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.

tention of non-protein nitrogen or creatinine. In the 6 old animals that gave evidence of renal impairment the urine contained a heavy trace of albumin and showed the presence of broad, hyaline and finely granular casts. Fatty casts were not present. The 2-hr output of phenolsulphenonephthalein by these 6 old animals varied from 21 to 43%. The non-protein nitrogen of the blood was between 61 and 82.7 mg and creatinine between 2.1 and 3.8 mg per 100 cc.

The experimental procedure consisted in the removal from the kidney of a wedge-shaped piece of tissue without the use of an anesthetic of the methane series. The use of such an anesthetic body even for a very short period eliminates the validity of any deduction concerning the amount of lipoid material occurring naturally in such tissue.[4] For the operative interference procaine was used supplemented at intervals by nitrous oxide-oxygen inhalations. Renal tissue so obtained was cut into two portions, one piece of which was immediately frozen for sectioning while the remaining tissue was fixed in 10% formalin for future study. The sections were stained for lipoid material by Herxheimer's Scharlach R. method. A study of such sections from the 3 groups of animals indicates the following differences in distribution and in amount of lipoid material that can be demonstrated in the renal nephron. In Group I (puppies and young dogs), such material has been observed in the proximal convoluted segment of the nephron in only 4 of the older animals of this young group. In this location such material appears as fine dust-like granules of a more brick-red than orange-red hue, the latter being the usual order of color determination when such material is present in tissue in any considerable amount. Such granules are located on the lumen side of the cells of this segment of the nephron. They have not been observed in the cytoplasm immediately surrounding the nucleus of such cells. In the remaining animals of this young group, 32 in number, there has been no microchemically detectable lipoid material in the cells of this segment of the nephron. In this Group I animals as well as in the adult and in the older and senile animals, stainable lipoid material occurs as a normal constituent for both the descending and ascending limbs of Henle's loop. Such material in these locations exists as coarse granules of a brick-red color. The incidence of the presence of this material in the loops of Henle becomes more marked as the animals advance in age. In the old and very old animals, with the exception of the 6 animals in Group III with evidence of renal damage, there is a striking difference in the amount and location of stainable lipoid material appearing in the renal nephron. There is associated with the ageing of the animals and not with a state of renal disease the constant appearance of stainable lipoid material in the cells of the proximal convoluted segment of the nephron. This observation is in marked contrast to the absence of such material in the same segment of the nephron in most of the puppies and young adult animals of Group I. In the adult, aged and senile dogs of Group II and III this material makes its appearance in the convoluted tubule cells as definite droplets or fused masses of an orange-red color which not infrequently obscures the nucleus of such cells. In general, it would appear that increments in such material first take place between the nuclei and the free lumen border of the cells.

Reference has been made to 6 animals of the old and senile group (Group III) that gave evidence both in the blood and urine of the existence of some order of chronic renal injury. Frozen sections from such tissue stained with Scharlach R, as well as sections stained with hematoxylin and eosin, have shown these dogs to have an advanced chronic glomerular nephritis with an associated repair process to the epithelium of the proximal segment of the nephron which has resulted in the formation of a changed type of cell, abnormal for this segment of the nephron. These cells, or if cell masses show poor cell differentiation, areas which are syncytial in configuration, the cells are formed of flattened, evenly and deeply staining cytoplasm, containing at irregular intervals prominent and intensely staining nuclei. This epithelial repair process has resulted in the formation of an embryonic order of tissue to replace the cuboidal, highly specialized cell

[4] MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1920, **15**, 249.

units normal for this segment of the nephron. A study of sections of this tissue stained with Scharlach R has demonstrated the occurrence of stainable lipoid material in the cells of the loops of Henle, which material is comparable in amount to that found in the remaining old and senile dogs of this group (Group III) which failed to give evidence of a renal injury. Other than the glomerular injury in the 6 nephritic animals of the group, and the changes in the configuration of the epithelial structure, the observation of importance is that stainable lipoid material cannot be demonstrated in this embryonic order of replacement epithelium, while in animals of the same advanced age group in which no injury has existed to provoke an atypical repair process in the convoluted tubule segment the cells of this portion of the nephron are heavily loaded with stainable lipoid.

A similar order of change in epithelial type has been observed for the liver in certain senile animals[5] associated with a reduction in certain manifestations of hepatic function and furthermore in other animals[6] not senile in which, following a severe injury from uranium nitrate, the liver underwent a process of atypical epithelial repair comparable to that described for the kidney in the present series of animals. With this order of repair for the liver, the cells and syncytial structure effecting the repair fail to show the presence of stainable lipoid material. This type of epithelium is resistant to subsequent intoxications by uranium nitrate and is also resistant to chloroform.

The 110 dogs which have been the subject of this discussion were not on a constant diet of known composition. Variations, if persistent, in the chemical nature of such material might with some readiness influence the amount of stainable lipoid material in the tissues of the animals. In order to minimize, if not eliminate, this factor 38 dogs between the ages of 4 months and 12 1/6 years have been maintained over periods of 3 months on Purina Dog Chow. No limit was placed on the amount of such material consumed. The water intake was not restricted. Studies of the blood and urine of this group of animals, as has been outlined for the dogs of the previous series, indicated the animals were free from renal injury. Kidney tissue was obtained from these animals by the usual technic and frozen sections stained by the previously described method. The distribution and apparent amount of stainable lipoid material in the cells of the renal nephron of these animals on a constant diet of known composition have been similar to those for the first and larger group of animals in which the diet was not controlled. Puppies of 4 months and the younger animals up to 1 year of age failed to show the presence of stainable lipoid in the specialized cells of the proximal segment of the nephron. Such material was present in the cells of the loops of Henle. As the animals advanced in their age segments such stainable material made its appearance as small and larger droplets and as fused masses in the cells of the proximal convoluted segment.

Conclusions. 1. Microchemically demonstrable lipoid material is not present in the epithelium of the cells of the proximal convolution of the nephron in puppies varying in age from 6 weeks to 4 months. Young adult animals under 1 year of age have rarely shown such material as fine dust-like granules. 2. As the dog ages, stainable lipoid makes its appearance in the cells of the proximal convoluted segment and increases in amount in this location as the animal advances in years. 3. In nephritic senile animals that have repaired the epithelial injury in the proximal segment by the formation of an atypical, flattened order of epithelial structure stainable lipoid material cannot be demonstrated by the use of a similar technic which does demonstrate its presence in cells of a normal order in this location. 4. Such variations in the occurrence of stainable lipoid material are not due to qualitative variations in the food intake. 5. Intracellular chemical manipulations would appear to become so modified by the factor of age as to influence the amount and distribution of stainable lipoid material in the cells of the proximal convolution of the nephron.

5 MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1936, **56**, 383.

6 MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1936, **56**, 373.