was made in these experiments to control bacterial contamination, it was thought that a part of the change in these control cells might have resulted from bacterial action. In another series of experiments, however, in which aseptic technics were followed it was also observed that the time for hemolysis of control cells decreased with lapse of time (Hunter and Larsh).[2] Results of this nature might have been predicted in view of previous experiments. Jacobs and Parpart[3] have shown that erythrocytes are quite sensitive to environmental changes while Harris[4] showed that under certain conditions of standing, erythrocytes lose potassium.

First a series of experiments were performed in which blood was allowed to stand at 37.5°C and time for hemolysis in glycerol was measured at varying intervals of time. Aseptic technic was observed throughout and tests were made for bacterial contamination as before (Hunter and Larsh).[2] Four stock suspensions were made. (1) Two cc of freshly drawn, heparinized chicken blood, (2) 1 cc of blood plus 1 cc of Ringer Locke, (3) 1 cc of cells plus 1 cc of plasma and (4) 1 cc of cells plus 1 cc of Ringer Locke. The results obtained using whole blood are shown in Fig. 1. Essentially similar results were obtained with all 4 solutions except the Ringer Locke appeared to exert a "protective effect" especially in (4). A similar series was run using blood which had been in the refrigerator for 27 hours. Hematocrit readings indicated that these cells had swollen although the initial hemolysis times and subsequent

TABLE I.
Effect of Standing on Hemolysis Time and Volume.

Time of exposure in hr	Time in sec for 50% hemolysis	Cell volume in μ^3
0	285	90
12⅓	245	104
0	395	112
23½	200	123
0	300	118
29¾	160	138

* The authors are indebted to the Faculty Research Fund of the University of Oklahoma for grants in aid.

1 Hunter, F. R., *J. Cell. and Comp. Physiol.*, 1947, in press.

2 Hunter, F. R., and Larsh, Howard W., to be published.

3 Jacobs, M. H., and Parpart, A. K., *Biol. Bull.*, 1931, **60**, 95.

4 Harris, J. E., *J. Biol. Chem.*, 1941, **141**, 579.