TABLE II.

	Thoracic duct lymph		Cervical duct lymph	
White cells/cmm	21,250 ± 1,580*	(37)†	7,065 ± 1,725	(10)
Lymph flow/hr	0.39 ± 0.03 cc	(27)	38.5 ± 3.75 mg	(12)
Protein conc. (whole lymph)	4.18 ± 0.11 (g %)‡	(19)	3.07 ± 0.18 (g/100 g)§	(9)

* Mean ± S.E.
† Figures in parentheses indicate number of animals.
‡ Copper sulfate gravity method. (Avg specific gravity of 19 specimens was 1.0187±0.00014)
§ Micro-Kjeldahl. (N × 6.25) (not corrected for N P N)

difference in the albumin-globulin ratio, but that there is a definitely higher content of gamma globulin in the cervical lymph as compared with blood plasma.

Summary. The quiescent anaesthetized rat exhibits a free flow of lymph from the cervical lymphatic trunks. A method of collecting cervical lymph in the rat is described. The average rate of flow is approximately 40 mg/hr, the lymphocyte count averages 7,065/cmm, and the average protein content is 3.07 g %. These figures are compared with similar data for thoracic duct lymph.

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Comparison of the Toxicity of Antiseptics for Embryonic Tissue and Bacteria.

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(Introduced by A. C. de Graff.)

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This report describes a procedure for the determination of the toxicity index of antiseptics which appears to have certain theoretical and practical advantages over those previously presented.

The evaluation of antiseptics has long been a difficult problem, largely because of the many types of chemical compounds which have been found useful in this field. To date, no entirely successful procedure has been devised for comparing these drugs except when tests are made of closely related compounds. The use of a toxicity index (the ratio of the dilution of an antiseptic which will produce some readily determined toxic effect on tissue to that which will kill bacteria tested) permits interclass comparisons to some extent. The reliability of the results can be enhanced further by inclusion of a suitable reference standard to control shifts in tissue or bacterial susceptibility. In addition it is generally recognized that since the toxicity of an antiseptic is frequently modified by the presence of organic matter, the tissue or bacteria should be implanted in the medium before contact with the antiseptic is permitted. In a recent paper Salle and coworkers[10] have described a method which takes into account all of the foregoing considerations. They suspended chick heart fragments in serum and embryonic extract, added antiseptic, and then, after 10 min exposure, cultured the tissue in Carrel flasks. In a corresponding series of experiments, bacteria were exposed for 10 min followed by subculture in beef broth.

Salle's work and that of other investigators[1–10] in this field has been based on either

1 Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.
2 *ibid.*, *J. Am. Med. Assn.*, 1916, **67**, 1300.
3 Lambert, R. A. and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.
4 German, W. J., *Arch. Surg.*, 1929, **18**, 1920.
5 Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

TABLE I.
Toxicity of Phenol for Embryonic Tissue and Bacteria.

Dilution	Fraction of heart fragments showing growth 48 hr after implantation 1st trial	2nd trial	3rd trial	Dilution	Growth of *Staphylococcus aureus* 1st trial	2nd trial	3rd trial
1:535	0/10	0/10	0/10	1:205	—	—	—
1:666	0/10	0/10	0/10	1:256	—	—	—
1:840	10/10	7/10	10/10	1:321	+	+	+
1:1042	10/10	10/10	10/10	1:400	+	+	+
1:1315		10/10	10/10				

TABLE II.
Toxicity of Antiseptics Towards Tissue Cells and Bacteria and Derived Toxicity Index.

Antiseptic	Dilution inhibiting growth of approximately 50% of fragments (A)	Dilution lethal to *Staphylococcus aureus* (B)	Toxicity index (Ratio A/B)
Strong solution of Iodine, USP XII	1:1315	1:4570	0.29
Azochloramide	1:65,000	1:101,700	0.64
Phenol	1:782	1:288	2.7
Zephiran	1:73,000	1:21,200	3.4
Merthiolate	1:202,000	1:52,000	3.9
Saponated solution of Cresol, USP XII	1:1163	1:285	4.1

the Carrel flask or the hanging drop method. It was found that neither of these procedures was convenient for a quantitative method. Both methods depend upon passive diffusion alone as the means of exposing the biological material to the antiseptic. In the case of the hanging drop technic accuracy in measurement of media is difficult.[5]

Moreover, the use of a 10 min exposure period[10] would seem to be an arbitrary condition imposed upon the experiments. This criticism is justified since not all antiseptics have the same wetting powers and it is impossible to plant pieces of tissue of exactly the same size and shape.

In the work reported below, Gey's method of cultivating tissue in roller tubes[11,12] has been adapted to the study of antiseptics. The continual movement imparted to cells and media seems to provide a closer approach to *in vivo* conditions. Luxuriant growth can be obtained and accuracy in measurement of media and antiseptic solutions is possible.

Procedure. The most convenient tube was found to be a vial 100 mm long by 25 mm wide with a neck 15 mm in diameter. It was sealed with a paraffined cork. With the aid of a pipette about one cc of chicken blood plasma was transferred to the tube and the tube gently rotated until the plasma was spread uniformly over the inner surface. The excess was removed with a pipette after allowing the tube to stand upright for a few minutes. Ten to 15 such tubes were prepared by this process called "lining" by Gey.[11] The tissue cells or the bacteria were then introduced into separate series of tubes. If tissue was being studied ten fragments of heart ventricles 0.5 to 1 cu mm in volume from 9-day embryonic chicks were set in the plasma lining. Where bacteria were being studied one 4 mm loopful of a 24-hr beef heart infusion culture of *Staphylococcus aureus* was streaked up the sides of the lining. In either case the tubes were stoppered and placed horizontally in a motor-driven drum rotating at a speed of 10 revolutions per hour in an incubator at 38.5°C and the plasma was allowed to clot. Each tube

[6] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665, 937, 1057, 1119, 1481.

[7] *ibid.*, 1935, **33**, 8, 393.

[8] *ibid.*, 1936, **34**, 371.

[9] Salle, A. J., McOmie, W. A., Sheehmeister, I. L., *J. Bacteriology*, 1937, **34**, 267.

[10] Salle, A. J., McOmie, W. A., Sheehmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.

[11] Gey, G. O., *Am. J. Cancer*, 1933, **17**, 752.

[12] Gey, G. O., and Gey, M. K., *ibid.*, 1936, **27**, 45.

was then removed momentarily from the incubator and 0.75 cc of embryonic extract was added followed by 0.75 cc of one of a series of antiseptic dilutions and the tube was agitated gently so as to assure complete mixing. In this way the gutter of embryonic extract and antiseptic extended the length of the lined surface and bathed the tissue cells approximately once every 6 min. All tubes were left in the incubator for a period of 48 hr. In the case of a tube containing tissue fragments, growth or no growth of each of the ten pieces of tissue was checked with the low power binocular microscope and recorded at the end of this period. In the case of a tube containing bacteria a loopful of the fluid material was transferred to a tube containing 10 cc of beef broth and incubated for an additional 24 hr. At the end of this time each tube was examined for the presence of staphylococci. Gross evidence, such as turbidity or lack of it, was supplemented by microscopic examination of stained smears. Routinely the beef broth tubes were saved for an additional 2 or 3 days as a check on possible bacteriostasis.

The stock solutions of antiseptics were as follows: saponated solution of cresol USP XII, strong solution of iodine USP XII, zephiran aqueous concentrate 10%, 1% merthiolate solution prepared from powder, 1% azochloramide solution prepared from the commercial tablets and 5% phenol solution prepared from the pure crystals. The dilutions were made from the above stock solutions with Tyrode solution containing 10% embryonic extract. The embryonic extract presumably acts as a protective colloid and prevents the precipitation which would otherwise take place in the case of cresol.†

† The saponated solution of cresol causes a flocculent precipitate of calcium and magnesium soaps when added to Tyrode solution. In the presence of embryonic extract these insoluble soaps remain finely divided and do not interfere mechanically with the procedure. The soap may play a considerable part in the antiseptic action of the phenolic antiseptics, especially when they are used in the presence of serum or exudate. Soap is, of course, necessary to maintain the phenol homologues dispersed in water.

The first experiments were of necessity orienting or range finding. In these the antiseptic dilutions were varied by a factor of 2 as 1:100, 1:200, and 1:400. After determining the range of toxicity further dilutions were made which differed by a factor of 1.25. In other words, each successive dilution differed by 25% from the preceding. It was possible to differentiate with ease tissue in which no appreciable migration of fibroblasts had taken place, from tissue showing unmistakable growth along the periphery. Border line cases were so rare as to permit scoring a group of fragments in terms of an all or none response. In the case of the tissue growth experiments six dilutions were usually set up for each drug so that those causing zero and 100% growth inhibition fell as nearly as possible in the middle of the group of 6. The median toxic dilution, that is, the dilution inhibiting growth in 50% of the heart fragments, was estimated as the midpoint between the zero and 100% values. Solutions for the study of bacterial growth were set up in like manner and the bactericidal dilution was taken as the average of a dilution which fails to kill and another dilution 25% smaller which is lethal. Phenol was used as a standard of comparison and a series of dilutions were tested with every group of experiments either with tissue or bacteria.

Results. The reproducibility of the results may be judged from Table I. It is evident that the data are entirely consistent and no shifts occurred in the susceptibility of tissue or organisms. In most instances the slope of the curve relating dilution to percent inhibition for tissue was quite steep so that by using a smaller factor between dilutions and larger groups of heart fragments, a median inhibiting dilution with its standard error might be readily determined.

In Table II are given the dilutions of the antiseptics toxic to tissue and lethal to bacteria and the toxicity indices derived from them. The toxicity indices have been arranged in ascending order; the larger the value the less favorable is the index. It will be evident that, only where the index is less than one, will the dilution of the antiseptic which is lethal to staphylococci be greater than the dilution which inhibits the growth of tissue.

The results are in agreement in order of magnitude with those of Salle[6-10] and Lambert.[1-3] The phenolic compounds and merthiolate have indices considerably greater than one, whereas iodine, with an index of 0.29, is by far the best of all the drugs studied. Of the two drugs which have not been studied previously, zephiran has an index of the same order as that of the phenolic compounds, whereas azochloramide has an index approaching that of iodine.

The interpretation of these results in terms of various clinical applications must be made with caution. The toxicity indices have been determined here under conditions similar to those which obtain when an antiseptic is applied to denuded tissue in the presence of serous exudate. When an antiseptic is applied to intact skin or mucous membrane the index will probably be different, and there is no reason to expect parallelism with the values reported above.

Summary. A method has been described for quantitative comparison under identical physiological conditions of the toxicity of antiseptics for tissue and bacteria. The technic is simple and the results are reproducible. The toxicity indices were found to be as follows: iodine, 0.29; azochloramide, 0.64; phenol, 2.7; zephiran, 3.4; merthiolate, 3.9; saponated solution of cresol USP XII, 4.1.

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Occurrence of Stainable Lipoid Material in Renal Epithelium of Animals Falling in Different Age Segments.*

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In previous publications[1-3] observations have been recorded of variations in the occurrence of stainable lipoid material in different segments of the renal nephron. The factor of age associated with these variations has been noted. The present study permits an extension and amplification of certain of these observations.

The first series of experiments represented by 110 dogs includes animals varying in age from 6 weeks to 18 years and 3 months. On the basis of age these animals have been divided into 3 groups. Group I, composed of 31 dogs between the ages of 6 weeks and 1 year; Group II, 52 dogs varying in age from 1 to 7 years; and Group III, 27 animals between 8 and 18 years and 3 months of age. Such a grouping permits observations of a cytological order that concern themselves with the tissues of puppies and young animals, Group I; adult animals, Group II; and older or definitely senile animals, Group III.

The dogs of the first series of experiments were not given any specialized diet but were allowed feedings once a day of scrap material from a large dining hall which usually contained scraps of meat, pieces of bone, bread and fats in the form of butter and tissue fat. Neither the food nor water intake was restricted. On 2 occasions, before the commencement of any experimental procedure, the blood and the urine of the animals were studied in order to eliminate the existence of a naturally acquired nephritis or some other form of renal injury. With the exception of 6 of the oldest dogs, in Group III, the urine was normal, the elimination of phenosulphonephthalein in a 2-hr period was within normal percentage limitations, and there was no re-

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1 MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1921, **17**, 289.

2 MacNider, Wm. deB., PROC. SOC. EXP. BIOL. AND MED., 1922, **19**, 222.

3 MacNider, Wm. deB., *Problems of Ageing: Biological and Medical Aspects*, Williams & Wilkins Co., Baltimore, 1939.