The observations suggest that in the dog the liver plays an important part in the metabolism of vitamin C. The changes in the output of the vitamin seen after induction of hepatic injury are much larger than the normal daily variations or those observed after the administration of a wide variety of substances studied as possible precursors of vitamin C, such as proteins, amino acids, carbohydrates and various vegetable and animal oils. It is likely the increase in vitamin C output following chloroform anesthesia was in part due to asphyxia and subsequent depletion of tissue glycogen.

The function of the liver that is concerned with vitamin C metabolism is not known, but the investigations on shivering and iodoacetate reported in an accompanying paper[2] suggest that the glycogenetic function is the one involved.

11695 P

Effect of Shivering, Iodoacetate, and Epinephrine on Vitamin C and Creatine Excretion in Fasting Dogs.*

A. T. MILHORAT, J. D. HARDY, W. E. BARTELS AND V. TOSCANI.

From the Russell Sage Institute of Pathology in Affiliation with the New York Hospital and Departments of Medicine and Psychiatry, Cornell University Medical College, New York City.

In a previous report[1] the excretion of vitamin C by the fasting dog was discussed, and it was shown that hepatic injury can increase the urinary output of the vitamin. In the following experiments, the effect of severe shivering, epinephrine, and iodoacetate on the excretion of various urinary substances was investigated.

Experimental. The technic was similar to that described previously.[1]

Shivering was induced by anesthetizing the animals with intravenous nembutal and then placing them in a refrigerator at 12°C. In most instances the body temperature fell to around 31°C. Although additional nembutal kept the animals in an unconscious state, severe and almost constant shivering occurred.

2 Milhorat, A. T., Hardy, J. D., Bartels, W. E., and Toscani, V., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 397.

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

1 Milhorat, A. T., Bartels, W., and Toscani, V., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 394.

Observations. Data in typical experiments are shown in Table I. Shivering produced considerable increase in the excretion of vitamin C and creatine, an increase in urinary nitrogen and urea and a decrease in creatinine.† Following periods of shivering lasting from 24 to 72 hours, the animals developed rigid paralysis of the extremities which persisted until food was given. One dog that was not fed had severe stiffness and paralysis of the extremities for 7 days when it died apparently with weakness of the muscles of respiration. In all animals muscular paralysis was accompanied by large output of urinary creatine and vitamin C.

The effect of nembutal in animals kept at warm room temperatures was determined. Complete anesthesia associated with slight shivering induced definite increase in urinary nitrogen but only a slight increase in vitamin C excretion and was without effect on the creatine.

Iodoacetate. Because of the similarity between the rigid paralysis induced by severe shivering and that seen in animals poisoned with iodoacetate, the effect of iodoacetate was studied. The data presented in Table I show that the administration of iodoacetate induced definite creatinuria but did not change the output of vitamin C.

TABLE I.
Effect of Shivering and of Iodoacetate on Daily Urinary Output.

Day of fast	Total N, g	Urea N, g	Creatinin, mg	Creatin, mg	P, mg	Vitamin C, mg
15	1.66	1.22	165	20	138	48
16	1.46	1.17	188	5	105	48
17*	Urine lost					
18*	1.44	1.14	96	56	134	124
19†	1.89	1.41	127	155	63	148
20†	2.38	1.91	152	165	90	120
21†	1.80	1.39	171	80	49	110
22†	1.76	1.36	156	43	74	60
17	2.52		310	36	132	60
20	2.17		357	5	139	68
21‡	3.05		375	28	222	64
22	3.42		327	160	94	62
23	2.55		307	84	96	40
24	1.76		284	0	88	40

*Severe shivering for 24 hrs.
†Rigid paralysis of extremities.
‡Iodoacetic acid 100 mg intrav.
Blood sugar level (mg per 100 cc):

Before shivering	55
1st day of shivering	108
2nd day of shivering	58

† The variations in the different experiments were: Vitamin C, from 80 to 200 mg; creatine, 70 to 140 mg; total nitrogen, 0.8 to 1.8 g; urea N, 0.7 to 1.7 g; creatinine, 70 to 110 mg.

Epinephrine. The administration of epinephrine increased the urinary excretion of nitrogen, vitamin C, and creatine.

Discussion. It is of interest that both severe shivering and epinephrine increased the urinary output of vitamin C and creatine whereas iodoacetate given in amounts large enough to induce definite creatinuria did not change the excretion of vitamin C. Both shivering and epinephrine are known to remove glycogen from the muscles (Külz,[2] Junkersdorf[3]), whereas iodoacetate interferes with the glycogen-lactic acid cycle at the hexose monophosphate stage but appears not to affect the glycogen store itself. In these experiments shivering and epinephrine increased the blood sugar and induced glycosuria. The large output of creatine probably was the result of impaired resynthesis of creatin-phosphoric acid secondary to the defect in the glycogen-lactic acid cycle. In some experiments the urinary excretion of phosphorus was increased during the first day. On subsequent days, despite appreciable creatinuria, the phosphorus output was not increased. It is probable that some of the phosphoric acid was retained as hexosephosphate esters (Cori and Cori[4]).

The data suggest that the metabolism of vitamin C is related significantly to that of glycogen. Moreover, the stages in glycogen metabolism that are involved in this relationship appear not to be those in the lactic acid cycle that are at or beyond the hexosemonophosphate stage, since factors that remove muscle glycogen increase the urinary vitamin whereas iodoacetate is without effect. This formulation would explain the findings of other investigators on the effect of ether anesthesia (asphyxia) (Bowman and Muntwyler,[5] Zilva,[6]) and insulin (Ralli and Sherry[7]) on vitamin C excretion.

---

2 Külz, E., C. Ludwig's *Festschrift,* Marburg, 1891, p. 119.

3 Junkersdorf, P., and Török, P., *Pflugers Arch. gesam. Physiol.,* 1926, **211**, 414.

4 Cori, G. T., and Cori, C. F., *J. Biol. Chem.,* 1936, **116**, 119.

5 Bowman, D. E., and Muntwyler, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 437.

6 Zilva, S. S., *Biochem. J.,* 1935, **29**, 2366.

7 Ralli, E. P., and Sherry, S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 669.