sion produced also some prolongation of the duration of estrus. A number of rats and mice were sacrificed during the later period of the estrus and the uteri examined histologically. They presented hypertrophy of the muscular layer and submucosa. The changes were more pronounced in the rats and in these the effect was more marked in the animals injected with the aqueous suspension than with the suspension in oil.

Inspection of the places of injection when the aqueous suspension was used showed no irritation, nor were deposits visible. In addition to the convenience of administration, the freedom of oil eliminates the possibility of reactions as they occasionally occur in patients sensitive to oil. Clinical investigation in humans, using the same aqueous suspension of estrone, corroborates the results in experimental animals.[7]

Conclusions. The duration of estrus in castrated rats and mice was measured after injection of equal amounts of estrone as an aqueous suspension, as a suspension in oil, and as a solution in oil. The suspensions, particularly the aqueous ones, by far outlasted the effect of the solution. The aqueous suspension of estradiol was also followed by a prolonged stage of estrus. This method offers a convenient way to produce prolonged effects with free estrogens without resorting to surgical implantation of crystals.

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Effect of Vitamin C Deficiency on Action of Different Types of Barbiturates.

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Generally, the liver is assumed to be of importance for the destruction of the so-called short-acting barbiturates, but not for the long-acting group. Recent work by Scheifley and Higgins[1] using partial hepatectomy, and by us[2] employing chemical liver damage, brought the unexpecteed result that the liver does not seem to be equally important for the destruction of pentothal sodium, a widely used ultra-short-acting intravenous anesthetic. Simultaneously, we

[7] Freed, S. C., *J. Clinic. Endocrin.*, in press.

[1] Scheifley, C. H., and Higgins, G. M., *Am. J. Med. Sci.*, 1940, **200**, 264.

[2] Richards, R. K., and Appel, M., *Anesthesia and Analgesia*, 1941, **20**, 66.

found that the type of liver damage appeared to be of importance, as there was little difference in the action of the various types of barbiturates if severe fatty infiltration of the liver in rats was produced by dietary means.

In pursuance of the possibilities of nutritional factors in the detoxification of barbiturates, we studied the rôle of vitamin C in this connection.

While it is well known that this vitamin affords a certain protection against some poisons, most of the recent publications concern the influence of drugs upon the vitamin C excretion. Here, the barbiturates were found to increase vitamin C output in the urine (Ritz, Samuels, and Addiss,[3] Svirbely,[4] Longenecker, Fricke and King.[5]

Our approach was to study the effect of vitamin C depletion upon the action of different barbiturates. Barbital sodium was used as a long-acting drug, pentobarbital sodium (Nembutal) as a short-acting hypnotic, and the ultra-short-acting pentothal sodium, on account of its different chemical structure, as a thiobarbiturate, and for the reasons mentioned before. These compounds were administered intraperitoneally in groups of guinea pigs and their sleeping time observed. This was repeated after 7 to 10 days, and then the animals were placed on a vitamin C-free diet on which they remained up to 33 days.

During this period the injection of the barbiturate was repeated at intervals and the sleeping time noticed. (See Table I—Barbital Sodium.)

Four groups of 9 to 13 animals were studied with barbital sodium. The average sleeping times did not show a consistent or significant increase even after prolonged vitamin C depletion which was fatal for some animals.

The next table shows similar experiments with Nembutal. (See Table II—Nembutal.) Here, the difference between the normal and C-depleted animals was striking. One week of C-free diet was without influence (Group A), but after 2 weeks in some groups (B and D), later in others (C), the sleeping time is markedly prolonged. Injections of 50 mg vitamin C subcutaneously daily for 3 to 4 days with (Group C) or without (Group D) replacement of normal diet promptly restored normal sleeping times.

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[3] Ritz, N. D., Samuels, G. T., and Addiss, G., *J. Pharm. and Exp. Ther.*, 1940, **70**, 362.

[4] Svirbely, J. G., *Abstracts Meet. Am. Chem. Soc.*, Oct., 1938.

[5] Longenecker, H. E., Fricke, H. H., and King, C. G., *J. Biol. Chem.*, 1940, **135**, 497.

TABLE I.
Barbital Sodium Intraperitoneally in Guinea Pigs.

Group	No. of animals	Days between tests	Sleeping time: Avg hr	Avg min	Range hr min	Range hr min	Diet and remarks
A	10		3	38	3 14	- 3 56	Normal
	10	7	2	48	1 50	- 4 15	"
	10	7	4	35	3 49	- 5 49	C-free 7 days
	10	7	2	55	2 19	- 4 04	" 14 "
B	13		2	52	1 05	- 4 48	Normal
	13	8	3	42	1 58	- 5 15	"
	12	14	3	51	1 39	- 5 47	C-free 14 days
	10	10	4	00	2 18	- 6 10	" 24 "
	9	7	4	15	1 55	- 5 33	" 31 "
C	9		5	10	1 36	- 6 42	Normal
	10	7	4	06	2 15	- 5 18	"
	10	20	6	09	5 09	- 8 39	C-free 14 days
	9	8	5	30	5 04	- 7 02	" 22 "
D	10		5	15	3 35	- 6 26	Normal
	9*	7	4	47	3 01	- 7 27	"
	10	28	5	51	4 14	- 7 57	C-free 23 days

*One animal failed to sleep.
Groups A and B received 100 mg/kg. Groups C and D, 130 mg/kg.

The significance of the differences in the sleeping time occurring on normal and on C-free diet was proven statistically on Group A and Group B. In Group A, the longest sleeping time on a normal diet, 4 hours, 57 minutes, was compared with the shortest one on a C-free diet, 7 hours, 14 minutes. The standard error ϵ for the normal experiment was 10 minutes and for the C-free one 32 minutes. The significance of the difference was calculated by the formula, $\frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$, where M_1 and M_2 are the averages of the two groups. The value found is 4.0 which makes the result statistically highly significant.

A similar calculation was performed in Group B where the normal sleeping time of 4 hours, 47 minutes was compared with 6 hours, 29 minutes on the C-free diet. Here, also, the highly significant value of 9.4 was obtained.

Since the differences in Groups D and E are practically identical to those in Groups B and C, no calculations were performed for Groups D and E.

Addition of 5 mg of vitamin C to a normal diet in non-depleted guinea pigs (Group E) did not exert a significant influence.

The following series comprises the experiments with pentothal sodium. (See Table III—Pentothal Sodium.) The results are

TABLE II.
Nembutal (30 mg/kg) Intraperitoneally in Guinea Pigs.

Group	No. of animals	Days between tests	Sleeping time: Avg hr	Avg min	Range hr	Range min	Range hr	Range min	Diet and remarks
A	10		4	57	4	00 -	5	45	Normal
	10	7	4	01	3	40 -	6	08	"
	10	7	4	20	2	39 -	5	26	C-free 7 days
	8	13	7	14	5	08 -	8	37	" 20 "
	8	7	7	44	5	51 -	9	20	" 27 "
B	11		3	53	2	44 -	5	58	Normal
	11	8	4	47	3	58 -	5	49	"
	11	15	6	55	6	21 -	7	09	C-free 14 days
	10	10	6	29	5	55 -	7	03	" 24 "
	5	7	6	26	5	41 -	6	50	" 31 "
C	10		3	25	2	44 -	4	23	Normal
	10	8	3	52	2	24 -	5	25	"
	8	30	7	13	5	40 -	8	36	C-free 24 days
	4	8	4	49	4	33 -	5	12	Normal diet 8 days; 50 mg ascorbic acid daily 3 days
D	12		4	38	3	05 -	6	13	Normal
	12	8	4	33	3	09 -	6	16	"
	12	14	7	06	6	44 -	7	23	C-free 14 days
	9	10	4	16	3	39 -	7	17	" 24 " ; 50 mg ascorbic acid daily last 4 days
	9	7	4	58	3	54 -	6	21	Normal diet last 7 days
E	10		4	27	2	49 -	5	25	Normal
	10	11	4	20	2	52 -	5	10	"
	10	7	3	30	2	14 -	4	41	" ; 5 mg ascorbic acid daily last 5 days
	9	16	3	48	3	27 -	5	34	Normal

similar to those with barbital, since vitamin C-depletion does not markedly influence the sleeping time. In Groups D and E the animals were first tested during C-depletion and addition of ascorbic acid did not change the effect in Group D, and only slightly shortened it in Group E. We had the impression that, in general, animals subjected to repeated pentothal injections were less resistant against vitamin C-deficiency than those on barbital and nembutal. Animals showing extreme loss of weight or inability to walk as a result of progressive vitamin C-depletion, were not included in these experiments.

Since it is known that vitamin C depletion causes glycogen deficiency in the liver (Altenburger[6]), we investigated whether or not the low glycogen may be responsible for the prolonged sleeping time. Therefore, experiments were conducted with all 3 barbiturates in guinea pigs after 36 hours' starving. With all 3 drugs sleep

[6] Altenburger, E., *Klin. Wschnschr.*, 1936, **15**, 1129.

TABLE III.
Pentothal Intraperitoneally in Guinea Pigs.

Group	No. of animals	Interval between tests, days	Sleeping time: Avg hr	Avg min	Range hr min	Range hr min	Diet and remarks
A	10		6	08	5 41	6 40	Normal
(male)	10	10	5	10	4 18	5 35	"
	8	33	4	08	2 01	9 31	C-free 24 days, 6 animals died within following week
B	10		4	06	1 31	5 40	Normal
(male)	5	28	5	05	3 46	6 10	C-free 28 days
C	12		3	41	1 18	4 41	Normal
(male)	12	22	5	11	2 41	7 00	"
	11	9	4	45	3 03	6 04	C-free 9 days
D	8		2	53	1 26	4 48	C-free 2 weeks
(male)	8	7	3	38	3 12	5 29	" 3 "
	8	6	3	09	2 21	3 29	Normal + 50 mg ascorbic acid daily last 5 days
E	12		4	42	2 17	6 58	C-free 32 days
(female)	10	7	4	07	2 57	6 33	" 39 "
	10	4	2	44	1 54	3 47	Normal + 50 mg ascorbic acid daily 4 days
	10	6	3	24	1 16	4 47	Normal

Group A received 30 mg/kg. Group B, 45 mg/kg. Groups C, D, E, 35 mg/kg.

lasted, as an average, one hour longer than in unstarved animals, which is not a very marked change of the effect. A certain prolongation of sleep in starved rabbits has also been observed by Blackberg and Hrubets[7] in their experiments with pentothal. Inanition as a reason for the prolonged sleeping time can also be excluded. We observed a marked prolongation of sleeping time in the nembutal experiments at a time when the C-depletion had not yet progressed sufficiently to cause a loss of weight, while, on the other hand, the loss of 100-150 g in similar experiments with barbital was not followed by prolongation of sleep. In this connection the work of Spellberg and Keeton[8, 9] is also of importance inasmuch as they found marked fatty changes in the liver of guinea pigs after vitamin C-depletion. We have, therefore, conducted numerous fat determinations in the liver of guinea pigs in different stages of vitamin C-depletion by means of Soxhlet extraction. Normal guinea pigs were found to have a liver fat

[7] Blackberg, S. N., and Hrubetz, C., *J. Lab. and Clin. Med.*, 1937, **22**, 1224.

[8] Spellberg, M. A., and Keeton, R. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 570.

[9] Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sci.*, 1940, **200**, 688.

TABLE IV
Relationship Between Sleeping Time and Liver Fat in Guinea Pigs.
(25 mg/kg nembutal given intraperitoneally.)

Days on C-free diet	% fat in liver	% glycogen in liver	Sleeping time hr	Sleeping time min	Remarks
21	6.8	—	8	04	
28	6.6	0.21	7	32	
28	9.6	0.93	7	32	
28	13.2	—	7	09	
28	9.1	—	9		
28	6.2	—	9		
23	5.4	1.33	2	02	100 mg Vit. C last 3 days before nembutal
23	24.7	0.42	2	12	
23	5.1	2.81	2	26	
23	8.0	2.02	2	37	
33	8.3	—	4	43	
33	10.8	—	4	17	
33	4.0	—	3	12	100 mg Vit. C last 4 days before nembutal
33	15.1	—	4	04	
33	3.9	—	3	11	
Normal diet	16.2	—	3	35	Starved 36 hrs

content of 2.6 to 4.2%. In our animals, fat accumulation in the liver was not as pronounced or as regular as in the experiments of Spellberg and Keeton. Values of 5.0 to 24.7% were found by us in guinea pigs after 3 to 4 weeks of C-free diet. (See Table IV.)

Table IV shows the relation between diet, fat content of the liver, glycogen and sleeping time after nembutal injection, and the influence of vitamin C administration. As can be seen, there was no consistent increase in fat content with prolonged C-depletion and, similarly, there was no significant correlation between the fat content and the sleeping time. The latter showed a marked increase in C-depleted animals as compared with normal sleeping times (Table II), while the fat content was frequently only little greater than in animals on a normal diet. Spellberg and Keeton[9] claim that addition of vitamin C to the C-free diet did not prevent the development of the fatty livers which they attribute to other factors in the diet. Table IV illustrates also that the addition of vitamin C was not regularly effective in restoring normal fat content, but, and this is significant, it restored normal sleeping time irrespective of the fat content. The lack of connection between sleeping time and liver fat is also illustrated in an animal starved for 36 hours which had a normal sleeping time in spite of a liver fat content of 16.2%.

Discussion. The results of our experiments demonstrate that only a certain type of barbiturate seems to be influenced in its duration

of effect by vitamin C-deficiency. This group is the so-called short acting barbiturate, of which nembutal is a representative, and we have no reason to assume that the other members of this group would behave differently. Since sufficient evidence has been brought forward that the liver destroys this type of barbiturates, one has, necessarily, to consider the possibility of liver damage as an explanation for the prolonged duration of action in the vitamin C-depleted animals. As we have demonstrated in this work, the appearance of fat due to the deficient diet or the low glycogen content is obviously not responsible. Neither were we able to find histological changes in the liver such as can be demonstrated after liver damage produced by drugs, as chloroform or carbon tetrachloride. We have, therefore, reason to believe that the vitamin C may be directly, or indirectly, connected with the destruction of these types of barbiturates. King[10] has recently mentioned the decrease in certain liver enzymes in the state of vitamin C-deficiency. Thus, it may be possible that the failure of liver enzymes accounts for the slower destruction of these drugs. Further support for this viewpoint is given by the fact that barbital is not significantly influenced in its duration of action by vitamin C-deficiency. This substance is known not to be destroyed in the body, but is excreted to a large extent unchanged by the kidney.

In addition, our experiments with vitamin C avitaminosis demonstrate further that pentothal does not behave as other short-acting barbiturates, and thus substantiates evidence brought forward by other authors, as well as ourselves, that this drug is not destroyed in the liver.

Summary. Vitamin C deficiency does not significantly alter the sleeping time of guinea pigs with either barbital or pentothal. The sleeping time with nembutal is markedly prolonged. This prolongation is not due to decreased liver glycogen, inanition, or increased liver fat, and can be promptly brought to normal by addition of vitamin C. We believe, therefore, that this vitamin must be connected in one way or another with the metabolism of nembutal and similar barbiturates. Furthermore, these experiments give additional evidence that the metabolism of pentothal is essentially different from that of nembutal and its related barbiturates.

[10] King, C. G., Biochem. Soc. Meet., Chicago, April, 1941.