borate, $Na_2B_4O_7 \cdot 10H_2O$, A.C.S., Fisher Scientific Co., 2% in water; tris(hydroxymethyl)aminomethane (Tham), purified, Fisher Scientific Co., 50 ml. 0.1 *M* solution adjusted to pH 9 with 0.1 *M* HCl and diluted to 100 ml. with water; phenylephrine hydrochloride, Winthrop Laboratories; Beckman DU spectrophotometer; and 1-cm. Corex cells were utilized.

Procedure Using 2% Sodium Borate Solution.—Three milliliters of an aqueous solution containing 150–450 mcg. of phenylephrine hydrochloride was pipeted into a 50-ml. volumetric flask. One milliliter of potassium ferricyanide reagent was added, and the solution was diluted to about 48 ml. with sodium borate solution. One milliliter of 4-aminoantipyrine reagent was added, and the volume was made up with borate buffer and mixed. The absorbance of this solution was determined immediately at 490 mμ against a reagent blank. The concentration of the sample was calculated by comparison with the color developed simultaneously on a standard solution of phenylephrine hydrochloride.

Procedure Using Tham Buffer and Isopropyl Alcohol.—Three milliliters of an aqueous solution containing approximately 150–450 mcg. of phenylephrine was pipeted into a 50-ml. volumetric flask. One milliliter of potassium ferricyanide was added, followed by 15 ml. of the buffer, 15 ml. of isopropyl alcohol, 1 ml. of 4-aminoantipyrine, and more of the buffer to volume. The contents of the flask were mixed after the addition of each reagent. The absorbance of the solution was determined at 490 mμ against a reagent blank 30 minutes after the development of color. The color developed simultaneously on a standard solution of phenylephrine hydrochloride was used to calculate the concentration of the sample.

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Acute Toxicity of Intravenous Sodium Lauryl Sulfate

By HELMUT F. CASCORBI, FRIEDA G. RUDO, and GORDON G. LU

Sodium lauryl sulfate (SLS) is an excellent emulsifying agent. An emulsion of methoxyflurane in oil, stable to autoclaving, and employing SLS as an emulsifier was prepared. The acute effects of intravenous SLS on red cells, electrocardiogram, vital organs of rabbits, dogs, and monkeys, and isolated hearts of rabbits and frogs were studied. The dose levels were 10 and 50 mg. per cent in 5 per cent glucose or in 3.5 per cent emulsion of methoxyflurane at administration rates of 6.2 ml./Kg./hr. intravenously. The hemolytic effects of SLS and its effect on the electrocardiogram are negligible. SLS evokes a precipitous transient depressor response in dogs. However, SLS has marked acute effect on lungs, kidneys, and especially liver. The hepatotoxicity of SLS seems to preclude its intravenous use in man.

SODIUM LAURYL SULFATE (SLS) has been used in dentifrices for years. Its pharmacologic and toxicologic properties have been studied by a number of investigators (1–5). Recently the Council on Drugs has directed attention to possible hepatosensitivity associated with erythromycin propionate lauryl sulfate (6).

The authors' work with intravenous anesthetic emulsions (7) prompted an investigation of the suitability of SLS as an emulsifier. SLS in concentrations of 2–5 mg. % proved to be an excellent emulsifier and produced a very stable emulsion that tolerated autoclaving for 19 minutes at 15 lb.

After we had established the usefulness of SLS as an emulsifier for emulsions of volatile anesthetics, we then proceeded to investigate its acute toxicity upon intravenous administration.

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The LD_{50} of SLS upon intravenous administration in rats and mice (kindly supplied to us by K. K. Chen) was found to be 118.2 ± 7.2 mg./Kg.

The properties that were of special interest to us were (*a*) lysis of the red cell membrane, (*b*) its influence on the electric activity of the heart, and (*c*) its acute toxic effects on liver, kidneys, and lungs.

METHODS

Hemolysis.—Two mongrel dogs were anesthetized with 25 mg./Kg. pentobarbital intravenously. The animals were then infused with a solution of 50 mg. % SLS in 5% glucose at a rate of 6.2 ml./Kg./hr. for 1 hour.

Prior to and after infusion, blood samples for hemolysis were taken. Samples were drawn through 17-gauge needles in plastic syringes, bubble free. Plastic centrifuge tubes and a well balanced centrifuge were used to avoid mechanical disrupture of the red cells.

TABLE I.—EFFECT OF SLS ON VITAL ORGANS OF DOGS

Dog No.	SLS, 10 Mg. %	Diagnosis		
		Lungs	Kidneys	Liver
12	Emulsion	Atelectasis, mild	Cloudy swelling	Cloudy swelling
15	Emulsion	Atelectasis pneumonitis	Acute pyelonephritis	Subhepatic necrosis
16	Emulsion	Acute congestion	Normal	Acute congestion
17	Glucose, 5%	Moderate congestion	Mild cloudy swelling	Normal
18	Glucose, 5%	Normal	Moderate cloudy swelling	Severe cloudy swelling. Hemorrhage focal necrosis severe.
19	Glucose, 5%	Normal	Minimal cloudy swelling	Moderate cloudy swelling

TABLE II.—EFFECT OF SLS ON VITAL ORGANS OF RABBITS

Rabbit No.	Lungs	Kidneys	Liver	Bone Marrow	Diagnosis
1 3/1/62	Focal inflammatory reactions throughout specimen; some in bronchi. Much of lung is air containing.	Normal tubules. Cloudy swelling of tubules with moderate glomerular congestion.	Pericentral necrosis of liver cells involving 3/5 of each lobule. Bile ducts, vessels, and triads are normal.	30% reduction with normal ratio of cells. (30% depression of cellulloid.)	Hyperplasia, bone marrow. Necrosis, pericentral; liver severe. Cloudy swelling, kidneys. Globular pneumonia acute.
2 3/1/62	No lesions	Cloudy swelling of tubules. Normal glomeruli.	Mild pericentral necrosis in 3/5 lobule which appears to be reversible. Normal triads and bile ducts.	Normal	Cloudy swelling, kidneys. Fatty necrosis pericentral liver.
3 3/1/62	Collapse and congestion with early inflammation near bronchi; early. Abundant aeration is still present.	Very mild cloudy swelling only with normal glomeruli and tubules.	Considerable pericentral necrosis and congestion involving 50% of each lobule. Bile ducts, triads, and periportal cells not affected.	No Specimen	Necrosis, pericentral; liver severe. Pulmonary congestion. Pneumonia, lobular.

Two additional mongrel dogs were treated in a similar manner, employing a 10 mg. % SLS in 5% glucose solution for infusion.

Electrocardiogram and Blood Pressure of Dogs.—Nine mongrel dogs and two monkeys were anesthetized with pentobarbital sodium (25 mg./Kg. i.v.) and infused in the foregoing manner.

Two dogs were infused with a solution of 50 mg. % SLS in 5% glucose, and seven dogs were infused with a solution of 10 mg. % SLS in 5% glucose for 1 hour each. One monkey was infused with a solution of 15 mg. % SLS in 5% glucose, and another monkey was infused with 20 mg. % SLS in 5% glucose, each for 30 minutes with 6.2 ml./Kg./hr. Lead II of the electrocardiogram was monitored in all animals.

Three mongrel dogs under ether anesthesia were injected intravenously with varying amounts (3.5 mg./Kg. to 7.0 mg./Kg.) of SLS in a concentration of 70 mg. %, and the blood pressure was monitored from the carotid artery.

EKG recordings (Lead II) were obtained at the same time.

Acute Toxic Effects On Liver, Lungs, and Kidneys.—Three mongrel dogs were anesthetized with thiopental sodium, and a control liver biopsy was obtained through a midline incision. Anesthesia was then continued with an emulsion of methoxyflurane (3.5 ml. methoxyflurane, 3.0 ml. cottonseed oil, 1.0 ml. ethanol, 0.5 Gm. Pluronic F 68, 4.2 Gm. glucose and water *q.s. ad.* 100.00 ml.) to which 10 mg. % of SLS had been added after emulsification. The dogs were anesthetized with the emulsion for 3 hours. The dose was determined by the individual susceptibility of the animals to the anesthetic. The dose was 10.8, 9.1, and 6.6 ml./Kg./hr., respectively. After 3 hours of anesthesia, biopsies of liver, lungs, and kidneys were taken, and the animals were sacrificed.

Three more dogs were anesthetized with pentobarbital sodium (25 mg./Kg. i.v. with subsequent doses if necessary), a biopsy of the liver was obtained, and then an infusion of 10 mg. % SLS in 5% glucose administered at the rate of 6.2 ml./Kg./hr. for 3 hours. After this time another biopsy of the liver and biopsies of the lungs and kidneys were obtained, and the animals were sacrificed.

The organ specimens were fixated with 1% $CaCl_2$ in 10% formalin and sent to Dr. John A. Wagner of the Department of Neuropathology for the examination.

Five rabbits were anesthetized daily with 3.5%

emulsion of methoxyflurane by intravenous injection into the marginal ear vein. The emulsion contained 10 mg. % of SLS. Anesthesia was maintained for 30 minutes and repeated five times. The average dose was 16 ml./Kg./hr.

Isolated Rabbit's Heart.—The modified Langendorf procedure was used (8).

Perfused Frogs' Hearts *In Situ.*—The Greene frog-heart perfusion was used.

RESULTS

Hemolysis.—The plasma of three of the dogs was free of any visible hemolysis, whereas the plasma of one dog that received 10 mg. % SLS in 5% glucose showed a trace of hemolysis that was found to be below 30 mg. % hemoglobin.

Electrocardiograms.—The EKG tracings were remarkably devoid of significant findings. We observed peaking of *T* in two cases and an inversion of the *T*-wave in one case. The EKG of the monkeys was not changed.

Blood Pressures.—Dosage levels of SLS up to 3.5 mg./Kg. caused a fall in mean arterial pressure of 7–21% of the norm. Levels of 7 mg./Kg. evoked a 36–40% fall in blood pressure. Electrocardiographic changes were not significant.

Acute Toxic Effects On Liver, Lungs, and Kidneys.—The pathological diagnosis of the six dogs are shown in Table I. The table shows clearly that SLS causes cloudy swelling and congestion in lungs, kidneys, and liver with a preponderance on hepatic damage where even focal necrosis was observed.

Isolated Rabbit's Heart.—SLS dissolved in the perfusion fluid (60 mcg.) had no effect upon the amplitude of contraction of the rabbit's heart in two experiments. Levels of 120 and 240 mcg. caused a moderate depression of contractility.

Levels of 30 and 60 mcg. caused irregular transient coronary flow responses. Levels of 120 and 240 mcg. caused diminution of 35 and 80%, respectively.

Perfused Frogs' Hearts *In Situ.*—In three hearts 1 mg. % of SLS had no effect on the rate or amplitude of contraction. A level of 10 mg. % reduced the rate and amplitude of contraction in two hearts and in the third heart caused cardiac stoppage.

Rabbits.—The results of repeated administration of SLS in emulsion to rabbits are detailed in Table II. Two of the animals died before the study was completed.

DISCUSSION

We administered 10 mg. % SLS in 5% glucose solution to ascertain whether the hepatic toxicity that was found for 10 mg. % SLS in an emulsion could be ascribed to one of the constituents of the emulsion.

The recurrence of renal and especially hepatic changes compel us to ascribe these changes to the action of SLS. However, even at these levels hemolysis was not encountered. We believe that SLS should not be used intravenously in man.

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Modification in Sample Preparation for the Microbiological Assay of Vitamin B_{12}

By ELKO M. STAPERT, HELEN CRAIN, and ROBERT NEEDHAM

A modification of the U.S.P. XVI method for the assay of vitamin B_{12} eliminates some negative bias encountered with some products and permits quantitative recovery of the vitamin. Soft-elastic and hard-filled capsules are blended in the buffered sodium metabisulfite solution before autoclaving. This procedure is particularly necessary to assay older capsules. Products containing reducing sugars are protected with potassium cyanide instead of sodium metabisulfite.

BLENDING WHOLE CAPSULES BEFORE ASSAY

THE MICROBIOLOGICAL METHOD for assaying products containing vitamin B_{12} is described in U.S.P. XVI (1). In this method the product is placed into a buffered solution of sodium metabisulfite and autoclaved for 10 minutes during which all convertible B_{12} is changed to the more stable sulfite form. In assaying many types of products, lower values were obtained with samples stored at room temperature for 18 and 24 months by U.S.P. XVI method than by the assay described in the U.S.P. XIV, third supplement (2).

When whole capsules are placed in the sodium metabisulfite solution, a period of time is required before the contents are in contact with the sodium metabisulfite. A modification in sample preparation described in this paper permits quantitative recovery of the vitamin in contrast to the U.S.P. method where some B_{12} may be destroyed.

Experimental Methods

Sample Preparation with KCN.—All operations and ingredients were as directed in the U.S.P. XVI assay method except the sample preparation.

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