THE EFFECT OF ETHYL URETHANE ANESTHESIA ON THE ACID-BASE EQUILIBRIUM AND CELL CONTENTS OF THE BLOOD.*

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Ethyl urethane has been frequently employed when a prolonged mild anesthesia is desired and it has been found particularly useful for keeping animals quiet during long exposures to x-ray. Our intention had been to use it for this purpose but since the experiments were designed to determine the action of x-ray on the circulating leucocytes and the acid-base equilibrium of the blood it was deemed advisable first to test the influence of the anesthetic itself.

Technique.—The chemical methods used were those devised for determining the acid-base equilibrium of small animals. The carbon dioxide content of the whole blood was determined by the Van Slyke method[1] and the pH by Cullen's method as modified by Hawkins.[2] The relative pH values were corrected and a constant subtracted to obtain absolute pH values at 38°C.[3] During the experiments all the animals were kept under uniform conditions both as to diet and to temperature. 12 hours prior to the observations all food scraps were removed from the cages, and during manipulation the animals were handled carefully to avoid exciting them.

Experiment 1.—20 rabbits were bled from the heart and the CO_2 content and pH of the blood were determined. 16 of the 20 were then given intraperitoneally 0.2 cc., per 100 gm. of animal weight, of a 50 per cent solution of ethyl urethane in sterile physiological saline. This amount was sufficient to produce anesthesia in from 30 to 45 minutes, which lasted from 6 to 7 hours. CO_2 and pH determinations were made at 1 and 3 hours after the injection and 1, 2, and 3 days thereafter. The results are given in Text-fig. 1 and Table I.

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[1] Van Slyke, D. D., *Proc. Nat. Acad. Sc.*, 1921, vii, 229.

[2] Hawkins, J. A., *J. Biol. Chem.*, 1923, lvii, 493.

[3] The constants were Rabbit Blood 17, Rat Blood 14.

The pH and CO_2 content of the whole blood of the animals injected with urethane were found to be markedly increased at 1 hour and remained at the high level for 24 hours. The normal level was not reached till 72 hours afterwards. The control animals bled at the same intervals had values within normal limits throughout the experiment.

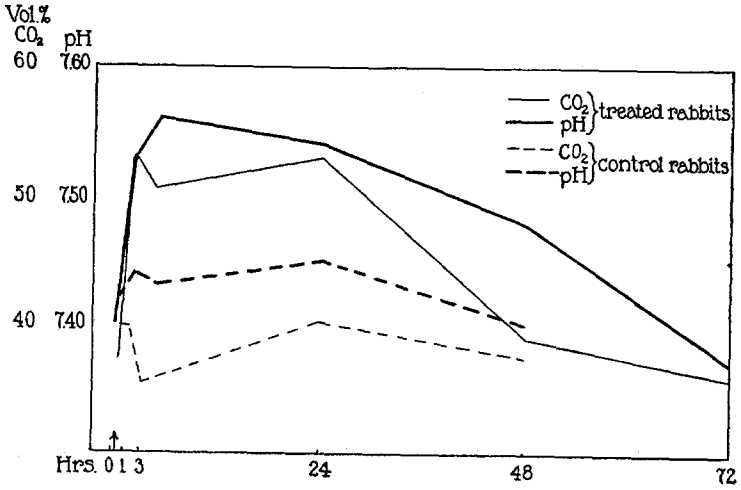
TABLE I.

Effect of Urethane on the pH and CO_2 Content of the Whole Blood of Rabbits.

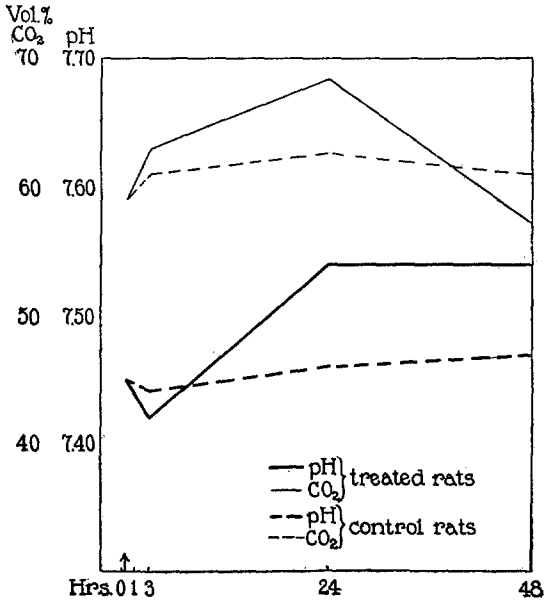
No.	0 hr.		1 hr.		3 hrs.		24 hrs.		48 hrs.		72 hrs.	
	CO_2	pH	CO_2	pH	CO_2	pH	CO_2	pH	CO_2	pH	CO_2	pH
1	35.2	7.30	48.5	7.52			42.0	7.52	39.5	7.53	44.4	7.46
2	29.7	7.19	52.2	7.52			55.2	7.49	28.7	7.27	36.2	7.38
3	39.6	7.39	64.2	7.48			56.8	7.59	38.5	7.45	31.6	7.31
4	34.2	7.34	44.2	7.45			58.8	7.57	35.7	7.41	28.4	7.28
5	44.2	7.43	59.7	7.58			74.8	7.58	43.6	7.52		
6	25.4	7.28	51.1	7.47			58.5	7.50				
7	40.5	7.45	48.6	7.50	39.3	7.62	50.2	7.58			30.2	7.32
8	49.7	7.49	59.1	7.57	71.8	7.62	59.5	7.56			40.3	7.42
9	48.6	7.55	62.8	7.61	61.8	7.59	61.0	7.56				
10	31.8	7.49	39.7	7.59	43.3	7.53	42.2	7.57	47.9	7.57		
11	39.5	7.49	55.5	7.55	44.6	7.53	45.9	7.51	44.9	7.52		
12	32.9	7.48			50.7	7.54			40.6	7.55		
13	39.1	7.46			63.1	7.58	46.2	7.55			42.0	7.51
14	24.8	7.31			36.6	7.51	39.1	7.39				
15	46.9	7.43			54.0	7.55						
16	30.3	7.35			40.7	7.52						
Average...	37.0	7.40	53.2	7.53	50.6	7.56	53.1	7.54	39.9	7.48	36.0	7.37
Controls.												
1	42.4	7.49	38.9	7.50	38.9	7.49	38.4	7.47				
2	41.0	7.40	39.6	7.42	33.5	7.42	42.4	7.42				
3	43.3	7.42	45.6	7.44					37.7	7.44		
4	35.6	7.39	35.4	7.39	34.0	7.38			37.4	7.37		
Average...	40.6	7.42	39.9	7.44	35.4	7.43	40.4	7.45	37.5	7.40		

CO_2 content expressed in volumes per cent.

Experiment 2.—As control the CO_2 content and pH of the whole blood of 10 rats were determined. The specimens were obtained by decapitating the animals and allowing the blood to drip under oil into a small glass cone. The determinations were made by the methods already described. 30 rats were now given intraperitoneally 1 cc., for every 100 gm. weight, of a 10 per cent solution of ethyl urethane



TEXT-FIG. 1.



TEXT-FIG. 2.

in sterile physiological saline. This amount was equivalent to that given to the rabbits and produced an anesthesia in 20 to 30 minutes lasting from 6 to 7 hours. At 3, 24, and 48 hours, respectively, after the injection, 10 treated and 5 normal animals were killed and CO_2 and pH determinations made on their blood. The results are shown in Text-fig. 2 and Table II.

TABLE II.

Effect of Urethane on the pH and CO_2 Content of Rat Blood.

0 hr.		3 hrs.		24 hrs.		48 hrs.	
pH	CO_2	pH	CO_2	pH	CO_2	pH	CO_2
7.49	53.4	7.36	65.8	7.53	73.0	7.42	56.4
7.42	62.1	7.41	55.6	7.52	75.6	7.47	60.4
7.41	61.3	7.38	60.0	7.53	70.4	7.49	58.5
7.44	58.4	7.40	69.3	7.56	76.2	7.49	62.0
7.40	56.7	7.43	63.8	7.51	74.3	7.49	54.2
7.43	65.7	7.41	62.7	7.51	57.9	7.56	54.9
7.51	65.4	7.44	61.0	7.52	59.2	7.55	60.4
7.46	57.3	7.53	58.2	7.51	64.4	7.70	56.2
7.47	58.9	7.36	66.2	7.55	58.3	7.63	61.7
7.47	61.8	7.43	58.8	7.56	58.5	7.64	44.5
Average...7.45	59.1	7.42	62.2	7.53	66.8	7.54	56.9
Controls.		7.41	62.7	7.38	63.8	7.57	59.7
		7.44	61.0	7.47	61.9	7.44	66.0
		7.53	58.2	7.48	63.3	7.51	61.0
		7.36	66.2	7.50	63.7	7.40	56.7
		7.43	58.8	7.46	60.7	7.42	62.1
Average.....		7.44	61.4	7.46	62.7	7.47	61.1

CO_2 content expressed in volumes per cent.

The CO_2 content of the whole blood of the animals injected with urethane was found to be increased at 3 hours and reached a maximum at 24 hours after injection. The pH was normal at 3 hours but had increased to a high level at 24 hours and remained at this level at 48 hours. The CO_2 content and pH of the whole blood of the control animals remained within the normal limits throughout the experiment.

These experiments show that a definite alkali excess exists in the blood of both rats and rabbits after injections of ethyl urethane. This is in accordance with the results of Cushny and Lieb[4] who have shown

[4] Cushny, A. R., and Lieb, C. C., *J. Pharmacol. and Exp. Therap.*, 1915, vi, 451.

that in rabbits under deep urethane anesthesia the reaction of the respiratory center to the blood gases is so altered that an increase in CO_2 or a decrease in O_2 has less accelerating action than is the case normally, while CO_2 accumulates in the blood since the rate of breathing is not accelerated to remove the excess CO_2. The CO_2 is retained in the course of the adjustment to restore the alkaline reaction to normal.[5]

The Action of Urethane Anesthesia on the Circulating Leucocytes.—In the course of a study of the action of x-ray on the blood, it was observed that destructive doses of this agent bring about a condition of uncompensated alkalosis.[6] When the same degree of alkalosis was induced by the injection of sodium bicarbonate it was accompanied by the same changes in the circulating white cells which characterized the x-ray effect. As an extension of this observation, we have studied the effect of the alkalosis produced by the injection of urethane on the circulating white cells of the blood.

Experiment 3.—Blood counts were made on 15 normal rabbits and 10 of these were then given a dose of urethane in the same amounts as in the previous experiments. Counts were made on all the animals at 1 and 3 hours after the injections and repeated at daily intervals for a week. The results are given in Text-fig. 3 and Table III.

The average of the counts on the ten treated animals showed a decrease of about 20 per cent in the lymphocytes during the 1st hour, a still greater decrease at the end of the 3rd hour, and a maximum decrease of 60 per cent at the end of the 48 hour period. Thereafter the count slowly returned to the normal level, reaching it by the end of 1 week. On the other hand, the polymorphonuclear leucocytes increased rapidly after the urethane injection, reaching a maximum of 200 per cent above normal within 24 hours and thereafter slowly returning to normal.

Experiment 4.—Blood counts were made on 39 rats on 2 consecutive days, twenty-six of these being given urethane in the same amount as in Experiment 2. Counts were made on all the animals at 3 hours and at 1, 2, and 6 days after the injection of the urethane. The results are given in Table IV and Text-fig. 4.

[5] Van Slyke, D. D., *J. Biol. Chem.*, 1921, xlviii, 153.

[6] Hussey, R. G., *J. Gen. Physiol.*, 1922, iv, 511.

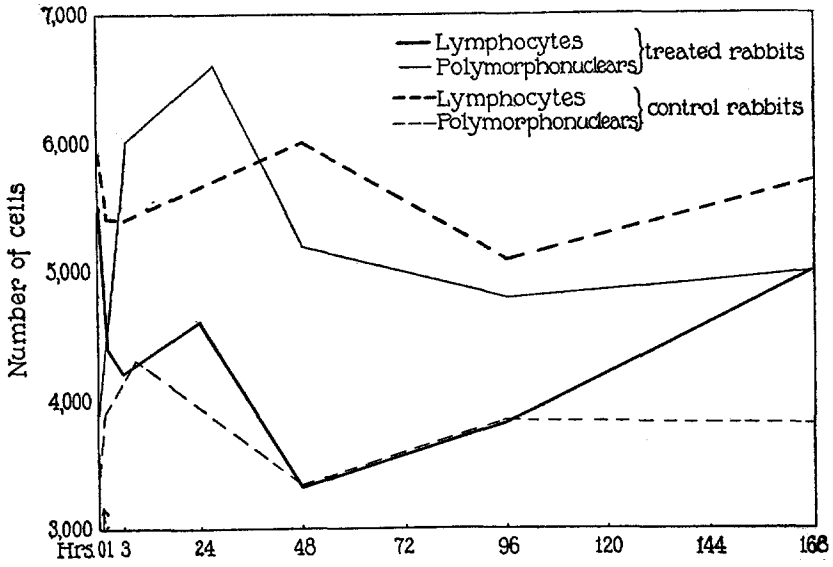
TABLE III.

Effect of Urethane on the Leucocytes of the Blood of Rabbits.

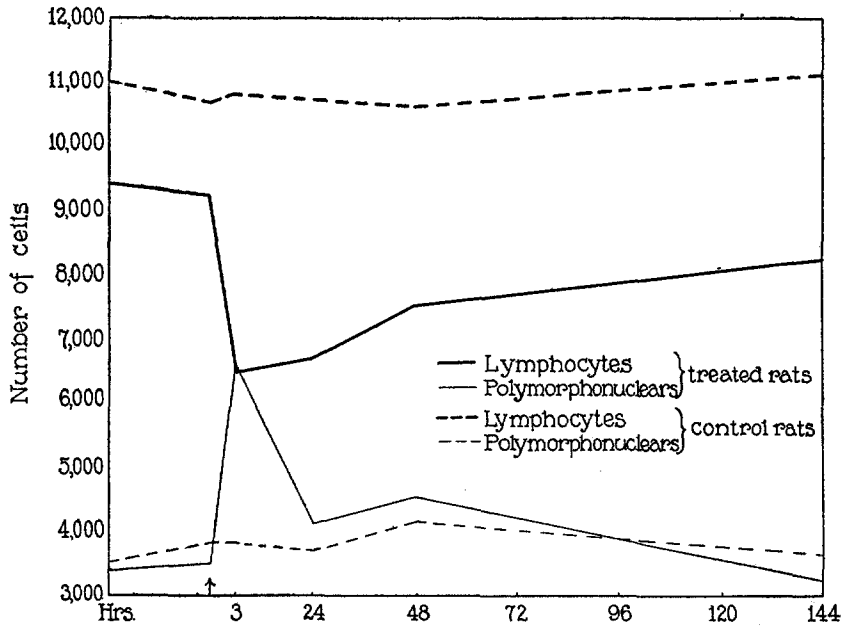
No.	0 hr.		1 hr.		3 hrs.		24 hrs.		48 hrs.		96 hrs.		168 hrs.	
	L.*	P.†	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.
	2,000	3,100	2,500	4,400	1,600	4,800	2,200	4,900	1,200	3,800	2,700	7,200	3,500	4,600
2	8,100	4,500	5,900	4,500	4,600	4,100	3,000	1,400	2,200	2,500	4,600	2,800	8,700	6,100
3	4,700	4,300	4,300	5,200	3,700	5,500	3,500	4,800	2,600	3,700	2,300	6,500	3,700	5,100
4	6,100	2,400	4,800	3,700	4,000	3,500	3,600	5,900	2,100	3,500	5,300	2,800	3,900	4,200
5	3,000	5,500	2,900	6,400	2,600	7,800	2,400	10,000	3,600	4,900				
6	5,800	3,400	2,400	2,800	4,000	7,600	9,300	10,700	4,800	6,100				
7	5,200	4,000	3,800	5,800	5,100	6,100	1,300	11,100	4,800	8,500				
8	7,200	3,400	5,200	4,100	3,900	5,800	6,800	5,500	3,400	7,500				
9	7,300	2,900	9,000	7,000	8,900	9,400	7,300	5,300	4,500	4,600				
10	5,700	5,000	3,300	5,900	4,900	5,100	6,800	6,200	3,800	6,700				
Average..	5,500	3,900	4,400	5,000	4,200	6,000	4,600	6,600	3,300	5,200	3,800	4,800	5,000	5,000
Controls.														
1	6,600	3,200			6,200	5,000			6,500	3,800			6,800	2,900
2	7,000	2,500			6,800	3,600			7,100	2,800			7,600	3,500
3	4,200	4,100			4,000	5,100			4,300	3,600	4,500	4,300	4,500	3,000
4	5,700	2,600	5,000	3,600	4,800	3,600			5,700	3,000	4,900	2,100	4,800	4,000
5	5,800	4,500	5,800	4,300			6,500	3,800	6,600	3,400	5,900	5,100	5,000	5,800
Average..	5,900	3,400	5,400	3,900	5,400	4,300	6,500	3,800	6,000	3,300	5,100	3,800	5,700	3,800

* Lymphocytes.

† Polymorphonuclear leucocytes.



TEXT-FIG. 3.



TEXT-FIG. 4.

TABLE IV.

Effect of Urethane on the Leucocytes of the Blood of Rats.

No.	0 hr.		0 hr.		3 hrs.		24 hrs.		48 hrs.		144 hrs.	
	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.	L.	P.
1	9,400	3,000	9,900	3,100	6,300	8,000	7,000	3,700	9,000	3,400	9,600	2,800
2	12,800	3,700	11,900	2,700	6,900	7,300	7,100	3,600	9,200	3,700	9,600	3,000
3	9,900	3,100	11,600	2,800	5,500	7,900	6,700	3,400	8,100	3,000	9,000	2,600
4	12,300	3,300	11,800	3,300	6,600	9,500	7,200	3,100	8,200	2,800	9,000	2,900
5	12,400	1,600	12,800	1,600	6,300	8,700	7,600	3,500	9,200	3,200	8,700	2,800
6	13,000	2,600	13,200	2,900	5,600	9,900	7,300	4,000	9,100	3,400	9,900	2,700
7	11,000	2,700	11,800	2,700	6,400	9,200	9,800	4,800	8,600	5,500	11,100	3,200
8	11,800	1,800	12,600	2,000	7,500	8,200	7,300	3,400	9,000	2,700	9,400	2,400
9	12,500	3,100	12,200	2,800	6,800	8,600	7,300	3,200	9,500	2,900	10,700	1,800
10	12,800	2,500	12,500	2,600	7,700	8,100	7,000	3,200	8,800	2,400	9,700	2,100
11	5,200	1,900	5,100	1,700	6,100	4,300	8,100	5,400	7,800	4,000	6,200	2,700
12	8,100	3,100	7,400	2,900	7,100	4,900	5,500	3,900	6,400	3,000	4,800	2,800
13	7,400	3,500	7,800	2,500	3,200	7,100	6,400	3,800	6,000	4,500	7,100	3,500
14	9,100	3,100	9,000	2,300	9,400	3,100	7,300	4,000	7,500	4,800	10,000	2,400
15	7,400	4,000	7,800	3,100	5,200	4,500	5,400	3,400	5,200	7,400	7,000	1,900
16	8,300	2,900	7,400	2,700	5,400	3,900	5,600	4,900	5,200	2,900	7,000	2,000
17	11,900	2,800	11,400	5,000	9,000	7,400	9,900	5,900	10,200	6,000	12,900	4,000
18	8,600	3,500	7,800	3,700	5,600	5,600	7,400	4,400	7,000	5,800	8,600	2,600
19	9,700	3,400	8,600	3,200	6,400	6,600	5,800	2,400	6,600	3,300	9,400	2,800
20	10,000	4,200	10,600	6,200	5,800	8,400	7,200	3,800	7,900	4,800	10,000	5,300
21	7,400	2,700	6,600	2,900	6,000	4,500	5,800	4,600	9,200	4,800	7,400	3,800
22	6,900	2,900	7,300	2,600	5,800	5,400	4,400	4,000	6,400	5,200	5,800	3,800
23	8,600	4,400	9,400	3,400	4,000	6,000	7,400	3,600	8,200	5,200	8,000	3,600

24	10,400	4,400	9,800	4,400	6,600	5,400	5,600	4,600	9,800	4,400	9,000	3,000
25	12,000	5,400	11,000	5,000	6,200	6,000	5,200	5,100	8,800	5,200	9,200	5,400
26	11,500	4,000	11,600	3,200	6,400	5,000	6,300	4,600	6,000	7,500	7,200	5,400
Average....	9,400	3,400	9,200	3,500	6,500	6,600	6,700	4,100	7,500	4,500	8,200	3,200
Controls.												
1	13,200	2,100	13,000	2,300	13,200	1,900	14,000	2,300	13,800	2,200	13,900	2,200
2	13,300	2,000	12,600	2,100	13,300	1,800	14,000	2,300	14,400	2,300	14,000	2,400
3	13,700	2,200	13,800	2,200	12,700	2,000	13,300	2,000	13,500	2,200	13,400	2,200
4	13,400	2,200	14,100	2,400	14,100	2,200	14,000	2,300	14,500	2,100	14,100	2,300
5	11,700	3,700	12,800	3,200	10,700	5,500	12,500	4,600	10,100	4,500	12,700	4,200
6	10,700	3,400	8,900	3,800	9,500	4,000	8,000	4,600	9,100	4,900	12,000	5,100
7	11,300	4,000	10,800	5,300	11,100	6,300	12,300	4,000	9,900	6,000	10,200	7,100
8	12,500	6,200	11,800	6,100	11,800	6,400	11,800	5,000	13,500	5,800	14,000	5,700
9	10,300	5,000	11,200	6,500	14,400	2,700	10,800	4,800	11,400	6,400	13,500	6,100
10	6,700	3,200	8,200	2,000	8,500	2,400	6,900	2,700	7,300	4,300	6,300	2,300
11	6,800	3,500	7,700	3,600	7,100	4,000	7,000	3,000	7,200	2,700	8,400	2,700
12	10,600	3,700	10,800	2,700	10,500	3,100	10,000	2,800	9,700	4,600	8,400	4,300
13	8,500	3,300	8,200	4,200	8,200	3,600	7,300	5,300	8,400	3,300	7,500	3,700
Average....	10,700	3,500	10,700	3,800	10,800	3,800	10,700	3,700	10,600	4,100	11,100	3,600

The average of these counts showed a decrease in the lymphocytes of about 50 per cent in 3 hours after the urethane, which persisted for 48 hours with an approximate return to the normal in 6 days. The polymorphonuclear leucocytes increased rapidly after the injection of urethane reaching a maximum of over 200 per cent at 3 hours and then slowly returned to normal.[7]

The marked and persistent alkalosis of the blood associated with urethane anesthesia eliminates the use of this agent in all experiments designed to study the acid-base equilibrium of the blood.

DISCUSSION.

The blood cell changes accompanying urethane anesthesia are of interest on account of the possible association of alkalosis in general with decreases in the lymphocytes. Work from this laboratory[6] has shown that a dose of x-ray sufficient to destroy the lymphoid tissues brings about a state of uncompensated alkalosis while the same degree of alkalosis when induced by the injection of sodium bicarbonate is accompanied by an identical change in the blood picture. These three observations are suggestive of a possible association between the reaction of the blood and the cell content but the processes involved are so complicated that perhaps even a tentative deduction would be unwarranted. However, the marked effect of urethane anesthesia revealed by the blood counts may explain some of the prevailing differences of opinion as to the action of x-ray on the circulating lymphocytes, since this method of anesthesia has been extensively used by some of the workers experimenting with x-ray exposures.

CONCLUSIONS.

When anesthesia is produced in rabbits or rats by injections of ethyl urethane, the CO_2 content and pH of the whole blood of the animal are more or less rapidly increased to a point where there is a marked uncompensated alkalosis which reaches its maximum at 24 hours and persists for 48 hours.

The increase in the CO_2 content and pH of the whole blood is accompanied by a decrease in the circulating lymphocytes and an increase in the polymorphonuclear leucocytes.

[7] It is of interest to note that, in some preliminary experiments, rats under urethane anesthesia, given an exposure to x-ray well below the lethal dose, invariably died between 7 and 14 days later.