TABLE II. Effect of Chondroitin Sulfate upon ACTH Induced Adrenal Ascorbic Acid Depletion, 3 Hr Post Injection. 5 animals in each series.

%	Vehicle	Dose ACTH, U.S.P. units	Adrenal ascorbic acid, mg/100 g adrenal gland
.9	Saline	0	371 ± 10
.9	"	1.0	273 ± 10
8.0	C.S.	1.0	155 ± 4
16.0	Gelatin	.1	236 ± 17
8.0	C.S.	.1	248 ± 7

preparations inhibiting hyaluronidase directly or by competitive inhibition may be ascribed to decreased absorption of the trophic material from the injection site. That the decreased rate of absorption from the site of injection may play a part in augmentation of pituitary gonadotrophic hormone was shown by the studies of Meyer and Hertz(15). They demonstrated that hourly injection of pituitary gonadotrophins over a 4½-day period produced a marked augmentation of response over that noted when the same dose was given at 12-hour intervals.

Summary. Anti-hyaluronidase agents, *i.e.* phosphorylated hesperidin, d-alphatocopherol phosphate, and chrondroitin sulfate were shown to be capable of increasing the effectiveness of administered pituitary trophic hormones such as follicle stimulating hormone (FSH) and corticotrophin. A four-fold increase in effectiveness of FSH was attained when the hormone was administered together with the anti-hyaluronidase agents as compared to the injection of the gonadotrophin alone. A similar degree of enhancement was obtained with corticotrophin. The augmentation induced by the anti-hyaluronidase compounds is attributed to the decreased rate of absorption of the hormone from the site of injection.

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Effect of Metabolites on Accumulation of Citric Acid in Fluoroacetate-poisoned Rats.* (20750)

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This study was undertaken in connection with our work on the effect of Krebs cycle inhibitors and metabolites on the performance and metabolism of isolated dog hearts, (heart-lung preparations). On adding dicarboxylic acids of the Krebs cycle into the blood stream in such preparations the problem of permeability arose. It cannot be assumed *a priori* that such highly charged dicarboxylate ions as are normally formed and metabolized within the cell will diffuse into the cell as readily as the glucose molecule. To gain more information on this point we have undertaken to study the organs of small animals *in situ,* using in the experiments here reported the fluoroacetate

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poisoned rat, which appears particularly suitable for such a study, since here the Krebs cycle is blocked at the citric acid stage and the latter accumulates in some organs of the animal. This accumulation of citric acid, as Peters(1) points out, is the best evidence we have that the Krebs cycle functions *in vivo*. Potter(2,3) has utilized the fluoroacetate poisoned rat successfully to study metabolic pathways and what he termed their "sequential blocking" *in vivo*. Potter reasons, that if in addition to the fluoroacetate block one places another block at the succinate stage by injecting malonate for example, a decrease in the amount of accumulated citrate is to be expected. Such a decrease was observed by him in the kidney, thymus, brain, and spleen though not in the heart, and this constitutes further evidence for the functioning of the tricarboxylic acid cycle *in vivo*. Now if it is assumed that in a fluoroacetate-poisoned animal a considerable fraction of the available C_4 member of the Krebs cycle is trapped in the accumulated citrate, we can expect upon injection of each of these C_4 fragments, or their precursors, to observe an increase in citrate over and above that which is observed after injecting fluoroacetate alone, provided that the necessary amount of C_2 fragments is readily available. On the other hand, if an excess of the C_2 fragments or their precursors is injected, one may expect the excess of acetate to compete with fluoroacetate for coenzyme A, with a resulting decrease in fluorocitrate formation and hence a diminution in citrate accumulation. Although acetyl donors have been used as antidotes in fluoroacetate poisoning(4) no one appears to have demonstrated a decrease in the amount of accumulated citrate.

We have also performed preliminary experiments to test the effect of injecting the C_4 and also the C_2 members of the Krebs cycle and their precursors on the tissue level of citric acid in non-poisoned animals. Here, however, no predictions could be made on the basis of existing knowledge.

Methods. Male and female albino rats 6 to 7 months old were used. Although these rats are of unrecorded strain, they have been thoroughly inbred for a long period of time at this University. The metabolites were injected in a standard dose of 2.25 millimoles per 100 g body wt in a volume of 2 or 4 ml. Larger doses were tried in each case when the results were inconclusive. The aqueous solution of the acid after being neutralized with NaOH was injected subcutaneously in several places in the back of the animal. Thirty minutes after administration of the metabolite, 3 mg sodium fluoroacetate per kg were injected intraperitoneally. Two hours after the fluoroacetate injection the animal was sacrificed and the heart and kidney quickly removed. The ventricles and whole kidneys were used for analysis, these tissues being homogenized in two volumes of ice-cold isotonic KCl and rehomogenized after addition of enough 10% trichloracetic acid to make the final dilution 5%. After centrifuging, the clear liquid was used for citric acid determination by the pentabromacetone method as modified by Natelson *et al.*(5). We used Mallinckrodt purified petroleum ether B.P. 30-75°C, and omitted the evaporation step. None of the metabolites we studied interfered with the accuracy of the method. Control experiments in which the same volume of saline was injected instead of metabolite were always run simultaneously.

Results. The results obtained in fluoroacetate-poisoned rats are summarized in Table I. It can be seen that the standard dose (2.25 millimoles per 100 g body weight) of each of the C_4 members of the Krebs cycle caused a marked increase in the accumulation of citric acid in the heart, but no significant increase in kidney citrate.. 1-Glutamate and 1-aspartate produced a slight but significant increase in heart citrate, on the other hand they produced a small but significant decrease in kidney citrate. Pyruvate was less effective than the C_4 fragments in increasing heart citrate but it caused a significant decrease in kidney citrate. Of the C_2 fragments studied, acetate and ethanol caused a marked decrease in both heart and kidney citrate. The most striking results were obtained with propionate and propanol. The latter produced low citrate values that approached those found in normal *i.e.* non-poisoned animals. Valerate also caused a marked reduction in the level of accumulated citrate. Butyrate on the other hand, caused

TABLE I. Citrate Accumulation in Heart and Kidney of Fluoroacetate-Treated Rats after Injection of Various Metabolites. Unless otherwise stated, 2.25 millimoles of the metabolites were injected per 100 g body wt.

Metabolite	No. of animals	Sex	Citrate in heart (μg/g wet tissue), mean ± S.E.‡	Citrate in kidney (μg/g wet tissue), mean ± S.E.
α-Ketoglutarate	3	♀	2030	1510
Saline controls	3	♀	893	1307
Succinate	10	♀	1700 ± 108	1490 ± 45
Saline controls	10	♀	954 ± 38	1417 ± 129
Malate	10	♀	1937 ± 67	1680 ± 64
Saline controls	10	♀	1039 ± 32	1529 ± 58
Oxalacetate	3	♀	1952	1736
Saline controls	3	♀	1068	1592
l-glutamate	10	♂	1173 ± 64	1226 ± 58
Saline controls	10	♂	960 ± 53	1561 ± 99
l-Aspartate	10	♀	1353 ± 65	1284 ± 80
Saline controls	10	♀	983 ± 48	1534 ± 55
Pyruvate	4	♀	1380 ± 108	889 ± 30
" *	7	♀	1919 ± 61	750 ± 51
Saline controls	7	♀	1013 ± 62	1473 ± 53
Acetate	4	♀	914 ± 57	961 ± 55
" *	10	♀	149 ± 23	416 ± 36
Ethanol	5	♂	541 ± 40	367 ± 48
" †	10	♀	340 ± 26	85 ± 8
Propionate	7	♂	31 ± 4	273 ± 66
n-Propanol	5	♂	19 ± 5	94 ± 10
n-Butyrate*	10	♂	1644 ± 137	963 ± 88
Saline controls	10	♂	960 ± 53	1560 ± 99
Acetoacetate	10	♀	1240 ± 59	974 ± 48
Saline controls	10	♀	1010 ± 48	1479 ± 61
n-Valerate	7	♂	319 ± 58	466 ± 95

* 4.5 millimoles/100 g body wt.
† 22.5 " " " .

‡ Stand. error $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

a rise in heart citrate but an almost equal lowering of kidney citrate. Acetoacetate behaved like butyrate in increasing the heart citrate and decreasing the kidney citrate.

Table II summarizes preliminary results obtained when metabolites are injected into normal *i.e.* non-poisoned animals. Here it is seen that all metabolites produce a substantial rise in kidney citrate, ethanol being the least effective. On the other hand, there was no significant increase in heart citrate except where the double dose of acetate or butyrate was used.

A comparison of Table II with Table I reveals that the heart and kidney citrate levels obtained after injection of the double acetate dose as well as the standard propionate dose, are almost identical in the poisoned and in the normal animals; the same is true of the kidney citrate values with ten times the standard dose of ethanol. This suggests that under these conditions the metabolites mentioned have completely overcome the fluoroacetate block.

Discussion. In interpreting results obtained after injecting various substances into a whole animal, certain factors must be considered which may play little or no part in the study of homogenates, tissue slices or isolated organs. For, if the reaction of one organ is different from that of another, it does not always follow that the metabolic patterns are different. Differences in cell permeability, and above all, in vascularity may be important. Furthermore, in an intact animal the kidney does not excrete all substances at the same rate, and, finally, a substance may be metabolized in one organ

TABLE II. Citrate Level in Heart and Kidney of Normal Rats after Injection of Various Metabolites. Unless otherwise stated, 2.25 millimoles of the metabolite were injected/100 g body wt.

Metabolite	No. of animals	Sex	Citrate in heart (μg/g wet tissue), mean ± S.E.	Citrate in kidney (μg/g wet tissue), mean ± S.E.
None	9	♂	19 ± 2.6	40 ± 1.4
Malate	4	♂	42 ± 9.2	389 ± 27
Pyruvate	4	♂	81 ± 29	224 ± 33
Acetate	4	♂	35 ± 3	102 ± 33
" *	4	♂	145 ± 19	527 ± 45
Ethanol†	5	♂	27 ± 5	70 ± 6.2
Propionate	4	♂	26 ± 1	192 ± 4
Butyrate	4	♂	51 ± 10	189 ± 21
" *	4	♂	326 ± 27	921 ± 101

* 4.5 millimoles/100 g body wt.
† 22.5 " " " .

and delivered to another organ in a changed form. This difficulty in interpretation, however, does not render experiments on the whole animal worthless. On the contrary, results so obtained, provide a stimulus for further experimentation using less complicated systems in an effort to analyze the importance of the various factors mentioned.

Fluoroacetate may penetrate organs to a varying extent. Hagan *et al.*(6) found that rat liver contained less organic fluoroacids after feeding fluoroacetate than kidney, heart and brain, although Potter's(2) bioassay revealed no significant differences between tissues. Now it is known that no citrate accumulates in the liver of fluoroacetate poisoned rats. In the light of our results concerning the inhibition of citrate accumulation in the heart and kidney by excess acetyl donors, could it be that this lack of citrate accumulation in the liver, the chief acetylating organ, may be due, in part at least, to the presence there of excess C_2 fragments?

The observed decrease in citrate accumulation in the kidney and heart of fluoroacetate poisoned rats after the administration of acetyl donors may be of toxicological interest. All the compounds that caused such an inhibition of citrate accumulation have been found to be effective as antidotes for fluoroacetate poisoning in one species or another(4). The question naturally arises as to whether this method may be utilized to screen compounds for use as antidotes for fluoroacetate poisoning. Kandel and Chenoweth(7) are of the opinion, however, that the blockade of the tricarboxylic acid cycle by fluoroacetate is not related to the development of symptoms of poisoning.

Why propionate and propanol are more effective than acetate and ethanol in inhibiting citrate accumulation in poisoned animals is not clear. Propionate is thought to be metabolized via pyruvate(8) but our results do not seem to support such a conclusion, since the effect of pyruvate is qualitatively and quantitatively different from that of propionate. The behavior of valerate is that which may be expected on the basis of β-oxidation to yield acetate and propionate.

The effect of the C_4 members of the Krebs cycle in increasing the citrate accumulation in the heart, but not in the kidney of fluoroacetate poisoned animals is difficult to explain. The injection of the C_2 or the C_4 members into non-poisoned animals causes a greater increase in kidney than in heart citrate level. Furthermore, the resting citrate level in the kidney is higher than in the heart. These facts could be explained by assuming that the metabolic wheel turns at a faster rate in the heart than in the kidney. The high kidney citrate value in fluoroacetate poisoning speaks against this hypothesis but this in turn might be explained by assuming that the kidney has a greater supply of C_4 fragments, perhaps by way of the corresponding amino acids.

Butyrate, pyruvate and acetoacetate increase the heart citrate value in poisoned animals, behaving in this respect like the C_4 members of the Krebs cycle. In the kidney, however, they reduce the citrate level, although not to the same extent as the C_2

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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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

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