100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 μg/ml (ppm), respectively.

One set of experiments was executed in 50-ml Erlenmeyer flasks containing 4 ml of carrot medium solidified with 0.8% agar. A second set of experiments was conducted in pyrex side-arm test tubes containing 4 ml of liquid medium. Cultures in test tubes were aerated with air forced through a solution of 10% potassium dichromate in sulfuric acid, sterile water, and numerous sterile cotton plugs placed at intervals in the rubber tubing connecting the test tubes. The cultures did not become contaminated. Each concentration of oxalate, solid and liquid medium, was run in duplicate. Inoculation was made with sterile 5-mg pieces of carrot removed by a cannula from the cambial region of a carrot root. After inoculation the cultures were placed in the dark at room temperature.

At the end of 10 days the cultures were examined. Aerated cultures were especially friable and crumbled readily. Indeed, some cultures had spontaneously broken into large or small pieces while growing. Certain tubes and flasks contained cultures with free single cells. In both tubes and flasks lower increments of oxalate (100 μg/ml) allowed both proliferation and production of loose cells. These were easily dislodged from the flask cultures by a glass needle or could be readily sucked into capillary pipettes when the tissue mass was placed in aqueous medium. Higher oxalate increments retarded the growth of the cultures. Aerated cultures had shed many cells into the surrounding medium. The cells were of many shapes and sizes, some ranging up to several hundred μg in length. Most cells were bean- or banana-shaped, but a few had single emergences resembling embryonic root hairs. Many cells were disorganized and obviously dead, but many other cells were visibly living, with nucleus, vacuole, plastids, granules, etc., plainly evident upon microscopic examination.

Viability of the cells was tested in two ways. One method was by direct microscopic observation of normal cells. This was done by sucking cells aseptically into sterile micropipettes, whose ends were then sealed with sterile paraffin. This permitted repeated observation of individual cells. Cytoplasmic streaming could be seen because of the orderly movement of material suspended in the cytoplasm, and was especially evident in the strands of cytoplasm traversing the vacuole. Some cells showed cyclosis at the end of 10 days even under these relatively anaerobic conditions. The other method of testing for viability was by means of the colorless dye 2,3,5-triphenyltetrazolium chloride. This dye turns pink or red when reduced, and has recently been widely used to distinguish living from dead cells (*10*). Cytoplasmic granules of many single carrot cells subjected to 500 μg/ml of the dye for 12 hr were pink, thus demonstrating their reducing ability, a common property of healthy protoplasm. Dead cells did not change the color of the dye.

In a recent publication, White (*11*) has commented that not enough is known about plant hormones at the cell level. It is hoped that the facility of the method here reported and the present widespread use of plant tissue culture techniques will stimulate the study of single cells and eventually lead to a better understanding of the interrelationships of plant cell and hormone. The effects of various chemicals on single carrot cells is being investigated.

References

1. HABERLANDT, G. *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse.* Abt., **III**, 69 (1902).
2. WHITE, P. R. *Botan. Rev.*, **2**, 419 (1936).
3. SCHMUCKER, TH. *Planta*, **9**, 339 (1929).
4. WHITE, P. R. *Arch. exptl. Zellforsch., Gewebezücht*, **10**, 501 (1931).
5. HERBST, C. *Arch. Entwicklungsmech. Organ.*, **9**, 424 (1900).
6. BONNER, J. *Botan. Rev.*, **2**, 486 (1936).
7. HELLER, R. *Compt. rend. acad. sci., Paris*, **229**, 845 (1949).
8. PRINGSHEIM, E. G. *Planta*, **2**, 555 (1926).
9. WHITE, P. R. *A Handbook of Plant Tissue Culture*. Lancaster, Pa.: Jaques Cattell (1943).
10. ATKINSON, E., MELVEN, S., and FOX, S. W. *Science*, **111**, 385 (1949).
11. WHITE, P. R. In GEORGE S. AVERY, JR., Ed., *Survey of Biological Progress*. New York: Academic Press, Vol. 1, 267 (1949).

The Effect of Heated Linseed Oil on Reproduction and Lactation in the Rat[1, 2]

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A series of experiments carried out in this laboratory during the past 4 years has shown that diets containing 10% by weight, or more, of heated linseed oil are less nutritious than diets containing the same amounts of unheated linseed oil, the effects having been gauged by live-weight gains, efficiency of feed consumption, and time of survival (*1, 2*). In this connection "heated oil" signifies oil heated in the absence of oxygen at 275° C. Impairment of nutritive value has been observed with oils heated for as short a period as 4 hr. It has been shown in the test herein reported that reproduction and lactation are impaired in the female rat by diets containing 10% of the heated oil.

The experiment used 72 female albino rats distributed among 4 diet groups of 18 rats each. The rats received the experimental diets from the time of mating until the end of the subsequent lactation. Groups 1 and 3 received the heated oil diet, and Groups 2 and 4 the unheated oil diet. Rats of Groups 3 and 4 received in addition a supplement of 20 mg α-tocopherol per week.

Immediately before parturition, the females were put into individual wire-mesh cages. The cages were

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series No. 275.

[2] This test is a part of a larger project undertaken for the Committee on Edible Fats and Oils of the National Research Council of Canada, whose financial assistance is acknowledged. Paper No. 259 of the Canadian Committee on Food Preservation.

TABLE 1

Ingredient	%
Ground wheat	28
Dehydrated cereal grass	7
Fishmeal	15
Ground oat groats	10
Wheat germ meal	10
Ground corn	9.5
Soybean oilmeal	5
Irradiated yeast	3
Bone meal	2
Iodized salt	0.5
Linseed oil*	10

* Heated at 275° C in a current of CO_2 for 12 hr in the case of Groups 1 and 3.

fitted with a nesting box having a solid floor, and shavings were supplied for the nest. Feed and water were offered *ad lib*. Neither live weights nor feed intakes were recorded for the mothers, but litter weights were recorded for the 7th, 14th, and 21st days. On the 7th day litters of 9 or more were reduced in size to 8 rats each.

All diets used in this experiment had the general formula given in Table 1.

Table 2 shows the effect of heated linseed oil on the survival of young rats.

The first evidence of disturbed reproductive function was the smaller numbers of rats born to the females receiving the heated oil diets. In addition to this, the viability of the rats born to these groups was clearly inferior to that of the rats born to the groups receiving unheated oil diets.

Table 3 gives an analysis of variance for the number of young born alive, and of those still surviving on the 7th and on the 21st days.

It will be evident from Table 3 that the heated oil has had a highly significant adverse effect on the number of rats born alive and on their survival through the normal nursing period of 21 days.

Data for the changes in weight of individual rats are not available, since the litters were weighed as groups. The records for survival of individual young showed that they died over a period of about 2 weeks. Only 1 rat out of some 200 born to mothers on heated oil diets still survived on the 14th day. No valid comparison of weights can be made between groups because of the constantly diminishing numbers of survivors in many of the litters. Before the young on the heated oil diet died, they lingered for several days in a moribund condition. Another abnormal condition was also noted. Whereas on normal diets individuals in small litters (5 or 6 rats per litter) tend to be heavier than those in litters of 10 or 12 young, the animals in the small litters on the heated oil diets contained the smaller individuals. It has not been determined to what extent the death of the young was a consequence of starvation.

TABLE 2

Effect of Heated Linseed Oil in the Diet of the Female Rat on the Survival of Her Young

Lot No.	Vitamin E supplement	Linseed oil treatment	No. litters born	Total born alive	Average litter size at birth	Average no. young alive per litter at ages noted (days)			
						7	7*	14	21
1	Nil	Heated	14	118	8.4	2.1	2.1	0.1†	0.1†
2	"	Unheated	14	161	11.5	5.5	4.6	4.0	3.9
3	Vitamin E	Heated	12	99	8.3	2.8	2.7	0.1†	0.0
4	"	Unheated	15	160	10.7	8.0	6.2	6.1	5.9

* After reduction of litters to 8 rats/litter where applicable.
† Represents 1 rat in 1 single litter only.

TABLE 3

Analysis of Variance of the Survival of Young Rats

Source of variance	*D/F*	No. born alive per litter		Alive at 7 days		Alive at 21 days		Necessary *F* values	
		Variance	*F* ratio	Variance	*F* ratio	Variance	*F* ratio	5%	1%
All causes	54								
Treatments									
Heat	1	101.6	9.2	261.8	16.9	328.2	55.5	4.03	7.17
Vitamin E	1	1.9	—	46.8	3.0	24.4	4.1	4.03	7.17
Interaction	1	3.3	—	.9	—	6.9	1.2	4.03	7.17
Remainder (error)	51	11.1		15.5		5.9			

Differences in numbers of females that produced litters are probably entirely dependent on the numbers that conceived, since no evidence of abortion was found. Inasmuch as the rats were not placed on the test diets until they were mated, we do not believe the differences among the four groups in numbers of litters born are related to the experimental diets fed.

The supplement of α-tocopherol under the circumstances of this experiment did not measurably counteract the damage from the heated oil diets. The just significantly greater number of survivors at 21 days in the vitamin E group as compared to the number receiving no vitamin E is possibly explained on the basis of variability between tests, since all the animals receiving vitamin E were fed at one time and those receiving no vitamin E at a different time.

The proportion of young surviving or retained at 7 days, which were eventually weaned, is rather poor in the case of the rats receiving the unheated oil diet. We are inclined not to attribute this to any nutritional defect of the oil, since linseed oil included in the diet at levels of up to 30% has been observed to give rates of gain and feed efficiency comparable with those given by other edible oils when included in the diet at the same levels (*1*).

It is, however, quite clearly evident that heating the oil damaged its nutritive value. The reproductive and lactation responses of the female rat and her litter may offer a more critical test of thermal damage to oils intended for dietary use than does the growth of the weaned rat.

References

1. CRAMPTON, E. W., and LANGERMAN, H. L. The Effect of Heat Treatment on the Nutritional Value of Some Vegetable Oils. Presented at the Gordon Research Conference, New London, N. H. (Aug. 1949).
2. CRAMPTON, E. W., FARMER, F. A., and BERRYHILL, F. M. *J. Nutrition* (in press).

Reactions of Mercurial Diuretics with Mono- and Dithiols

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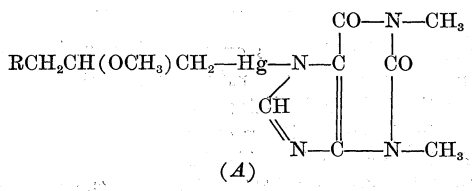
A recent review on renal transport mechanisms summarizes as follows: "There can be little doubt that the kidney is the principal site of the diuretic effect of the mercurial agents. . . . In general, mercurial agents combine with sulfhydryl groups, and this is responsible for their inhibitory effect on a number of essential cellular dehydrogenases. That the diuretic effect of mercury is attributable to the inhibition of such enzymes seems likely . . ." (*1*). The purpose of this communication is to describe certain reactions that occur *in vitro* between mercurial diuretics and thiols and to discuss their physiologic significance.

Prior to 1949 the mercurial diuretics used in medicine could be grouped under the general structure



(*A*)

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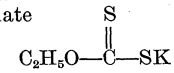
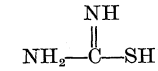
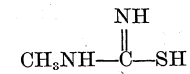
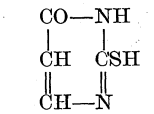
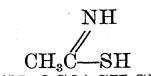
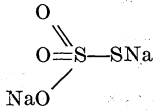
TABLE 1

RECOVERY OF THEOPHYLLINE FROM THE REACTION BETWEEN MERCUZANTHIN AND VARIOUS SULFHYDRYL COMPOUNDS

Monothiols		Mols theophylline recovered per mol mercury
Potassium ethyl xanthate		1.12
Thiourea		1.06
N-methyl thiourea		0.89
Thiouracil		1.20
Sodium thiosalicylate	$(NaOCO)C_6H_4SH$	1.17
Thioacetamide		1.23
Sodium thioglycollate	$(NaOCO)CH_2SH$	1.06
Sodium salt of cysteine	$(NaOCO)CH(NH_2)CH_2SH$	1.08
Sodium thiosulfate		1.06
Thio phenol	C_6H_5SH	1.12

where R is usually the sodium salt of a carboxylic acid residue to which the three-carbon side chain is attached through the nitrogen atom of a carbamyl group. It has been found that these drugs, represented by Mercuzanthin (Mercurophylline, U.S.P.), Salyrgantheophylline (Mersalyl with theophylline, U.S.P.) and Meruhydrin (Meralluride, N.N.R.), will react immediately at room temperature with a wide variety of monothiols according to the following equation:

$$RCH_2CH(OCH_3)CH_2Hg(\text{theophylline}) + R'S^- + H^+ \rightarrow RCH_2CH(OCH_3)CH_2HgSR' + (\textit{theophylline}) \quad (1)$$

where R'SH may be any simple sulfhydryl compound. If the reaction is carried out in concentrated solution and the rise in pH caused by removal of hydrogen ions is prevented by buffering with CO_2, the theophylline precipitates quantitatively.

To 10 ml of 0.2 *M* Mercuzanthin solution was added a mol equivalent of the various sulfhydryl compounds listed in Table 1. The solution was then saturated with CO_2, and the precipitated theophylline monohydrate was filtered off on a sintered glass filter, washed with a small volume of ice water, dried in a desiccator, and weighed. From the