to biotin in both ml and sub-μl volumes of total incubation mixture is represented in Fig. 3. It is apparent that, with the 0.010 μl droplets, a change of over 10% transmission per 1×10^{-15} g of biotin was obtained up to 50% transmission.

Summary. A technic for microscopic microbiological assay was elaborated in which droplets of total incubation mixture down to 0.010 μl volume were employed under oil. Measurement of bacterial growth by light scatter was used. The technic was applied to determination of biotin to 10^{-15} g with approximately the same percent accuracy and precision as the macrotechnic with ml-volumes.

TABLE IV. Biotin Assay of Fat-Free Dry Rat Liver by Light Scatter with 0.6 μl Droplets of Assay Mixture.*

Liver (10^{-10}g) /assay	Biotin (10^{-15}g) /droplet		Biotin (10^{-14}g) /μg liver	
	A	B	A	B
127	55	60	433	472
255	117	120	459	470
510	232	247	455	484

* Assay values mean of triplicate analyses of liver from two rats, A, B, respectively.

TABLE V. Recovery of Biotin Added to Hydrolyzed Rat Liver Measured by Light Scatter with 0.6 μl Droplets of Assay Mixture.*

Biotin (10^{-15}g)			
Hydrolysate	Added	Total found	% recovery
37	80	110	91
40	80	126	107
55	60	113	97
55	60	110	92
60	60	120	100
66	60	129	105
68	60	129	102
73	40	117	110

* Assay values mean of triplicate analyses.

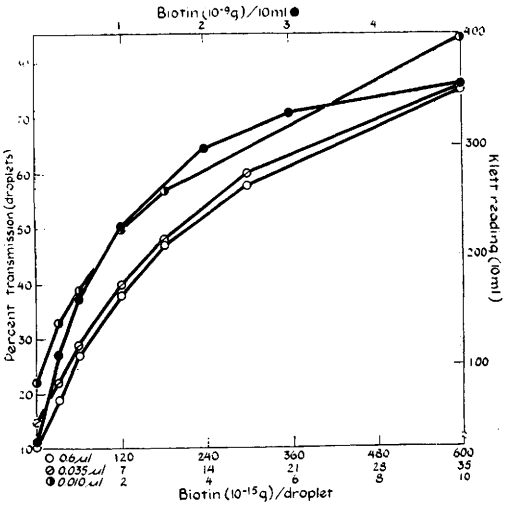

FIG. 3. Response of *Lactobacillus arabinosis* (17-5) to biotin in 10 ml and droplet assays. Sensitivity settings on the Photovolt photometer for droplets 0.60, 0.035, and 0.010 μl are 1000, 100, and 10×, respectively.

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Effect of Sulfated Mucopolysaccharides and of Endogenous Plasma Heparin(s) on Thromboplastin Generation. (27344)

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The first stage of clotting accomplishes the formation of plasma thromboplastin. Heparin delays the rate of thromboplastin production at a concentration as low as 1/80 unit/ml(1) and prevents its formation at 1/20-1/30 unit/ml(1,2). It therefore seemed desirable to evaluate the effect on thromboplastin generation of an extract of normal human plasma which we believe contains heparin(3). Since other acid mucopolysac-

TABLE I. Effect of Acid Mucopolysaccharides upon Plasma Thromboplastin Generation. Clotting time in seconds.

Inhibitor	Conc., mg/ml	Thromboplastin generation Incubation time* 2 min.	4 min.	6 min.
None†		16	13	15
Heparin	.01	45	51	64
"	.1	285	190	165
Heparin monosulfate	.01	18	13	16
"	.1	43	48	58
"	1.0	115	260	270
B-Heparin	.01	16	14	15
"	.1	26	20	18
"	1.0	58	74	88
Chondroitin sulphate	.01	25	15	16
"	.1	30	12	16
"	1.0	64	73	78

* .1 ml of each of reconstituted materials (platelet factor, serum, adsorbed plasma) and of 1/40 M $CaCl_2$ were incubated and .1 ml of the mixture added at 2, 4, 6 min. after recalcification to .1 ml reconstituted substrate plasma plus .1 ml $CaCl_2$.

† Control generation tube contained .05 ml distilled water; other tubes contained .05 ml of inhibitor.

charides would also have been removed from the plasma by the extraction procedure, their action on thromboplastin formation was also determined.

Materials. Normal human platelet-poor plasma samples were extracted as previously described(3). The method essentially consists of the tryptic digestion of the plasma proteins previously precipitated by methanol-acetone, with subsequent dialysis and lyophilization. The final product, which can be neutralized by protamine, is assayed for anticoagulant activity using recalcified sheep plasma (U.S.P. 14 technic) and a heparin standard. Commercial beef heparin was used as a reference standard.* The other mucopolysaccharides studied were B-heparin, heparin monosulfate and chondroitin sulphate-A.† In the present investigation commercially available thromboplastin generation test materials‡ were used to evaluate plasma thromboplastin formation and its inhibition.

Results. Table I shows that formation of plasma thromboplastin was markedly reduced by .0005 mg of heparin (.05 ml of a .01 mg/ml concentration) whereas .005 mg of heparin monosulfate was required to produce the same inhibitory effect. B-heparin and chondroitin sulphate only slightly delayed thromboplastin generation at .005 mg but decreased its formation when between .005 and .05 mg was present. Apparently B-heparin was slightly more inhibitory than chondroitin sulphate at the lesser concentration.

The results obtained using various combinations of the acid mucopolysaccharides are presented in Table II. The action of a small amount of heparin is reduced in presence of the other mucopolysaccharides. The relative effect of the 4 substances on the action of preformed thromboplastin is similar to that on its generation, namely heparin>heparin monosulfate>B-heparin>chondroitin sulphate (Table III).

Human plasma extracts (Table IV) reduced thromboplastic generation to approximately the same extent as .0005 mg of heparin (Tables I and II). We had previously assayed these samples for their anticoagulant activity and had calculated that their heparin content varied from 10-20 units % (.001-.002 mg/ml). Thus .00025-.0005 mg heparin was present in the .05 ml aliquot of the plasma extract that was used in the thromboplastin generation test.

The interference of other acid mucopolysaccharides with the inhibitory effect of a small amount of heparin upon thromboplastin generation raised the possibility of their masking the full anticoagulant action of the plasma heparin extract which probably also contains some of these substances. Accordingly this question was studied and the results are shown in Table V. When heparin is not present (vertical column 1) B-heparin and heparin monosulfate have a slight anticoagulant action. At higher concentrations of heparin (although still 1/5-1/10 the amount

* Heparin Standard 100 μ/mg.

† Obtained through the courtesy of Leon Freeman, Riker Laboratories. B-heparin was originally supplied by Dr. A. Winterstein, Hoffmann-LaRoche, Basle, Switzerland; heparin monosulfate by Dr. K. Brown, Veterans Admin. Hosp., Downey, Ill.

‡ TGTR—Warner-Chilcott.

TABLE II. Effect of Other Acid Mucopolysaccharides upon Inhibition of Thromboplastin Generation by Heparin. Clotting time in seconds.

Inhibitor				Generation tube incubation times		
Heparin, .01 mg/ml	Heparin monosulf., .01 mg/ml	B-Heparin, .01 mg/ml	Chond. sulph., .1 mg/ml	2 min.	4 min.	6 min.
* —	—	—	—	25	16	20
+	—	—	—	90	65	77
—	+	—	—	21	16	22
+	+	—	—	36	47	63
+	—	+	—	28	25	25
+	—	—	+	30	29	35
+	+	+	—	35	34	37
+	+	—	+	24	17	19
+	—	+	+	37	33	32
+	+	+	+	32	24	25

* Control generation tube contained .15 ml distilled water. .05 ml of inhibitors added to other tubes.

TABLE III. Effect of Acid Mucopolysaccharides on Preformed Thromboplastin. Thromboplastin generation mixture incubated for 4 min. after recalcification and then inhibitor added.

Inhibitor	Conc., mg/ml	Clotting time, sec.	
None*		17	
Heparin	.01	40	flocculation
"	.1	85	sediment, no clot
" monosulfate	.1	30	
" "	1.0	—	no clot in 3 min.
B-Heparin	.1	18	
"	1.0	—	no clot in 3 min.
Chondroitin sulphate	.1	17	
" "	1.0	60	flocculation

* Control clotting substrate tube contained .05 ml distilled water. .05 ml of inhibitor added to other clotting substrate tubes.

of the other acid mucopolysaccharides) little additive or inhibitory effect results from the presence of the other substances. We have not investigated whether this difference between the anticoagulant assay and the thromboplastin generation test is due to the differences between the 2 procedures or to the smaller amount of heparin used in the latter (Table II).

Discussion. The action of heparin and related substances has been compared in different clotting systems by various investigators (4-7). However, the effect of acid mucopolysaccharides other than heparin upon thromboplastin generation has not been evaluated except for B-heparin which has been found to inhibit thrombin generation slightly(7). Our results demonstrate that heparin monosulfate has approximately 10%, B-heparin from 1-10%, and chondroitin sulphate probably between 1-5% the inhibitory activity of heparin in formation of thromboplastin. These results are generally similar to those previously reported using other anticoagulant systems (4-7). However, the interference of all the mucopolysaccharides tested with the action of heparin on thromboplastin generation has not been noted heretofore.

The potent inhibitory activity of the extracts of normal human plasma on thromboplastin generation affords additional evidence of the presence of heparin in plasma, particularly since the other acid mucopolysaccharides

TABLE IV. Effect of a Human Plasma Extract on Thromboplastin Generation. Clotting time in seconds.

Plasma extract,* .05 ml in thromboplastin generation tube	Thromboplastin generation Incubation time 2 min.	4 min.	6 min.
2718	30	30	24
2772	88	100	110
2777	58	85	88
2781	59	65	79
2782	40	46	46
2787	51	68	90
Control generation tube with .05 ml H_2O	27	17	18

* 1 ml of final solution contains the material extracted from 5 ml of plasma. Heparin content of these samples varied from 10-20 units % (.1-.2 mg /100 ml of plasma), or .005-.01 mg in final 1 ml extract. Thus .05 ml contained .00025-.0005 mg heparin (1/40-1/20 unit).

TABLE V. Effect of Heparin and of Heparin plus Other Acid Mucopolysaccharides upon the Clotting Time of Recalcified Sheep Plasma.

Additional substances in clotting tubes*	Quantity of heparin in clotting tube (ml) 0	.05	.10	.15	.20
	Clotting times in min.				
None (heparin only)	5	12	21	40	60
B-Heparin, .1 ml	6	12	22	40	61
Heparin monosulfate, .1 ml	8	16	23	40	60
Chondroitin sulphate, .1 ml	5	12	21	41	62

* Inhibitors used in following concentrations: Heparin, .01 mg/ml; B-Heparin, heparin monosulfate and chondroitin sulphate at .1 mg/ml.

ides possibly present in the extract were found to be so much less effective than heparin as inhibitors of thromboplastin formation. The relative lack of interference of these substances with the anticoagulant assay of heparin using recalcified sheep plasma indicates that the plasma heparin levels we have reported(8) are substantially correct.

Summary. 1. Heparin monosulfate has 10%, and B-heparin and chondroitin sulphate less than 10% the inhibitory activity of heparin on thromboplastin generation. 2. This action of heparin is decreased in presence of other acid mucopolysaccharides. 3. Extracts of normal human plasma strongly inhibit thromboplastin formation thus confirming either the presence of heparin in plasma or of an unknown substance which acts identically with heparin. 4. The presence of other acid mucopolysaccharides does not substantially alter the assay of heparin when clotting time of recalcified sheep plasma is the method used.

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Effect of Thyroid Hormone on Protein Biosynthesis by Cell-Free Systems of Liver.* (27345)

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Thyroid hormone is required for normal growth and its presence is one of the prerequisites for the secretion and activity of growth hormone(1,2). The availability of cell-free preparations for study of metabolic pathways offered a possibility of localizing more closely one of the effects of thyroid hormone on growth, namely that on protein synthesis. In the following experiments the microsomal system of Zamecnik *et al.*(3), from the livers of normal and thyroidectomized animals was used to study the role of thyroid hormone.

Materials and methods. Chemicals. (ATP) adenosine triphosphate dipotassium salt was obtained from Sigma Chemical Corp. (FDP) fructose diphosphate, barium salt, from Nutritional Biochemicals Corp. was converted to

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