ney slices obtained from these dogs. This indicates that enough acetate or 2 carbon fragments had accumulated during the infusion period to stimulate the uptake of PAH to the same extent as 0.01M acetate added *in vitro* (compare Table I and III). The infusion of acetate further protected the kidney from NaFAc inhibition and the protection was roughly proportioned to the rate of acetate infusion.

The effect of fluoroacetate on oxygen consumption was relatively small compared to its effects on PAH accumulation. This is especially apparent in those experiments where no acetate was added. This indicates that only a small fraction of the total oxygen consumption is utilized for PAH transport. On the other hand fluoroacetate eliminated both the acetate induced increments in oxygen consumption and PAH uptake. This finding shows that acetate or the two carbon fragment is of primary importance in renal PAH transport(2).

Summary. Small doses of fluoroacetate mainly inhibit the ability of dog renal slices to respond to acetate stimulation as measured by PAH uptake and oxygen consumption. Larger doses of fluoroacetate produced inhibition of oxygen consumption and PAH uptake of renal slices in the absence of substrate. The effects on PAH uptake are more marked than are those on oxygen consumption.

1. Farah, A., Graham, G., and Koda, F., *J. Pharmacol. and Exp. Therap.*, 1953, v108, 410.

2. Cross, R. G., and Taggert, J. V., *Am. J. Physiol.*, 1950, v161, 191.

3. Weeks, J. R., Chenoweth, M. B., and Shideman, F. E., *J. Pharmacol. and Exp. Therap.*, 1950, v98, 224.

4. Farah, A., West, T. C., and Angel, R., *ibid.*, 1950, v98, 234.

5. Furchgott, R. F., *ibid.*, 1950, v99, 1.

6. West, T. C., Jones, D. M., and Loomis, T. A., *Am. J. Physiol.*, 1953, v172, 541.

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Stability of Serum Amylase.* (20598)

ROBERT L. MCGEACHIN. (Introduced by Warren S. Rehm.)

From the Department of Biochemistry, University of Louisville School of Medicine, Louisville, Ky.

Data available on the stability of pancreatic amylase in aqueous solution(1,2) indicate that highly purified preparations are quite stable but that crude preparations may lose their activity rapidly. However, a search of the literature disclosed a paucity of information regarding the stability of amylase in body tissues or fluids during storage. Scharles and Salter(3) state that aqueous extracts of the tumors of mouse sarcoma 180, preserved with toluene and kept in the refrigerator, retained their activity for several months. Fennel(4) states "urine or serum properly obtained and stored in the icebox at 4°C is trustworthy for 24, 48, or even 72 hours . . . the amylase content of serum remains remarkably constant in storage at 4°C; that of urine is not so stable." However, no quantitative data to support these statements were given in these papers and none was found elsewhere.

In clinical laboratories, it may be necessary to delay serum amylase determinations several days. Therefore, the question arises whether the amylase activity of stored sera remains constant.

Procedure. Samples of human blood were obtained from 14 normal, presumably healthy, staff members, technicians and secretaries of the University of Louisville School of Medicine. After clotting of the blood, the sera were immediately separated by centrifugation and analyzed for amylase activity by the method of Van Loon(5). Determinations of amylase activity were carried out immediately after separation of the sera (Day 1), one day

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TABLE I.
Amylase Activity of Human Sera, in 14 Subjects.

	Amylase activity of serum		
	Day 1	Day 2	Day 5
	87	82	77
	102	111	103
	86	89	86
	121	119	118
	61	74	86
	112	112	114
	64	66	81
	106	105	105
	105	104	103
	107	93	101
	76	85	93
	110	101	95
	39	44	48
	126	123	122
Avg	93.0	93.5	95.1
Stand. dev.		6.5	11.1
		9.2%	16.1%
Stand. error		1.8	3.0
		2.5%	4.3%
t		.28	.70
		.83%	1.30%

TABLE II.
Amylase Activity of Dog Serum in 5 Animals.

	Amylase activity of serum		
	Day 1	Day 2	Day 5
	1180	1190	1290
	2660	2370	2440
	900	1470	1450
	800	800	780
	940	940	940
Avg	1300	1350	1380

later (Day 2), and 4 days later (Day 5). The sera were stored in tightly stoppered test tubes in a refrigerator at 9-10°C between analyses. The results of these analyses are given in Table I. Sera obtained from 5 dogs were analyzed and treated in similar manner (Table II). Since the amylase content of dog serum is normally 10-20 times that of human serum, 1:100 dilutions of serum were used in these analyses instead of the standard 1:10 dilutions.

Results. The results shown in Table I were analyzed statistically using the method of paired variants. "Student's" t test was applied to the results using the hypothesis that no change in amylase activity occurred. Using the 5% value for t, which for 14 pairs of observations is 2.16, it was found that no change occurred. The t values in all cases were less than 2.16. Fiducial limits for Day 1-Day 2 were 0.5 ± 3.8 amylase units and 2.0 ± 5.3%; for Day 1-Day 5, 2.1 ± 6.5 amylase units and 5.5 ± 9.3%.

Discussion. In no case were the changes of such magnitude that they would lead to erroneous clinical diagnoses if serum amylase determinations were delayed. The increases in the amylase activities of the sera from P.M. and S.W. and from Dog 3 were significant but the mechanism of these increases is unknown. Work is now in progress testing the possibility of the formation of amylase activators or the presence of labile inhibitors. When the data on human sera were analyzed omitting the results for P.M. and S.W. there was no change in the general conclusion that the amylase activity of serum does not change during storage.

It is interesting that the serum amylase level of one subject, W.C., was definitely subnormal (normal range for Van Loon's method is 60-160) even though the subject showed no clinical symptoms of pancreatic insufficiency or hepatic disease.

Summary. The amylase activity of serum does not change significantly on a statistical basis when sera are stored at 9-10°C for 5 days. A few cases in which there were significant increases were noted, however.

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1. Meyer, K. H., Fischer, E. H., and Bernfeld, P., *Arch. Biochem.*, 1947, v14, 149.

2. Caldwell, M. L., and Kung, J. T., *J. Am. Chem. Soc.*, 1953, v75, 3132.

3. Scharles, F. H., and Salter, W. T., *Am. J. Cancer*, 1934, v20, 613.

4. Fennel, E. A., *Am. J. Clin. Path.*, 1944, v14, 89.

5. Van Loon, E. J., Likins, M. R., and Seger, A. J., *ibid.*, 1952, v22, 1134.

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