THE EFFECT OF ETHYLENE GLYCOL ON THE SERUM CALCIUM OF THE RABBIT.*

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A report of work done under the direction of C. L. Wible College of Pharmacy, University of Nebraska, in partial fulfilment of the requirement for the degree of Master of Science in Pharmacology.

Ethylene glycol, commonly known as glycol, is a diatomic alcohol which stands midway between the monatomic alcohol, ethanol, and the triatomic alcohol, glycerol. Because of this relation it has been suggested as a substitute for these two compounds. It has been used as a preservative of fruit juices and extracts, and in non-intoxicating beverages (1). It has also been introduced as a vehicle for certain medicinal compounds not easily soluble in water. Its use as a vehicle for iodobismuthite is an example of this.

Ethylene glycol when used in the above ways has been considered harmless. Reid Hunt (2), (3), however, has seriously doubted this, while Hanzlik (4) claims toxicity only in large amounts which are never given for therapeutic purposes.

Hunt claims that the toxic effects of ethylene glycol are due to oxalic acid formed by the oxidative processes of the body. This seems to be true because the presence of oxalic acid can be demonstrated in the urine after the administration of ethylene glycol (5), (6). Calcium oxalate is present in the renal calculi formed by the administration of small amounts of ethylene glycol given over a long period of time.

In this work it has been assumed that if oxalic acid is formed it will precipitate the calcium of the blood as an insoluble salt, and thus cause a fall in the calcium content of the blood.

EXPERIMENTAL.

The experimental work was divided into two parts. In the first part the calcium changes occurring as the result of the administration of toxic doses were determined. The rabbit was starved 24 hours to eliminate the changes due to ingested food. At the end of this period the first blood sample was taken. The glycol was then administered and the samples of blood taken at approximately 15-minute intervals for the first hour, and then at half or hour intervals until death occurred.

In order to determine the effect of excessive loss of blood on the calcium level a control rabbit was run for each experiment. Physiological saline was administered instead of ethylene glycol. Samples of blood were taken at the same intervals as in the experimental animal.

Four experiments of this sort were run. In two of them the glycol was administered intraperitoneally, in the other two the intravenous method was used.

All four of these experiments checked each other closely. The results of a typical experiment are embodied in Table I and Fig. 1.

In the second part of the experimental work the effects of small doses of ethylene glycol given over a period of time were determined.

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* Scientific Section, A. PH. A., Madison meeting, 1933.

TABLE I.—ACUTE TOXICITY.

Control Rabbit A-7.				Experimental Rabbit A-8.		
Weight—2.84 Kg.				Weight—2.32 Kg.		
Dose—4.5 cc. of sterile saline per Kg. by intravenous injection.				Dose—4.5 cc. of ethylene glycol per Kg. by intravenous injection.		
Time.	Calcium.	Hmgb.	Remarks.	Calcium.	Hmgb.	Remarks.
9:00	14.4 mg.	80		13.1 mg.	85	
9:10	Inject saline			Inject ethylene glycol		
9:30	13.7 mg.	80		12.8 mg.	85	Accel., resp.
9:45	13.8 mg.	80		12.5 mg.	80	.. ..
10:00	13.8 mg.	80		12.6 mg.	80	.. ..
10:30	14.2 mg.	80	No distinctive	13.2 mg.	80	Resp. norm.
11:00	14.1 mg.	75	changes from	12.8 mg.	80	
11:30	14.0 mg.	75	normal.	12.7 mg.	75	
12:00	14.2 mg.	70		13.2 mg.	70	Slight depr.
1:00	14.0 mg.	70		13.0 mg.	70	.. ..
3:00	14.5 mg.	60		13.2 mg.	60	.. ..
6:00	13.8 mg.	60		13.0 mg.	50	Depression
8:00	13.2 mg.	50		12.0 mg.	45	Coma
8:35				Dead		

No abnormal conditions observed in a gross examination of various organs.

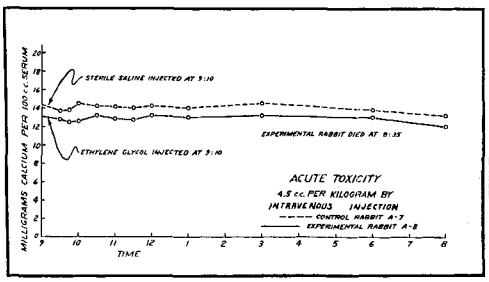

Fig. 1.

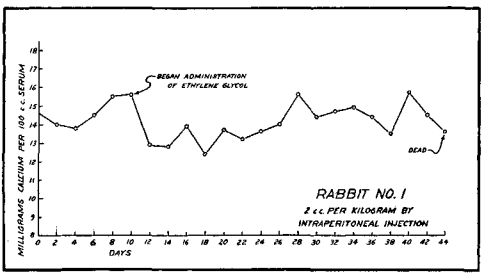

Fig. 2.

Calcium determinations were made on nine rabbits over a period of two weeks. These were made every other day. The daily administration of ethylene glycol was begun at the end of this two-week control period. The intraperitoneal method of injections was used. The rabbits were given doses varying from 3 to 4 cc. of glycol per Kg. per day.

Diet is the cause of much variation in serum calcium, and in order to keep this as constant as possible, a diet of cabbage and oats was fed. An excess of food was kept in the cage at all times. The animals were kept on this diet a few days before starting the experimental period.

A great deal of variations was encountered both in the control period and in the period during which the glycol was administered. The accompanying table (Table II) and graph (Fig. 2) may be considered typical of the results obtained in this part of the experimental work.

In order to determine whether or not any deleterious results might occur from the loss of blood, hemoglobin determinations were made at the same time that the blood was collected. In rabbits receiving the acute dose, the fall in the experimental animal was paralleled by a fall in the control rabbit. In experiments on

chronic intoxication the hemoglobin remained constant during the two weeks' control period. This indicates that the loss of blood required for the calcium determinations had no serious effect on the serum calcium. After the administration of the glycol the hemoglobin fell.

Serum calcium was determined by the method of Kramer and Tisdall (7), (8), using the modified washing technique of Clark and Collip (9). This is a volumetric procedure. Calcium is precipitated from the serum, after the removal of the proteins, as calcium oxalate. The precipitate is dissolved in sulphuric acid and titrated at 70° C. against 0.01*N* potassium permanganate.

TABLE II.

RABBIT No. 1.

Chronic Intoxication.

Days.	Calcium.	Hmgb.	Remarks.
0	14.6 mg.	..	
2	14.0 mg.	..	
4	13.8 mg.	..	
6	14.5 mg.	75	
8	15.5 mg.	80	
10	15.6 mg.	80	Begin admin. 2 cc. per
12	12.9 mg.	70	Kg. daily
14	12.8 mg.	70	
16	13.9 mg.	60	
18	12.4 mg.	55	
20	13.7 mg.	50	
22	13.2 mg.	50	
24	14.6 mg.	50	
26	14.0 mg.	50	Some depression
28	15.6 mg.	50	
30	14.4 mg.	50	Depression
32	14.7 mg.	45	
34	14.9 mg.	50	
36	14.4 mg.	45	
38	13.5 mg.	40	Weak
40	15.7 mg.	45	Weak
42	14.5 mg.	40	Very weak
44	13.6 mg.	40	Dead
			Postmortem showed no adhesions, viscera appeared normal. Stomach filled.

DISCUSSION.

Our experimental work seems to indicate that ethylene glycol has no marked effect on serum calcium. This is established with fair certainty in the work with toxic doses. Such variations as were present in the experimental animal were paralleled by variations over a similar range in the control.

In the work involving the continuous administration of small doses, extensive fluctuations were noted throughout the course of the experiment, both in the control and experimental period. These variations are probably due to diet. It was thought that this could be minimized by keeping the diet as constant as possible, but this proved difficult. McBorne and Campbell (10) state that normally the fluctuations of serum calcium may amount to as much as 5 or 6 mg. per 100 cubic centimeters of serum. Our variations over the entire period of the experiment amounted to 4 or 5 mg.

Naturally it follows that any change in calcium due to the effect of ethylene glycol must needs be greater than the range of normal variations in order to be detected. Therefore if there is any change, it is so small as to be covered by the normal variations.

Oxalic acid is undoubtedly formed because in no other way can the presence of oxaluria, and calcium oxalate in the renal calculi be easily explained. From our work we believe that the ethylene glycol is oxidized to oxalic acid so slowly that any change in serum calcium is too small to be easily detected.

CONCLUSION.

Variations in serum calcium of the rabbit do not depart from normal with the administration of ethylene glycol in toxic amounts or with small doses given over a period of time.

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A STUDY OF THE CONSTITUENTS IN CASCARA SAGRADA EXTRACT I. ISOLATION OF A RHAMNO-GLYCOSIDE OF EMODIN.*[1]

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For more than fifty years cascara sagrada extracts have been under investigation in order to determine the chemical nature of the compounds which are responsible for the characteristic cathartic properties. The extracts are usually classified as anthracene, anthraquinone or emodin cathartics, because the characteristic constituents separated have been derivatives of methyl anthraquinones. Dohme and Englehardt (1) reported finding a substance present in cascara which resembled frangulin (m. p. 237°), but their conclusion was questioned by Jowett (2). Beal and Tumminkatti (3) and Daels (4) have shown that the anthraquinone-type substances present are in part free and in part combined in a form which is liberated by hydrolysis. It is now generally accepted that the anthraquinone derivatives are present in part in a glycosidic type of linkage (5), (6). Thorpe and associates have reported finding a glycoside (frangulin) of rhamnose and emodin in Rhamnus frangula (7). Definite identification of the most active substance or substances remains to be accomplished, however. This study of the separation of the chemical constituents of cascara sagrada extract has been made to obtain further information leading toward the identification of the cathartically active constituent or constituents. A glycoside of rhamnose and emodin has been identified as one of the substances present in considerable quantity.

EXPERIMENTAL.

A procedure has been developed for the first stages of a separation of the chemical constituents in an alcoholic extract of the dry cascara bark. The various fractions thus obtained were representative of types of material present, providing a

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