brain extract since both substances contain an incompletely activated thromboplastin. The thromboplastin-like activity of the "T" fraction can be considerably enhanced by incubating it with serum and prothrombin-free plasma, as is done in the thromboplastin generation test(3). Fig. 1 shows that the "T" fraction becomes fully activated after incubation for 4 min. with serum, together with plasma treated with $BaSO_4$.

Discussion. One difference between the "T" fraction and brain thromboplastin is that the latter, when heated, has no effect on the prothrombin consumption of hemophilic blood (4). Another difference is that the "T" fraction can be centrifuged at 2000 *g* for 30 min. without an appreciable reduction in activity. A centrifugal force of 31,000 *g* for 90 min.—conditions similar to those used by Chargaff (5) to purify tissue thromboplastin—is required in order to precipitate this material. Biggs, Douglas and Macfarlane(6) have previously shown that brain extract can be activated by incubation with serum and prothrombin-free plasma—a phenomenon also demonstrable with the "T" fraction. Further studies are indicated in order to determine whether tissue thromboplastin is identical with urine thromboplastin.

Neither the physiologic significance nor the source of the urinary thromboplastic material is known. Using the present method for purification, it is possible to obtain thromboplastin from the very same individual on whom studies are to be performed. Thus, it is no longer necessary to resort to heterologous materials in studies of clotting.

Summary. A method is described whereby thromboplastic material is extracted from normal male human urine by adsorption on $BaSO_4$ and subsequent elution. The thromboplastic material is comparatively resistant to heat; its activity is enhanced by incubation with serum and prothrombin-free plasma. As little as 6 μg/cc of the thromboplastic material per cc of hemophilic plasma was found to restore the clotting properties of this plasma to normal.

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Metabolic Interrelationship between Ascorbic Acid and Pteroylglutamic Acid. (22322)

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A close interrelationship between metabolism of ascorbic acid and pteroylglutamic acid (PGA) was reported by various workers (1-4). Schwartz and Williams(5-7) observed that in PGA deficiency produced by feeding aminopterin, rats excreted less ascorbic acid in urine, and their liver ascorbic acid contents were diminished. This indicated that biosynthesis of ascorbic acid was dependent on PGA-nutrition of animals. Chloretone enhances the biosynthesis of ascorbic acid in rats(8). It was, therefore, of interest to find out if the increased synthesis of ascorbic acid, after chloretone treatment, was interfered with in rats treated with aminopterin. Ascorbic acid helps in the conversion of PGA to citrovorum factor (CF) in rats(9). It was also of interest to find out if this conversion was further stimulated in chloretonised rats.

Methods. Biosynthesis of ascorbic acid. Male rats weighing 100-170 g were fed mineralized whole milk with 2 drops of concen-

TABLE I. 24-Hour Urinary Excretion of Ascorbic Acid by Rats in mg. (Avg excretion of 12 rats.)

	1 day	3-4 day	6-7 day	8-10 day
Milk diet alone	2.5	2.4	2.5	—
+ a*	1.4	1.5	1.4	.7
+ c	1.9	10.4	11.1	11.0
+ c + a	1.9	10.6	12.0	10.4
+ s + c	1.9	9.7	13.2	12.0

* a = aminopterin; c = chloretone; s = sulfaguanidine.

trate of vitamin A and D (Adexolin-Glaxo), twice a week. The animals were divided into 5 groups of 12 animals each. Group I received milk without supplement. Group II were fed 40 γ aminopterin per day. Group III were fed daily 20 mg chloretone dissolved in .2 cc groundnut oil. Group IV were fed both aminopterin and chloretone in doses mentioned above, and Group V were fed daily chloretone and .5 g sulfaguanidine. Within 7 days rats fed aminopterin showed deficiency syndromes. Each animal was placed in a metabolism cage over a glass funnel and urine collected in conical flasks containing 2 cc of a 10% solution of oxalic acid and 1 cc toluene. Total ascorbic acid in 24 hour output of urine was determined by titration against a standardized solution of 2,6-dichlorophenol indophenol(10). Animals of the different groups were killed on tenth day of experiment and ascorbic acid present in liver, kidneys, adrenals, small intestine, testis and pancreas was extracted with 4% sulfosalicylic acid and determined by titration with 2,6-dichlorophenol indophenol(11). Dehydroascorbic acid was absent from these tissues. Thiamine, riboflavin and nicotinic acid contents of liver of all animals were determined(12-14). No significant change in these values in livers from different groups of animals could be observed. The results are given in Tables I and II. *Excretion of citrovorum factor and ascorbic acid.* Ten male rats were divided into 5 pairs, each placed in one metabolism cage and urines collected as described above except when analysed for CF. For CF estimation urines were collected under toluene only. Rats were fed mineralized milk supplemented with 100 γ PGA by stomach tube per animal daily for 2 weeks. Ascorbic acid and CF were estimated in urine samples on alternate days. CF was estimated by the method of Sauberlich and Baumann(15) using leucovorin (Lederle) as the reference standard. Results are given in Table III. Male rats of another group of 12 were similarly fed 100 γ PGA for one week and urinary excretion of CF and ascorbic acid determined. After one week these animals were fed 20 mg chloretone in addition to PGA and urinary excretion of CF and ascorbic acid were determined. The results are given in Table III. *Estimation of CF, ascorbic acid and glutathione contents of livers.* At the end of 2 weeks, rats from each group, described in previous section, were killed by decapitation, livers removed, chilled, weighed, ascorbic acid and glutathione estimated according to the method described previously (11) in aliquots of liver samples and CF according to the method of Doctor *et al.*(16) after incubating aliquots of liver homogenate in nitrogen. The results are given in Table IV.

Results. Administration of aminopterin diminished urinary excretion of ascorbic acid indicating interference in normal synthesis of ascorbic acid in PGA deficiency. Urinary excretion of ascorbic acid was stimulated by administration of chloretone either alone or in conjunction with aminopterin. PGA deficiency, therefore, did not hamper urinary excretion of ascorbic acid in chloretonized

TABLE II. Ascorbic Acid Values of Tissues of Rats (mg/100 g Fresh Tissue).

Rats on	Milk	Milk + a	Milk + c	Milk + c + a	Milk + c + s
Liver	31.2 ± .9*	23.8 ± .4	43.1 ± 2.3	35.8 ± 3.2	42.1 ± 2.2
Kidney	20.9 ± 1.1	15.3 ± 1.4	34.8 ± 1.6	23.5 ± 3.2	35.0 ± 1.7
Adrenals	413.7 ±17.5	274.0 ±14.2	408 ±34.4	294 ±44.5	392 ±46.4
Intestine	46.2 ± 1.2	35.6 ± 2.0	59.8 ± 2.8	45.9 ± 5.1	60.9 ± .6
Testis	24.4 ± .7	23.6 ± .7	26.4 ± 1.0	28.6 ± .1	27.5 ± .4
Pancreas	9.6 ± .4	8.0 ± .7	12.9 ± 1.3	10.4 ± .9	10.8 ± 0

* Stand. error.

TABLE III. 24-Hour Urinary Excretion of CF and Ascorbic Acid by Rats Fed 100 γ PGA per Day.

	Before chloretone		After chloretone	
	CF excretion/animal/day	Ascorbic acid excretion/animal/day	CF excretion/animal/day	Ascorbic acid excretion/animal/day
1st wk	468 ± 44 mγ	.62 ± .06 mg	—	—
2nd wk	566 ± 26	.65 ± .06	1572 ± 163 mγ	6.75 ± .63 mg

TABLE IV. CF, Ascorbic Acid and Glutathione Values of Rat Liver.

	Without chloretone	With chloretone	t
Ascorbic acid	24.3 ± 1.2 mg/100 g	34.6 ± 1.4 mg/100 g	4.7
Glutathione	233.7 ± 8.8 "	273 ± 13.9 "	7.5
CF incubated in nitrogen	151 γ/100 g	143 γ/100 g	

rats. Sulfaguanidine fed rats receiving chloretone also excreted in the urine increased amounts of ascorbic acid. The intestinal flora, therefore, may not be concerned in increased urinary excretion of ascorbic acid.

Ascorbic acid contents of liver, kidney, adrenals and small intestine significantly diminished in animals which received aminopterin. Ascorbic acid contents of these tissues increased when animals were fed chloretone. Administration of aminopterin in addition to chloretone increased ascorbic acid values of these tissues, but the values were at a lower level as compared to values in animals which received only chloretone. These findings indicated that aminopterin-induced PGA deficiency interfered with biosynthesis of ascorbic acid by normal and chloretone-fed rats. There was, however, no change in ascorbic acid contents of testes and pancreas in rats differently treated.

After administration of PGA in normal rats there was 6- to 8-fold increase in urinary excretion of CF. This excretion was further enhanced when animals were fed chloretone. Ascorbic acid and glutathione contents of liver were increased in chloretonized rats but CF contents did not change appreciably. The increased conversion of PGA to CF in chloretone-fed rats might be due to increased synthesis of ascorbic acid and glutathione. Ascorbic acid enhanced urinary excretion of CF in rats fed PGA(9).

Summary. The effect of aminopterin on ascorbic acid excretion and tissue contents of ascorbic acid was studied in both normal and chloretone-fed rats. Aminopterin decreased urinary excretion of ascorbic acid but did not decrease excretion in chloretone-fed rats. Sulfaguanidine also could not diminish the increased ascorbic acid excretion in chloretonized rats. Ascorbic acid contents of liver, kidney, adrenals and small intestine significantly diminished in both groups of animals receiving aminopterin alone or in conjunction with chloretone. Ascorbic acid contents of testes and pancreas of differently treated rats did not change. Chloretone feeding enhanced urinary excretion of ascorbic acid and CF and increased ascorbic acid and glutathione contents of liver.

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In vitro Cultivation of Porcine Kidney. (22323)

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In early tissue culture experiments, choice of a menstruum for proliferation of a virus was generally restricted to host and tissue normally parasitized by the given virus. It is now apparent that these restrictions are not so clearly defined. Enders and his co-workers (1) for instance, propagated poliomyelitis virus in non-neural tissues (kidney, skin-muscle and intestine) and Maitland and Laing (2) grew vaccinia virus on rabbit kidney.

Recent observations that epithelial cells of renal origin support multiplication of a number of viruses beyond the confines of host or tissue specificity prompted us to study the nutritive factors involved in culturing of kidney tissue. Kidney of porcine origin was used because of favorable economic considerations and its availability.

Materials. A. *Porcine Constituents.* 1. *Hog Embryo Extract (HEE).* Embryos (10-20 cm long) were removed from the gravid uteri of sacrificed sows and washed with cold physiological saline. The tails, hooves, heads, ribs, and intestinal tracts were removed and the remaining skin-musculature and organs were cut into small pieces and placed in the deep freeze. The dismembered embryos were thawed 24 hours later and homogenized in a Waring Blendor for 30 seconds. An equal volume of Hanks Balanced Salt Solution containing antibiotics was added and the mixture rehomogenized an additional 15 seconds. The homogenized embryo extract was clarified by cheese cloth filtration and by centrifugation (4,000 rpm for 45 minutes at 1°C). The HEE was sterilized by filtration through an EO (Ertel) pad and stored in the deep freeze in 50 ml amounts. 2. *Hog Serum (HS)* was Seitz filtered and stored in deep freeze in 10 ml amounts until used. 3. *Hog Serum Ultrafiltrate (HSU)* was prepared by forcing hog serum through Visking casing. The pH of HSU was adjusted to 7.3 by means of 0.1 M NaH_2PO_4. The HSU was then Seitz filtered and stored in deep freeze in 10 ml amounts. (Selection of kidney tissue and hog constituents should be made from healthy non-immune stock, otherwise, humoral antibody or endogenous viruses may interfere with virus proliferation.) B. *Bovine Constituents.* Beef embryo extract (BEE), serum (BS), and serum ultrafiltrate (BSU) were obtained from Microbiological Associates, Bethesda, Md. C. *Balanced Salt Solution (BSS)* used in preparation of the media was prepared in the manner specified by Hanks and Wallace(3). Just previous to use, 2.5 ml of a 1.4% solution of sterile $NaHCO_3$ was added/100 ml of BSS. The pH of BSS was approximately 7.4. D. *Synthetic Mixture #199 (SM #199),* com-