ments with leprosy have been undertaken to exclude such adventitious organisms. Of 50 filtrates from rat leprosy through tested Seitz, Berkefeld N and W, and Chamberland L_2 and L_3 filters 16 gave positive cultures, while of 2 filtrates of human leprosy through Berkefeld N and W candles, the one through N gave a positive culture. These cultures from filtrates were in every case a pleomorphic and facultative acid-fast organism identical with the one cultivable directly from leprous lesions of the rat and man.

While it has been demonstrated by direct microscopic examination of centrifugal precipitates of filtrates that an occasional acid-fast bacillus, among the myriads present in a suspension of the organism, may pass through the pores of tested bacterial filters,[1] the probability of a chance contaminant or a microscopically unrecognizable secondary invader passing the filter in such a large proportion of the filtrates seems remote. Therefore, the repeated cultivation of this facultative acid-fast organism from filtrates of leprous tissues of the rat and man appear to furnish some support of the etiologic and endemiologic theories of leprosy advanced by us.[2]

7484 C

Chronic Toxicity of Dinitrophenol: Renal Function.*

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In connection with the clinical use of alpha dinitrophenol (2-4),[1] it is important to know whether administration over long periods of time will produce changes in the functions of vital organs. Previous papers[2] from this laboratory contain limited data on this problem. This paper deals with the results of experiments designed to deter-

1 Walker, E. L., and Sweeney, M. A., *J. Infect. Dis.*, 1934, **54**, 182.

2 Walker, E. L., *J. Prev. Med.*, 1929, **3**, 167; Walker, E. L., and Sweeney, M. A., *J. Prev. Med.*, 1929, **3**, 325.

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1 Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 193; Tainter, M. L., Stockton, A. B., and Cutting, W. C., *J. Am. Med. Assn.*, 1933, **101**, 1472; Cutting, W. C., and Tainter, M. L., *J. Am. Med. Assn.*, 1933, **101**, 2099; Tainter, M. L., and Wood, D. A., *J. Am. Med. Assn.*, 1934, **102**, 1147.

2 Tainter, M. L., Boyes, J. H., and DeEds, F., *Arch. Internat. de Pharm. et de Therap.*, 1933, **45**, 235; Tainter, M. L., and Cutting, W. C., *J. Pharm. Exp.*

mine whether dinitrophenol in repeated subfatal doses injures the kidneys.

Two series of experiments were performed, the first, a quantitative study of the urinary sediment, and the second, an estimation of the phenolsulphonphthalein excretion. Adult male rabbits were used throughout.

Urinary sediment. Six rabbits were put on a diet consisting of rolled oats and water. This diet causes an acid urine, in which the casts do not dissolve. Each sample of urine was tested with phenol red to be sure it had the required acidity. Once or twice every 24 hours the rabbits were fastened to a board, catheterized, and the bladder washed out with physiological saline solution. They were then released, and after a measured interval of several hours, catheterized again, and the urine and bladder washings quantitatively collected. The washings were centrifuged and the casts, red blood cells, white blood and epithelial cells counted, according to Addis' method.[3] From these counts, the output of the formed elements in 24 hours was readily calculated. The cast count is claimed to be the most sensitive test for renal injury, since even slight degrees of renal damage are revealed by the increased numbers of casts. The other elements are also informative, although not so significant under our conditions, since slight trauma or infection from the frequent catheterization may increase the red and white cells in the urine.

The six rabbits were observed in this way for an 11-day control period, during which time from 11 to 14 counts of the sediments were made on each one. Then 4 of the rabbits were injected subcutaneously each day with a 2% solution of alpha dinitrophenol dissolved in 1% sodium bicarbonate solution. The dosage was increased slowly until a fatal dose was reached. During the first 6 days, 6.5 mg. were injected, then 10 mg. for 4 days, 15 mg. for 3 days, 20 mg. for 3 days, and 25 mg. for 3 more days (all doses per kilo body weight). The last one of these 4 rabbits died on the third day of the 25-mg. dosage. The 2 remaining rabbits were injected with similar volumes of the bicarbonate solution as controls.

The average results for the various doses of dinitrophenol are

Therap., 1933, **48**, 410; *ibid.*, **49**, 187; Cutting, C. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 97; Hall, V. E., Field, J., Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432; Emge, L. A., Wulff, L. M. R., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 152.

[3] Addis, T., *J. Am. Med. Assn.*, 1925, **85**, 163.

TABLE I.

Average 24 hour output of formed elements in the urine of rabbits receiving dinitrophenol subcutaneously each day. Figures in parenthesis are the range of distribution.

	Control	Dosage of Dinitrophenol 6.5 mg.	10 mg.	15 mg.	20 mg.	25 mg.
Casts (thousands)	3.3 (0-46.7)	2.7 (0-83.3)	2.7 (0-53.3)	0 (0-0)	2.8 (0-42.3)	0 (0-0)
Red blood cells (millions)	61.2 (0-1880.0)	3.4 (0-25.7)	17.1 (0-71.0)	4.8 (0-23.6)	1.0 (0-5.8)	1.2 (0-7.2)
White blood and epithelial cells (millions)	3.2 (0-50.1)	4.9 (0-33.0)	12.4 (2.3-88.0)	5.4 (0-29.0)	3.7 (0-13.0)	11.8 (1.2-26.8)

presented in Table I. It is seen that there were no significant changes in the amount of sedimentary elements, all averages being well within the range of normal daily variations. The same was also true for each individual rabbit. The control rabbits receiving only bicarbonate solution behaved similarly. Therefore, according to this test, which is the most sensitive available for renal injury, dinitrophenol, in repeated subfatal and fatal doses, did not damage the kidneys. The kidneys of these rabbits were also examined histologically by Dr. D. A. Wood, and were found to contain no pathological alterations.

Excretion of phenolsulphonphthalein. Six rabbits were tied on boards, catheterized, and their bladders washed out with normal saline solution. Then, with the catheters still in place, 10 mg. of phenolsulphonphthalein were injected into the ear vein of each rabbit. Exactly 30 minutes later, the urine was drawn off and the bladder washed out as before. The urine and washings were combined, alkalinized, made up to a volume of 1 liter, and the dye-concentration estimated by comparing with a set of standards. This procedure was carried out 10 times on each rabbit during a 25-day control period. The average output of dye in the 30 minutes was 63%, which was taken as the average control value for these rabbits. Four of the rabbits were then injected with 20 mg. of alpha dinitrophenol per kilo subcutaneously each day for 77 days. The other 2 rabbits were injected with sodium bicarbonate solution as controls. The dye-output of each rabbit was estimated each week during the dinitrophenol period, or 11 times in all. The outputs averaged 62% during the injections, practically identical with that during the control period. It was also just as high at the end of the experimental period as it was at the beginning. Therefore, daily administration of just subfatal doses of dinitrophenol for 11

weeks failed to perceptibly diminish the excretory function of the kidney for phenolsulphonphthalein.

It is obvious that the results of these 2 series of experiments on rabbits were consistent with each other in showing that large repeated doses of dinitrophenol did not impair the functional efficiency of the kidneys.

Conclusions. 1. Daily subcutaneous injections in rabbits of alpha dinitrophenol in doses up to and including fatal ones, and for as long as 77 days, did not modify renal function as indicated by quantitative counts of the formed elements in the urinary sediment, according to Addis' method, and by excretion of phenolsulphonphthalein. 2. Therefore, it is unlikely that dinitrophenol, in ordinary clinical dosage, will injure the kidneys.

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Sex-Limitation of Cilia in Body Cavity of the Frog (R. pipiens).

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The occurrence of ciliary systems in the peritoneal lining of the body cavity of the frog has long been known (Gray[1]) but, so far as the writer is aware, the fact that they are sex-limited and appear only in the female has hitherto been unrecognized. However a comparative study of the peritoneal lining of the body cavities of 20 female and 20 male frogs *(R. pipiens)* has shown that such ciliation occurs only in the female and may be regarded as being strictly sex-limited. This study was made on frogs taken during the breeding season (April-May) at which time the ovaries of the females were greatly enlarged. Strips of the thin peritoneal lining were removed and temporarily mounted in frog Ringer for examination. The cilia show up clearly when illuminated by unfiltered light and with the diaphragm of the microscope almost completely closed. An 8 mm. objective combined with a No. 18 eyepiece proved to be the most adequate lens combination for this type of work. In the female frog ciliary systems are present throughout the ventral and lateral areas of the peritoneum, extending the entire length of the body cavity and including the pericardial region; they are particularly numerous in the peritoneal tissue immediately adjacent to the

[1] Gray, J., Ciliary Movement, 1928, Cambridge University Press, p. 163.