quate cortin. Animals allowed to go more than a day or two without adequate cortin must be brought back to normal with cortin before they can be used for assay because more cortin is required to bring them up if they have lapsed. Injections should be made twice daily, preferably 10 to 12 hours apart because some individuals will not grow normally with one injection.

To assay, one uses 3 or more groups containing sufficient numbers to rule out individual variation. A range of doses is employed, one for each group. This makes it possible to determine the minimum dosage which will maintain normal growth. Anything less than this will result in a loss in appetite, a retardation of growth, or even a loss in weight. This becomes apparent within 24 to 72 hours because growing animals are so sensitive. The test can be carried over a period of 3 or more days if necessary. After an assay, the animals used may be quickly brought back to normal weight and growth by giving an adequate amount of cortin. Therefore, the same animals may be used for assay many times.

If rats weighing 75 to 150 gm. are used, it is not necessary to vary the amount of cortin in regard to the weight.

We suggest as a tentative unit for cortin the amount necessary to be injected twice daily for the maintenance of normal growth in an adrenalectomized rat.

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Pathological Lesions Produced in Rabbits Following Intravenous Injection of Concentrated Scarlet Fever Toxin.

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Duval and Hibbard[1] reported in numerous publications the production in rabbits of acute glomerulo-nephritis following the intravenous administration of the bacteria-free toxic principle of *Streptococcus scarlatinae,* which they characterized as endotoxic in nature. Their histopathological material was considered analogous to that observed in human scarlatinal glomerulo-nephritis. Reith, Warfield

[1] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, **44**, 567; *J. Am. Med. Assn.*, 1926, **87**, 898; *South. M. J.*, 1926, **19**, 858; *New Orleans M. and S. J.*, 1927, **79**, 669; PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 876; *ibid.*, 1928, **25**, 529; *J. Exp. Med.*, 1927, **46**, 379.

and Enzer[2] after repeating these experiments concluded that identical renal lesions also occurred in normal rabbits as well as in those injected with suspensions of non-scarlatinal streptococci. They averred that none of the renal lesions produced were typical of human acute glomerulo-nephritis. Rich, Bumstead and Frobisher[3] were able to produce glomerular damage in rabbits by the intravenous injection of bacteria-free filtrates of fresh broth cultures of a virulent strain of *Streptococcus viridans* isolated from the blood in a case of subacute endocarditis with renal involvement. Their histopathological material was typical of acute hemorrhagic glomerulo-nephritis.

Recent attempts to concentrate scarlet fever toxic filtrates suggested that a more potent toxic principle than that available in the unconcentrated 72 to 96 hours broth cultures might exert a more constant and specific destructive action on the kidneys and possibly be productive of a minimum lethal dose of scarlet fever toxin useful as test material in the standardization of scarlet fever antitoxin. Two years ago, Hartley[4] prepared such a concentrated substance by dialyzing the scarlet fever toxin through collodion bags, in the manner of Walpole.[5] A large part of nitrogen was thus removed and the dialyzed toxin was subsequently precipitated with increasing quantities of normal acetic acid. The precipitate was finally dissolved in normal saline solution. The resultant solution was reduced to one-fortieth of the original filtrate, having a minimum lethal dose for rabbits of "more than 0.2 cc. but less than 0.25 cc." and contained from 0.17 mgm. to 0.21 mgm. of total nitrogen per cubic centimeter. Pulvertaft[6] simultaneously reported the results obtained on chinchilla rabbits by the intravenous injection of scarlet fever toxin concentrated by precipitation with 5 volumes of absolute alcohol. The strength of Pulvertaft's concentrated toxin was more than 10 times that of the original filtrate, contained 90% less of the nitrogenous constituents of the crude toxin and proved fatal in doses varying from 0.5 cc. to 0.1 cc., death usually occurring within 24 hours. By means of this procedure he asserted that the therapeutic efficiency of scarlet fever antitoxins could be estimated inasmuch as complete protection was afforded against the concentrated toxin by the streptococcal antisera.

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[2] Reith, A. L., Warfield, L. M., and Enzer, N., *J. Inf. Dis.*, 1930, **46**, 42.

[3] Rich, A. R., Bumstead, J. H., and Frobisher, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 397.

[4] Hartley, P., *Brit. J. Exp. Pathol.*, 1928, **9**, 259.

[5] Walpole, G. S., *Biochem. J.*, 1915, **9**, 284.

[6] Pulvertaft, R. J. V., *Brit. J. Exp. Pathol.*, 1928, **9**, 276.

More recently Shinn[7] reviewed the claims of Hartley and Pulvertaft and reported that a lethal dose of scarlet fever toxin for rabbits had not been established. Although rabbits were injected intravenously with more than 7 times the dosage of scarlet fever toxin designated by Hartley as the lethal dose for rabbits, Shinn failed to demonstrate a specific lethal action for the toxin and concluded that rabbits were not susceptible for standardization of scarlet fever toxin and antitoxin. Parish and Okell[8] made a rejoinder to Shinn's report by restating their original contention that large rabbits (2 kilo) yielded results analogous to human tests with the Dick toxin and were useful as test animals for estimation of the therapeutic efficiency of antitoxins. Our purpose in this communication was two-fold: firstly, to study the degree of toxicity of the concentrated scarlet fever toxin in chinchilla and the mixed breed of stock rabbits, and secondly, to carefully scrutinize the specific destructive action of the concentrated scarlet fever toxin on the rabbit renal structures.

Experimental: Our 2 lots of toxic filtrates were prepared by incubating Douglas' broth cultures of *Streptococcus scarlatinae,* Dochez S.F-NY.5, for 72 hours at 37° C. Following incubation, the cultures were passed through Berkefeld V candles and the filtrates tested for sterility and total number of skin test doses of toxin per cubic centimeter. Pulvertaft's method of concentrating the crude toxic filtrate by precipitation with 5 volumes of absolute alcohol was carefully followed. Both the filtrate and alcohol were cooled to about 2° C. before being mixed and the whole was allowed to remain in the refrigerator over night, after which the supernatant fluid was decanted. The precipitate was then dissolved in normal saline solution, the final volume being one-tenth that of the original filtrate. The solution was then placed on the water-bath at 37° C. for 1½ hours and frequently shaken. The solution was finally passed through a Berkefeld V candle and according to Pulvertaft's advice was immediately injected intravenously in rabbits in gradually increasing doses. A quantitative estimation was made of the total nitrogen, total solids and total number of skin test doses of toxin contained in each cubic centimeter of the original crude toxic filtrate and the final concentrated product. Table I shows the results obtained with 2 lots of scarlet fever toxin. It is noted that more than 90% of the nitrogenous constituents and total solids of the original crude toxin have been removed and that a 9-fold con-

[7] Shinn, L. F., *J. Inf. Dis.,* **1930, 46, 77.**

[8] Parish, H. J., and Okell, C. C., *J. Pathol. and Bact.,* 1930, **33,** 527.

centration of the total skin test doses of toxin was attained. Estimation of the latter was made on 3 individuals susceptible to the Dick toxin.

TABLE I.
Degree of Concentration by Alcoholic Precipitation of *Streptococcus scarlatinae* Toxic Filtrates.

	Original Crude S. F. Toxin (Dochez SF. NY. 5-1) Lot 1	Concentrated S. F. Toxin (Dochez SF. NY. 5-1) Lot 1	Reduction %
Volume, cc.	1,000	100	90
Skin Test Doses per cc.	14,000	125,000	
Total nitrogen per cc.	2.365 mgm.	0.195 mgm.	91.8
Total solids per cc.	36.20 "	3.08 "	91.5
	Lot 2	Lot 2	
Volume, cc.	1,000	100	90
Skin Test Doses per cc.	14,000	120,000	
Total nitrogen per cc.	2.350 mgm.	0.193 mgm.	91.8
Total solids per cc.	36.20 "	2.67 "	92.6

Degree of Toxicity: Table II shows the results obtained with the intravenous injection in chinchilla and the mixed breed of stock rabbits of doses of unconcentrated and concentrated scarlet fever toxin varying from 0.05 cc. to 10 cc. Although the chinchilla rabbits showed a relatively greater susceptibility to the lethal action of both the unconcentrated and concentrated filtrates, a minimum and specific lethal dose was scarcely discernible either in the chinchilla or in the mixed breed of stock rabbits. The doses causing death varied irregularly between 0.6 cc. and 5 cc. of the concentrated toxin, although one rabbit survived the injection of 5 cc. and another 10 cc. without any apparent toxic effects. The stock rabbits which lived 24 hours after the injection were killed at that time and studied for macroscopic and microscopic lesions. The chinchilla rabbits which survived the first injection were given a second injection 2 weeks later. With one exception these animals died from 12 to 18 hours after the intravenous injection of the concentrated scarlet fever toxin varying from 2 to 5 cc. Autopsies were performed on all animals within a few hours after death. Blood and peritoneal exudate cultures were made on all the animals at time of autopsy and were regularly found to be sterile. No remarkable macroscopic lesions were found in any one animal.

Kidney Histopathology: Although minor pathological differences were observed in individual animals the histopathological picture as a whole was uniform and the differences showed no correlation with

TABLE II.
Lethal action of intravenous injection of unconcentrated and concentrated scarlet fever toxic filtrates in chinchilla (C) and stock (S) rabbits.

Rabbit	Weight kilo.	First injection		Result	Second injection (2 weeks later)		Result
		Dose, Concentrated S.F. Toxic Filtrate			Dose, Concentrated S.F. Toxic Filtrate		
		cc.	Total Nitrogen, mgm.		cc.	Total Nitrogen, mgm.	
C.294	1.9	0.3	0.0822	Survived	4.0	1.096	Died 18 hrs.
C.295	1.8	0.6	0.1644	Died 16 hrs.	—	—	—
C.296	2.5	1.0	0.2740	Survived	5.0	1.370	Died 18 hrs.
C.297	1.9	2.0	0.5480	"	5.0	1.370	" 12 "
C.298	1.8	5.0	1.370	Died 12 hrs.	—	—	—
C.299	1.9	10.0	2.740	Survived	5.0	1.370	Died 16 hrs.
S.281	2.2	0.05	0.0137	Survived			
S.280	2.0	0.08	0.0219	"			
S.279	2.1	0.1	0.0274	"			
S.278	1.9	0.2	0.0548	"			
S.277	1.9	0.5	0.1370	"			
S.276	1.6	1.0	0.2740	"			
2.275	1.9	2.0	0.5480	Died 18 hrs.			
S.274	2.2	5.0	1.370	Survived			
S.273	1.9	10.0	2.740	Died 12 hrs.			
		Unconcentrated Toxin					
C.290	2.4	1.0	2.365	Survived	2.0	0.548	Died 18 hrs.
C.291	2.2	2.0	4.730	Died 16 hrs.	—	—	—
C.292	2.2	3.0	7.095	" 18 "	—	—	—
C.305	1.9	4.0	9.460	Survived	2.0	0.548	Died 12 hrs.
C.306	2.1	5.0	11.825	"	2.0	0.548	Survived
C.307	2.2	10.0	23.650	Died 12 hrs.	—	—	—
S.287	2.1	1.0	2.365	Survived			
S.286	1.8	2.0	4.730	"			
S.285	2.0	3.0	7.095	"			
S.284	2.1	4.0	9.460	"			
S.283	1.9	5.0	11.825	"			
S.282	2.1	10.0	23.650	"			
S.309	1.9	20.0	47.300	"			
S.310	2.3	30.0	70.950	"			

dosage of the unconcentrated or concentrated filtrates, number of injections or type of rabbit employed. The renal vessels showed a moderate hyperemia, especially of the capillaries and smaller veins. Congestion of the glomerular capillaries was fairly marked in some sections, while only moderately in others. No hemorrhage was observed. The dominant picture was excessive tubular swelling, especially pronounced in the convoluted tubules. The cytoplasm of the latter was markedly granular and the nuclei were only faintly stained. No definite necrosis not attributable to post-mortem changes was seen. The tubular swelling was so severe in several

sections that the tubular ends were pushed into the space intervening between the glomerulus and Bowman's capsule. No tubular casts were observed. The glomeruli were in general hyperemic without showing the least trace of hemorrhage. A faint-staining amorphous substance, resembling albuminous exudate, often distended Bowman's capsule. No glomerular adhesions were seen and no cellular reaction of polymorphonuclear or round cell types was found in the interstitial substance, glomeruli or tubules.

Thymus: In several of the chinchilla rabbits the thymus showed similar histopathological changes, consisting in an intense vascular engorgement throughout the thymic substance.

Conclusions: A minimum and specific lethal dose of the concentrated scarlet fever toxic filtrate was not discernible in rabbits, although the chinchilla breed displayed a relatively greater susceptibility to the lethal toxic effects of scarlet fever toxin. The renal changes observed in the rabbit following intravenous injections of unconcentrated and concentrated scarlet fever toxic filtrates were not those of acute hemorrhagic glomerulo-nephritis but rather analogous to lesions observed in milder forms of tubular damage.

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Attempted Production of the Scarlatinal Syndrome with Whole and Filtered Scarlet Fever Faucial Exudate.

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The rôle of *Streptococcus hemolyticus* in scarlet fever is generally admitted to remain as unsettled as the question about the actual cause of this disease.[1] Both Ciuca[2] and Satake[3] failed consistently to produce experimental scarlet fever in a large series of Dick positive human volunteers by swabbing or injecting their tonsils with freshly isolated cultures of scarlet fever streptococci or scarlet fever blood. Specificity of the Dick toxin has not been granted by Bür-

[1] League of Nations. Health Committee, 1929, **3**, Nr. 7, 11; Freund, R., *Immunität, Allergie u. Infektionskr.*, 1929, **2**, 118.

[2] Ciuca, M., and Gheorghiu, I., *Compt. rend. Soc. da Biol.*, 1927, **97**, 1427.

[3] Satake, T., *Bull. de l'Office Internat. d'Hyg. Publ.*, 1927, **19**, 1627.