fragments.

Summary. In fluoroacetate-poisoned rats, the C_4 members of the Krebs cycle further increase the accumulation of citrate in the heart but not in the kidney. C_2 members in appropriate doses may completely overcome the fluoroacetate block, a finding which may be of significance in antidotal therapy. In non-poisoned rats both C_4 and C_2 members of the cycle increase the citrate level in the kidney. The bearing of these findings on the metabolic patterns of various organs is discussed.

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Received November 25, 1953. P.S.E.B.M., 1953, v84.

Toxicity Studies on Hydroquinone.* (20751)

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A non-injurious chemical which significantly reduces the deterioration of food fats (oxidative rancidity) will be significant in reducing food deterioration, and in protecting human and animal health, since spoilage or rancidity of food fats destroys the palatability and the fat soluble vitamins in foods. The employment of hydroquinone as a food preservative has been advocated because of its effectiveness as an antioxidant(1-7). However, despite evidence to the contrary(8), the widespread consumption of this compound has not been considered safe(9). While hydroquinone (HQ) is definitely toxic at high dosages, this in itself does not necessarily militate against its use, providing the largest quantities that would be normally ingested prove innocuous. The purpose of this report is to present additional data on the chronic and acute toxicity of hydroquinone in rats, together with supplementary observations that were gathered on dogs, cats, and men.

Methods. Chronic experiments. Rats. Sprague Dawley male and female rats of weaning age (23 to 24 days old) were placed on a basic diet consisting of 23.7% dried skimmed milk, 9.24% lard, 66.0% ground whole wheat, and 1.0% iodized salt(10). Each kilogram of feed was supplemented with 4,800 international units of vit. A and 250 international units of vit. D. Fresh batches of food were prepared weekly and the rats were allowed to feed *ad lib*. Also, each animal received 5 g of fresh lettuce and 5 g of fresh lean beef each week. In Exp. 1, 4 groups of male and 4 groups of female rats, 10 rats in each of the 8 groups, were placed on the basic diet containing 0.0%, 0.1%, 0.5%, and 1.0% hydroquinone. In Exp. 2, the hydroquinone was heated together with the lard to 190°C for 30 minutes before incorporation into the food mix. Eight groups of rats, with 16 to 23 rats in each group, were fed the basic diet contain- 0.0%, 0.1%, 0.25%, and 0.5% hydroquinone. Finally, (Exp. 3) 8 groups of rats, with 20 rats in each group, were maintained on the basic diet containing both hydroquinone (0.0%, 0.1%, 0.5%, 1.0%) and citric acid (0.1%). The weights of the animals were recorded periodically for 103 weeks. After the first 6 months had elapsed, some of the males and females in Exp. 1, 2, and 3 were mated. The number of offspring for 2 successive litters

* This work was done under a grant from the Tennessee Eastman Co., and from the Douglas Smith Foundation for Medical Research of the University of Chicago.

was tabulated. At various periods during the experiment and at the end of 103 weeks, the following histological sections were prepared and examined: liver, omentum, kidney, spleen, heart, lung, bone marrow, stomach wall, pancreas, adrenal, subperitoneal and intramuscular abdominal fat. In addition to the studies initiated on immature animals, one group of 14 adult rats, together with an equal number of controls, were maintained for 9 weeks on the basic diet containing 5% hydroquinone.

Dogs. Three female and 2 male mongrel pups 4 months of age from 2 litters were placed on "Pard" (Swift), supplemented by "Esbilac" (Borden). Two of the animals served as controls while one of the other 3 received 16 mg hydroquinone/kg/day for 80 weeks. The other two dogs ingested 1.6 mg hydroquinone/kg/day for 31 weeks and 40 mg/kg/day for the succeeding 49 weeks. Also, 5 adult male dogs received 100 mg/kg/day of hydroquinone for 26 weeks. The compound was administered to the animals in the form of sugar-coated tablets that were mixed in with the food. Routine analyses of the blood and urine were made periodically. After the experiment had terminated the animals were sacrificed and autopsied, and sections were prepared as described above.

Men. Two males ingested 500 mg of hydroquinone daily for 5 months, and 17 subjects, both male and female ingested 300 mg/day for 3 to 5 months. The compound was taken in 3 divided doses with meals. During a control period for one month and while the experiment was in progress, the following analyses were performed on the blood: % hemoglobin, hematocrit or red blood cell count, differential white blood cell count, sedimentation rate, platelet count, coagulation time and icteric index. Urinalysis included albumin, reducing sugars, white and red cell counts, casts and urobilinogen.

Acute experiments. Rats. The LD50 of hydroquinone was determined in Sprague-Dawley, Priestly, and Wistar rats. The compound, dissolved in glycerin, propylene glycol, or water, was administered in varying amounts by stomach tube. Animals that were fasted were deprived of food for at least 18 hours before the hydroquinone was administered. In each experiment 6 groups of 10 to 40 animals apiece were employed in each determination and the LD50 was calculated by the method of Bliss(11). *Dogs and cats.* The LD50 of hydroquinone was also determined in these two species. Twenty-eight unselected dogs and 10 cats were employed in this study and the compound was administered in sugar-coated tablets together with a small amount of meat.

TABLE I. Final Weights of Rats after 104 Weeks of Diets Containing Hydroquinone (HQ).

	Sex	% HQ in total diet .0%	.1%	.25%	.5%	1.0%
H*	♂	527	534		460	467
	♀	300	379		300	248
H & citric acid	♂	502	401†		448	414†
	♀	273	326		320	271
H preheated with lard	♂	472	541	476	436	
	♀	280	269	263	226†	

* H = Hydroquinone.
† Significantly lower than controls at the 5% probability level.

Cumulative toxicity. Experiments. Rats. Six groups of 20 to 48 rats each were given doses of 500, 750, 1000, 1250, 1500, and 1750 mg of hydroquinone/kg by stomach tube 9 times in 12 days. An additional group of 16 animals received 500 mg/kg 101 times in 151 days. The temporal course of the fatalities was noted, and at the end of the experiment, the survivors were autopsied and histological sections were prepared as described above.

Results. Chronic Experiments. Rats. The final weights of the experimental animals after 2 years on diets containing up to 1.0% hydroquinone were generally not statistically different from the controls (Table I). However, during the first month of the experiment hydroquinone usually produced a definite decrement in the rate of growth of animals maintained on diets containing 0.5 and 1.0% hydroquinone. On the average, control males and females gained 27 and 22 g per week respectively, while rats reared on diets containing 1.0% HG gained only 16 g per week. This effect was not observed in the experiment where hydroquinone was previously heated with lard before incorporation into the diet. Hematological analyses of the experimental rats, including R.B.C., % hemoglobin, and differential W.B.C.

counts, disclosed no statistically significant deviations from the control values. The histopathological findings were also negative. The average number of off-spring of control and experimental females was almost identical for 2 successive litters. These offspring, reared on diets containing 0.0%, 0.1%, 0.25%, 0.50% hydroquinone that was previously heated with lard, grew at the normal rate. Adult rats maintained on the basic diet containing 5% hydroquinone suffered a 46% loss in weight over the course of 9 weeks, and developed aplastic anemia. Examination of the bone marrow revealed an average decrease of 66% in cellularity with marked atrophy of the hematopoietic elements. Pathological studies also revealed atrophy of the liver cord cells, lymphoid tissue of the spleen, adipose tissue, and striated muscle together with superficial ulceration and hemorrhage of the stomach mucosa.

Dogs. Pups that had received 1.6, 16, and 40 mg of hydroquinone/kg/day for 80 weeks grew normally, and adult dogs received 100 mg/kg/day for 26 weeks maintained their body weight. The gross autopsy findings, urinalyses, and hematological analyses were negative, and no pathological changes were observed in the experimental animals which could not be seen in the controls. However, hemosiderosis was usually more marked in the spleens, livers, and marrow of the control animals.

Man. Analyses of the urine and blood of 19 human subjects that ingested 300 to 500 mg of hydroquinone daily for 3 to 5 months revealed no abnormal findings.

Acute experiments. Rats. The LD50's of hydroquinone (approximately 1050 mg/kg) in different strains of rats treated under a variety of conditions is tabulated in Table II. Fasting the animal for 18 hours previous to the administration of the hydroquinone produced a 2- to 3-fold increase in the observed toxicity. Animals that survived 101 administrations of hydroquinone in 151 days, at a dose level of 500 mg/kg grew at the same rate as did the controls. Here again the autopsy findings were essentially negative, although more than half of the experimental animals succumbed after the first 2 months. In experiments where 6

TABLE II. LD_{50} of Hydroquinone (HQ) in Rats.

Strain	Carrier	Condition	LD_{50} (mg/kg) ± S.E.
Priestly	Glycerin	U*	1295 ± 2
	"	U	1005 ± 2
Sprague-Dawley	Propylene glycol	U	1090 ± 2
	Distilled water	U	1182 ± 2
	Glycerin	U	1081 ± 2
	Propylene glycol	F	323 ± 2
Wistar	" "	U	731 ± 2
	" "	F	298 ± 2

* U = Unfasted; F = Fasted.

groups of rats received from 500 to 1750 mg/kg of hydroquinone nine times in 12 days, it was found that 71% of the total mortality rate occurred within 24 hours after the initial dose. During the succeeding 11 days in which the remaining 8 administrations were made, the mortality rate averaged less than 5% per day. *Cats and dogs.* The LD50 of hydroquinone in dogs was found to be 299 mg/kg. Too few cats were employed to obtain a statistically accurate estimate of the LD50, but it apparently lay somewhere between 42 and 86 mg/kg.

Discussion. The LD50 is of little practical value for the estimation of the chronic and potentially cumulative toxicity of noxious substances, and the inferences of such a study are often misleading. For example, in fasted rats, dicumarol is less toxic than hydroquinone on an acute basis (LD50 of 310 mg/kg, in contrast to 540 mg/kg), while it produced an 80% mortality in animals maintained for 30 days on a diet containing .01% dicumarol(12). Assuming that each 250 g rat consumed 4 g of food daily, during the entire 30-day period each animal ingested less than 1/10 of the LD50 dose. Also, on the basis of the LD50, mapharsen was originally rejected in favor of salvarsan while it has since been demonstrated that the former drug produces far fewer delayed reactions than either arsphenamine or neoarsphenamine(13).

However, the acute determinations have divulged one fairly basic aspect of hydroquinone toxicity, *i.e.,* the increased susceptibility of fasted animals. The LD50 of unfed rats was 310 mg/kg in contrast to an average of 1064 mg/kg observed in normal animals. This finding is possibly related to both the enhanced

rate of absorption of hydroquinone from the empty gastrointestinal tract and to the diminished detoxification potential of the fasted, glycogen-poor liver. In this connection it is interesting to note that the LD50 of hydroquinone when administered via stomach tube to fasted rats closely approximates the lethal dose observed when the compound is injected subcutaneously(14). Of course, if the chemical was ingested as part of the normal diet (as it would be if incorporated in food commercially), these considerations would not generally be applicable.

Rats subsisting for 2 years on diets containing from 0.1% to 1.0% hydroquinone generally grew and reproduced normally, although at the 0.5 and 1.0% levels there was a definite retardation in growth observed during the first month on the diet. Blood, tissue and urine analyses were negative. However, when adult animals were kept on a diet containing 5.0% of hydroquinone, a definite and progressive loss in body weight was observed together with generalized pathological alterations. The adverse findings were due at least in part to inanition, for these animals ingested less food. Hydroquinone has a discernable taste at this concentration in the diet. The results obtained with immature rats during the first month of the experiment may also be related to this observation, for when the hydroquinone was previously heated with the lard the animals grew at the normal rate.

Young dogs that received 1.6 to 40 mg hydroquinone kg/day for 80 weeks grew normally, and mature dogs that received 100 mg/kg/day for 26 weeks maintained their body weight. No consistent pathological findings could be demonstrated.

Contrary to reports in the literature(15), there was no evidence to indicate that hydroquinone possesses a cumulative toxicity, at least when given by stomach tube. At a dose of 500 mg/kg, (one-half the LD50), it was administered 9 times in 12 days to rats and the observed mortality rate was only 1.0% per day. Furthermore, rats which survived 101 administrations in 151 days grew at the same rate as the controls although more than half of the experimental rats had died over this period. The mortality rate was 0.4% per day.

The daily ingestion of 300 to 500 mg of hydroquinone for 3 to 5 months by 10 human subjects caused no observable alterations in the blood and urine. Since these quantities of accidentally ingested material have been reported to produce toxic symptoms(16,17), it may be significant to note that the compound was taken in 3 divided doses at meal time, so that the rate of absorption was undoubtedly diminished under these circumstances. This factor may also account for the absence of eye lesions in dogs that received 100 mg of hydroquinone/kg/day mixed with their food, such as were observed by Woodard(18).

It has been pointed out(2) that hydroquinone is widely distributed as a plant component. When ingested as part of the plant it has never been found to have toxic effects. Finally, it should be emphasized that the quantities of hydroquinone employed in these studies were relatively enormous. Assuming that a 70 kg man ingests one gram of fat per kilogram per day, and that all the fat was commercially treated with 0.01% hydroquinone, our human subjects did consume daily from 40 to 70 times the normal quantities of hydroquinone for 3 to 5 months, and on the same basis, our rats ingested daily up to 1000 times the calculated commercial dose. This tremendous safety factor demonstrated in these chronic studies strongly militates agains the exclusion of hydroquinone as a preservative in food fats.

Summary. Chronic toxicity experiments with hydroquinone have been carried out in rats, dogs, and men at dose levels greatly exceeding those to be expected if hydroquinone were utilized commercially as a preservative in food fats. It was found that relatively tremendous quantities of the substance had to be ingested before demonstrable physiological pathology occurred in the experimental animals. Acute studies on rats revealed that the LD50 of fasted animals was approximately one-third that of fed controls. The relatively large amount of hydroquinone that could be administered with impunity indicates that the compound could be used safely as a food preservative.

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Received October 14, 1953. P.S.E.B.M., 1953, v84.

Effect of N-Allyl Normorphine on Excretion of C-14-Labeled Morphine in Mice. (20752)

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The use of N-allyl normorphine in the treatment of respiratory depression resulting from overdosage of morphine or one of its synthetic substitutes(1,2) has been indicated by the many favorable responses which have been obtained both experimentally and clinically. The practical application of this antagonism, however, precedes any reasonable explanation of the mechanism involved in the spectacular recoveries reported in the literature. The experiments reported here indicate that N-Allyl normorphine is capable of producing at least two secondary effects in intact, morphinized animals, in addition to central stimulation. These results were obtained in the course of studies on the excretion of C-14-labeled, biosynthesized(3) morphine, utilizing small amounts of the drug; the quantity of alkaloid was not sufficient to cause central depression of remarkable degree.

Morphine has been shown to produce an antidiuretic response in experimental animals (4); the mouse shares this property of the alkaloid. This finding and other data suggested that a partial explanation of N-Allyl normorphine's action might rest in an increased ability to excrete morphine, free or conjugated, by activation of renal or other mechanisms, thereby decreasing the time that the drug could exert its effects.

Methods. Eight mice weighing 20 g each were injected intraperitoneally with 0.070 mg of C-14-labeled morphine base, each dose equivalent to 2100 counts (the specific activity of the compound isolated from Papaver somniferum was 30000 counts per mg per minute). Four of the animals were then injected intraperitoneally with 500 γ of N-allyl normorphine ("Nalline", Merck). The Straub reaction appeared in each animal approximately 2 minutes after injection of the C-14 morphine.

The urine was collected hourly in glass metabolism cages and suitable aliquots of each sample were plated on copper discs and counted in open window, gas-flow counters; results are expressed as counts recovered per hour. As the most significant findings appeared within 6 hours after injection, only