

HAEMOGLOBINURIA CAUSED BY PROPYLENE GLYCOL IN SHEEP

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Haemoglobinuria occurred in sheep anaesthetized by an intravenous injection of pentobarbitone sodium containing propylene glycol: an equivalent dose failed to cause haemoglobinuria in rabbits. Intravenous injection of an aqueous solution of 20% propylene glycol caused haemoglobinaemia and haemoglobinuria in sheep. Neither distilled water nor 20% glycerol in water administered under identical conditions produced these effects.

Haemoglobinuria occurred on some occasions when an aqueous 20% solution of propylene glycol was administered to sheep after an injection of saline: it never occurred when a solution of 20% propylene glycol prepared with physiological saline was injected. It is suggested that saline may protect against the haemolytic action of propylene glycol in sheep and that propylene glycol should be avoided as a menstruum for pharmaceutical preparations to be used for injection into the blood stream of these animals.

Propylene glycol has been used for some time as a vehicle for medicinal and pharmaceutical preparations because of its solvent properties and apparently low toxicity (Seidenfeld and Hanzlik, 1932; Hunt, 1932). In a pharmacological study of the effects of propylene glycol on dogs, chickens and rabbits, Lehman and Newman (1937) found that an intravenous injection of a 33.3% solution of propylene glycol in physiological saline produced haemolysis and haemoglobinuria. Hanzlik, Newman, Van Winkle, Lehman and Kennedy (1939a) reported that 25 ml. of a 50% solution of propylene glycol in saline injected intravenously at a rate of not less than 0.5 ml./kg. body weight/min. was lethal to dogs. However, McGavack and Vogel (1944) gave daily intravenous injections of propylene glycol in relatively large doses to dogs and humans without any signs of abnormal effects.

Examination of the effects on metabolic activities made by Hanzlik, Newman, Van Winkle, Lehman and Kennedy (1939b) indicates that propylene glycol can increase glycogen storage in rats. Laug, Calvery, Morris, and Woodard (1939) in a systematic study of the toxicology of glycols consider that propylene glycol can be safely tolerated in small doses by the animal body since it can be oxidized and probably converted to a normal body constituent. It thus resembles ethyl

alcohol and glycerol and, as with these substances, large quantities are toxic.

Among the pharmacological preparations which contain propylene glycol are various solutions of pentobarbitone sodium used to produce hypnosis and general anaesthesia in animals. Preliminary observations indicated that such preparations could produce haemoglobinuria in sheep when used to induce general anaesthesia. A systematic study was therefore carried out to see how often haemoglobinuria followed the administration of pentobarbitone sodium to sheep, and if the use of propylene glycol in proprietary preparations of the anaesthetic contributed to the haemoglobinuria.

METHODS

Altogether 19 Merino ewes aged from 2 to 10 years in which the weights varied from 35 to 50 kg., and two male Chinchilla rabbits weighing 1.5 kg., were used. Ten observations were made on six sheep which were anaesthetized for varying periods of up to 2 hr. with proprietary solutions of pentobarbitone sodium. Four of these sheep were anaesthetized with a freshly prepared 6% (w/v) solution of pure pentobarbitone sodium in water and four others received this freshly prepared solution to which had been added propylene glycol to make a final concentration of 20% (w/v).

In most experiments anaesthesia was induced in the sheep with an initial dose of 15 ml. which contained

900 mg. pentobarbitone sodium (26.4 mg./kg. body weight) injected at an approximate rate of 3 ml./min. and maintained thereafter with an injection of 2 ml. (120 mg.) every 10 min. Because of differences in individual response, the dose and the rate of administration varied slightly. An indwelling catheter was used to obtain urine samples from the bladder.

To determine the effect of propylene glycol in the same and other unanaesthetized sheep, the glycol was prepared as a 20% (w/v) solution in water and an initial volume of 15 ml. was injected intravenously at a rate of 3 ml./min. followed by 2 ml. every 10 min. for a total period of 2 hr. The propylene glycol was purified by distillation to prevent the possibility of impurities affecting the result. Urine samples were obtained by catheter before the injection and thereafter at half-hourly intervals. The urine samples were tested qualitatively for haemoglobin by the benzidine reaction and if positive were confirmed by spectroscopy. From six of the sheep, urine samples were taken for an additional 3 hr. following cessation of the glycol injection. Blood samples from these animals were collected by venipuncture of the external jugular vein, meticulous care being exercised to prevent extravascular haemolysis. Samples of urine were obtained from the bladder at the same time. The blood was centrifuged and the amount of haemoglobin in the plasma and urine samples was determined by a modification of the method of Bing and Baker (1931) as outlined by Dacie (1956) and adapted for spectrophotometric estimation using a Unicam spectrophotometer at a wavelength of 510 m μ . Haematocrit readings were determined on the blood samples from two of the animals. Tests for haemosiderin were performed on the urine samples taken during the collection period and 24 hr. later, to ascertain whether iron had been excreted in the urine as a result of reabsorption of haemoglobin by the kidney tubules.

To assess the importance of osmotic effects in the blood stream, the propylene glycol was injected at the same rate into two sheep as a 20% (w/v) solution in physiological saline (0.85%) instead of water, and an equivalent volume of distilled water was injected in the same manner as the propylene glycol into two other sheep. In addition an initial volume of 150 ml. physiological saline was given to four sheep 5 min. before the injection of 20% propylene glycol in water and the effect of this priming saline injection was noted.

The effect of administering glycerol (which like propylene glycol is a polyhydric alcohol) and ethyl alcohol was also observed in two sheep. The animals received no food or water for the period during which the urine samples were obtained.

Quantitative experiments on haemolysis were carried out *in vitro* on 10 ml. samples of blood, carefully withdrawn from the external jugular veins of four sheep, and added with gentle agitation to dry glass centrifuge tubes which all contained heparin and equivalent amounts of physiological saline,

distilled water, 20% glycerol in water, 20% propylene glycol in water, 20% propylene glycol in physiological saline or physiological saline followed after 5 min. by 20% propylene glycol in water. The volumes of the respective solutions, to which the samples of blood were added, were calculated from the amount of propylene glycol usually required to produce haemoglobinuria in a sheep with a blood volume of 3.5 l.

Two rabbits were deeply anaesthetized with the same proprietary preparations of pentobarbitone sodium as used for the sheep. The anaesthetic was injected into the marginal ear vein and the urine was analysed for haemoglobin during the subsequent 4 to 5 hr.

RESULTS

Pentobarbitone Sodium.—Eight out of ten observations made on six sheep which received proprietary preparations of pentobarbitone sodium intravenously indicated that there was haemoglobin in the urine. In the other two it is possible that insufficient pentobarbitone sodium was injected to produce the effect.

Haemoglobinuria was also observed in all the animals which received a freshly prepared 6% solution of pentobarbitone sodium containing 20% propylene glycol. There was no haemoglobinuria when propylene glycol was omitted from this solution.

Two rabbits anaesthetized with the same proprietary preparations of pentobarbitone sodium as the sheep showed no haemoglobinuria.

Propylene Glycol (20% in Water).—Haemoglobinuria with concurrent intravascular haemolysis was usually produced when 15 ml. of a solution of 20% propylene glycol in water followed by 2 ml. every 10 min. was administered to sheep as a slow steady intravenous injection. The amount of haemoglobin in the plasma was always increased in the sample taken 30 min. after the initial injection of propylene glycol, and this increase continued until the glycol injection was discontinued. Thereafter the plasma haemoglobin gradually decreased until the pre-injection value was reached about 5 hr. after the initial injection of propylene glycol.

Haemoglobin usually appeared in the urine when the plasma haemoglobin began to increase, but sometimes this effect was delayed. On these occasions haemoglobinuria did not appear until 60 to 90 min. after the propylene glycol injection had commenced. The haemoglobin had disappeared from the urine at the end of 5 hr. in all but two animals, and only small amounts remained in the urine of the latter.

The concentrations of haemoglobin in the plasma and urine of five sheep after the administration of the propylene glycol are shown in Table I. One sheep, No. 56, to which 20% propylene glycol was administered as described in Table I showed no signs of haemoglobinuria even though there was some haemolysis. A subsequent injection of the same quantity of 30% propylene glycol increased the haemolysis and haemoglobin then appeared in the urine.

Haemosiderin was not detected in the urine either during the period when haemoglobin was present or 24 hr. later: it thus appears that tubular reabsorption of haemoglobin by the kidney tubules may not have occurred during this period.

Graphs showing the haemoglobin concentrations in the plasma and urine of sheep No. 04, together with the concurrent haematocrit determinations, are given in Fig. 1. It can be seen from these curves that the degree of haemolysis and haemoglobinuria varies inversely with the ratio of blood cells to plasma, an indication of intravascular haemolysis as a result of the propylene glycol administration.

Apart from the resulting haemoglobinuria, the sheep did not appear to be affected unduly by the propylene glycol.

Propylene Glycol (20% in Saline).—Propylene glycol, when injected into sheep as a 20% solution prepared in physiological saline and administered in the same manner as 20% propylene glycol in water, never produced haemoglobinuria.

Propylene Glycol (20% in Water) after Physiological Saline.—The results of the same quantities of 20% propylene glycol in water administered 5 min. after an intravenous injection of physiological saline were varied. Two sheep which received 150 ml. physiological saline before the glycol exhibited no haemoglobinuria, while the urine of one contained haemoglobin after the

severe diuresis, which followed the injection of the saline, had ceased. Haemoglobinuria without diuresis appeared in the other animal that received only 100 ml. physiological saline.

Water, Glycerol or Ethyl Alcohol.—An intravenous injection of distilled water, glycerol solution (20% in water) or ethyl alcohol (10% in water), when given under the same conditions as the 20% propylene glycol in water, did not produce haemoglobinuria in any sheep. The results of administering different solutions are summarized in Table II.

TABLE II
APPEARANCE OF HAEMOGLOBIN IN BLOOD AND URINE OF SHEEP DUE TO INITIAL INTRAVENOUS INJECTION OF 15 ML. OF VARIOUS SOLUTIONS AT 3 ML./MIN. FOLLOWED BY 2 ML. EVERY 10 MIN. FOR 2 HR.

Treatment	No. of Experiments	Haemoglobin-aemia	Haemoglobin-uria
Proprietary solutions of pentobarbitone sodium	10	8	8
Pentobarbitone sodium—6% (w/v) in water	4	—	—
Pentobarbitone sodium—6% (w/v) in water	4	4	4
Propylene glycol—20% (w/v) in water	6	6	5
" " —20% (w/v) in water	2	—	—
" " —20% (w/v) in 0.85% saline	2	—	—
Propylene glycol—20% (w/v) in water after 150 ml. 0.85% saline	4	2	2
Distilled water	2	—	—
Glycerol—20% (w/v) in water	2	—	—
Ethyl alcohol—10% (v/v) in water	2	—	—

In vitro Experiments.—Little information was gained from adding freshly and carefully collected blood to solutions of propylene glycol, saline and glycerol. There was haemolysis in only half the samples obtained when blood was added to an aqueous solution of 20% propylene glycol, but when blood was mixed with saline and then added to the propylene glycol solution haemolysis always occurred. Haemolysis was not detected when blood was mixed with saline, with glycerol or with a 20% solution of propylene glycol in saline.

TABLE I
HAEMOGLOBIN CONCENTRATIONS IN PLASMA AND URINE OF SHEEP
The initial injection of 15 ml. of propylene glycol was followed by 2 ml. every 10 min. for 2 hr.

Animal No.	Weight (kg.)	Conc. of Propylene Glycol in Water (%)	Haemoglobin in Plasma (g./100 ml.)								Haemoglobin in Urine (g./100 ml.)							
			Hr. after Initial Injection								Hr. after Initial Injection							
			0	½	1	1½	2	2½	3	5	0	½	1	1½	2	2½	3	5
52	42	20	-01	-16	-24	-45	-55	-42	-21	-06	—	—	-14	-46	-50	-49	-57	—
14	38	20	-01	-26	-44	-48	-50	-68	-54	-16	—	-49	1.4	4.0	5.9	8.5	5.7	—
02	40	20	-02	-16	-23	-26	-25	-22	-14	-04	—	-01	-40	-60	-60	-70	-32	-01
25	40	20	-03	-26	-18	-16	-13	-09	-08	-02	—	-62	-66	-22	-18	-07	—	—
56	46	20	-02	-03	-05	-05	-07	-04	-03	-01	No haemoglobinuria							
56	46	30	-03	-06	-05	-19	-50	-37	-24	-06	—	—	—	-07	-30	-98	-70	—

DISCUSSION

Haemoglobinuria was observed in sheep during the intravenous injection of an aqueous solution of 20% propylene glycol administered at 10 min. intervals for 2 hr. Proprietary preparations of pentobarbitone sodium, which contain propylene glycol in similar proportions, caused haemoglobinuria when used to produce anaesthesia in sheep.

The haemoglobinuria is invariably accompanied by intravascular haemolysis. There was considerable individual variation in the degree of haemolysis and haemoglobinuria, and from Table I it may be seen that, although haemoglobinuria was produced in most sheep by 20% propylene glycol in water, the concentration of the glycol had to be increased to 30% to cause haemoglobinuria in sheep No. 56. The difference in individual response is also seen in Fig. 1, which shows that the haemoglobinaemia in sheep No. 04 was relatively great, yet the amount of haemoglobin measured in the urine was less than that of the sheep in Table I. The low concentration of haemoglobin present in the urine was not due to diuresis

because the volume of urine passed by sheep No. 04 during the experimental period did not exceed that passed by the other sheep. Tubular reabsorption of haemoglobin from the kidneys might also be discounted as haemosiderin was not found in the urine up to 24 hr. after haemoglobin appeared there.

Lehman and Newman (1937), in a report on the haemolytic action of a 33.3% solution of propylene glycol dissolved in normal saline and injected into the blood stream of dogs, rabbits and chickens, suggested that the blood cells are destroyed osmotically in passing through the glycol at the site of the injection. If this were the case in the sheep, the first sample of plasma taken after the initial injection of 15 ml. would be expected to contain more haemoglobin than the subsequent samples which were taken when only 2 ml. of the glycol was being injected at 10 min. intervals. This was not so, and there was no haemoglobinuria after intravenous injections of similar quantities of distilled water and 20% glycerol in water. These facts indicate that propylene glycol had some action on the blood cells other than a simple osmotic effect.

The anomalous *in vitro* effects of mixing whole blood with 20% propylene glycol in water seem to indicate that the red cells of individual sheep vary in their sensitivity to propylene glycol, and this may explain the variation in extent of haemolysis that was observed after propylene glycol had been injected.

It was interesting to observe that when physiological saline was substituted for water in the preparation of the 20% propylene glycol haemoglobinuria did not occur. However, when the sheep received an aqueous injection of 20% propylene glycol 5 min. after an initial injection of 100 to 150 ml. of physiological saline, haemoglobinuria was sometimes observed, particularly if a transient diuresis followed the saline injection. In view of the evidence cited against osmotic effects, this suggests that saline may exert some sort of protective action against the haemolytic effects of propylene glycol.

It was also interesting that haemoglobin was not found in the urine of rabbits, unlike that of sheep, when the animals were anaesthetized with an equivalent amount of a solution of pentobarbitone sodium containing propylene glycol.

It thus appears that the sheep may be particularly susceptible to the haemolytic action of propylene glycol, and the incorporation of the latter in pharmaceutical preparations for intravenous administration to sheep would appear to be unwise.

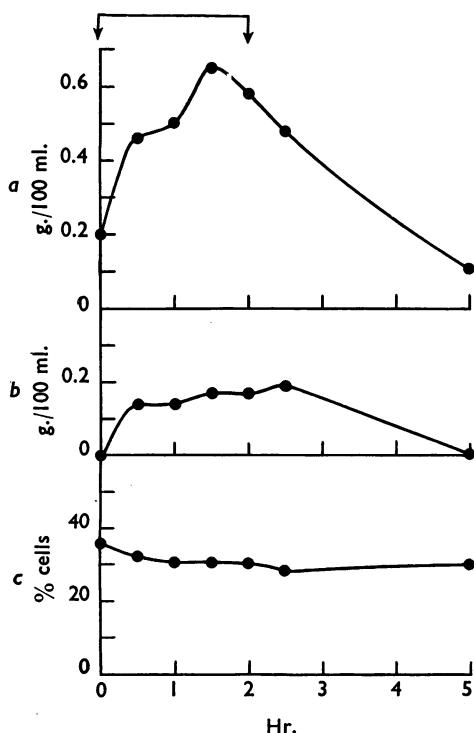


FIG. 1.—Haemoglobin concentration in plasma (a) and urine (b) and haematocrit (c) of sheep No. 04. 15 ml. of 20% propylene glycol was injected at first arrow, 2 ml./10 min. until second arrow.

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