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Maximum Biliary Excretion of Bilirubin and Sulfobromophthalein During Anesthesia-Induced Alteration of Rectal Temperature.* (32080)

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The influence of temperature on various physiological functions such as bile flow, biliary bilirubin concentration and the excretion of dyes such as sulfobromophthalein (BSP) has been recognized for many years(1). Brokaw and Penrod(2) found that BSP uptake from the blood of dogs was diminished during hypothermia. Archdeacon *et al*(3) showed that in the anesthetized rat low temperature exerted a definite inhibitory effect on bile production. Brauer *et al*(4) using intact rats and isolated perfused rat livers have shown that bile flow and the excretion of BSP falls during hypothermia, while there are only rather minor changes in other bile constituents.

The impairment of thermoregulation as a consequence of anesthesia and/or restraint has also been reported(5,6). It would be expected, therefore, that anesthesia-induced hypothermia could reduce the functional capacity of the liver. Anesthesia, as well as restraint, is routinely employed in experiments in which maximum excretion patterns of the liver are determined. The intent of this report is to demonstrate the magnitude of thermoregulatory loss which can occur under conditions employed in maximum biliary excretion (Tm) studies, and to establish the effect of such changes in body temperature on the apparent Tm of bilirubin and BSP.

Materials and methods. Male, Swiss-Webster mice (35-40 g) and male, Sprague-Dawley rats (300-400 g) were used throughout the study. The animals were housed 10 per cage and maintained unrestricted on laboratory diet and tap water until use. Bilirubin (Sigma Chemical Co., St. Louis, Mo.) for infusion in the rat was prepared by dissolving 40 mg of bilirubin in 10 ml of isotonic Na_2CO_3-NaCl solution (0.5 g Na_2CO_3, 0.53 g NaCl per 100 ml). For the mouse 50 mg of bilirubin was dissolved in 10 ml of isotonic solution and the pH adjusted to approximately pH 8 with 5 N HCl. The bilirubin solutions were infused at a rate of about 0.07 and 0.02 ml/min in rats and mice respectively. BSP (Hynson, Wescott and Dunning, Baltimore, Md.), 50 mg/ml, was diluted in sufficient 0.9% NaCl to allow an infusion of about 0.03 ml/min.

Rectal temperature experiments. Rectal temperatures were continuously recorded prior to and following pentobarbital sodium-induced anesthesia (rats, 45 mg/kg, i.p.; mice, 80 mg/kg, i.p.) using a thermistor probe (YSI 401) and a Grass Polygraph recorder. Arterial blood pressure was also measured in these animals using a Statham pressure transducer (P23AA).

Biliary excretion experiments. Rats and mice were anesthetized with pentobarbital sodium (rats, 45 mg/kg, i.p.; mice, 80 mg/kg, i.p.) and restrained in the supine position on a metal surgical board. The bile duct was then cannulated with PE-10 tubing. Infusions were accomplished by cannulation of the femoral vein in the rat (PE-50) and the external jugular vein in the mouse (PE-10). In the rat, bilirubin was infused at a rate of 0.3 mg/min following a priming dose of

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20 mg/kg. Bilirubin was infused at a rate of 0.1 mg/min in experiments with mice. In the BSP experiments in rats, an infusion rate of 2.5 mg/min/kg was employed.

Bile volume and bile concentration measurements of bilirubin or BSP were done on samples collected during periods of maximum excretion(7,8). In rats the sample used for analysis was collected over a 15-minute period, beginning 15 minutes after the start of the infusion. In mice the bile sample was collected for 30 minutes beginning 30 minutes after the start of the infusion. Bile volume of rats was measured by means of pipet graduated in 0.01 ml; bile volume of mice was determined gravimetrically. Bile bilirubin concentrations were determined using the method described by Weinbren and Billing(9). BSP concentrations were determined after alkalinization(8).

In order to maintain the desired rectal temperature a heating lamp and/or an electric fan were used in conjuction with a Thermistemp temperature controller (Yellow Springs Instruments Co., Yellow Springs, Ohio).

Statistical analysis. The data were analyzed by an analysis of variance. The best-fitting lines shown in Fig. 2-4 were calculated by the method described by Steel and Torrie(10).

Results. Illustrated in Fig. 1 is the fall in rectal temperature in mice and rats following pentobarbital-induced anesthesia. Each point on the graph represents the mean $\pm$ S.E. of a group of 3 animals. The time represents the period beginning with the administration of the anesthetic. The mice showed a more rapid and greater fall in rectal temperature than the rats, dropping approximately 10°C in 60 minutes. The rats showed a drop of 2.5°C over the same period. The room temperature during these experiments ranged between 23 and 25°C.

Fig. 2 shows the influence of rectal temperature on the Tm of the liver for bilirubin and BSP excretion. Each point represents the mean value derived from separate groups of 3 rats each. The percent decrease in bilirubin Tm was approximately 5% per °C decrease in rectal temperature (40 μg/kg/min), while BSP Tm decreased approximately 7% per °C(100 μg/kg/min). No significant changes in blood pressure were seen in these experiments.

The influence of temperature on bile flow in these same animals is shown in Fig. 3. Bilirubin-infused animals showed a decrease in bile flow of approximately 6% per °C (5 μl/kg/min), while bile flow in BSP-infused animals decreased approximately 8% per °C (8 μl/kg/min). By an analysis of variance the calculated lines shown in Fig. 2 and 3 were found to have significant regression and no deviation from linearity ($P<.05$). Bile concentrations of bilirubin and BSP calculated from these data showed no significant regression ($P>.05$). There was however a tendency for the concentration of bilirubin and BSP to increase with a decrease in temperature.

Fig. 4 is a summary of data collected from separate groups of mice infused with bilirubin at different rectal temperatures. Part A shows the Tm values obtained at the various rectal temperatures. The Tm for bilirubin decreased approximately 4% per °C (10 μg/kg/min) over the temperature range studied. Bile flow (Part B) on the other hand showed an approximate decrease of 8% per °C (6 μl/kg/min), resulting in an increase in bile bilirubin concentration (Part C) of approximately 6% per °C (0.3 μg/μl). All these lines showed significant regression and no deviation from linearity ($P<.05$).

Discussion. The importance of realizing the influence of temperature on various physiological and biological activities has been pointed out(5,6,11). The results obtained in the present investigation emphasize the need for monitoring body temperature in yet another situation; that is, during biliary Tm studies. According to the results the decrease in rectal temperature which occurs in a period of time (Fig. 1) corresponding to the duration of a normal Tm experiment, is sufficient to cause a decrease in the apparent Tm for bilirubin and BSP. This is especially true in the mouse. In light of this, one might envision the possibility that a loss of thermoregulation, in response to certain experimental treatments, could result in an alteration in the hepatic transport maximum, but this effect could be erroneously attributed to an effect of the treatment on the hepatic paren-

chyma itself. Such an interpretation could lead to conclusions contradictory to those of others.

The bile flow and biliary Tm data for bili-

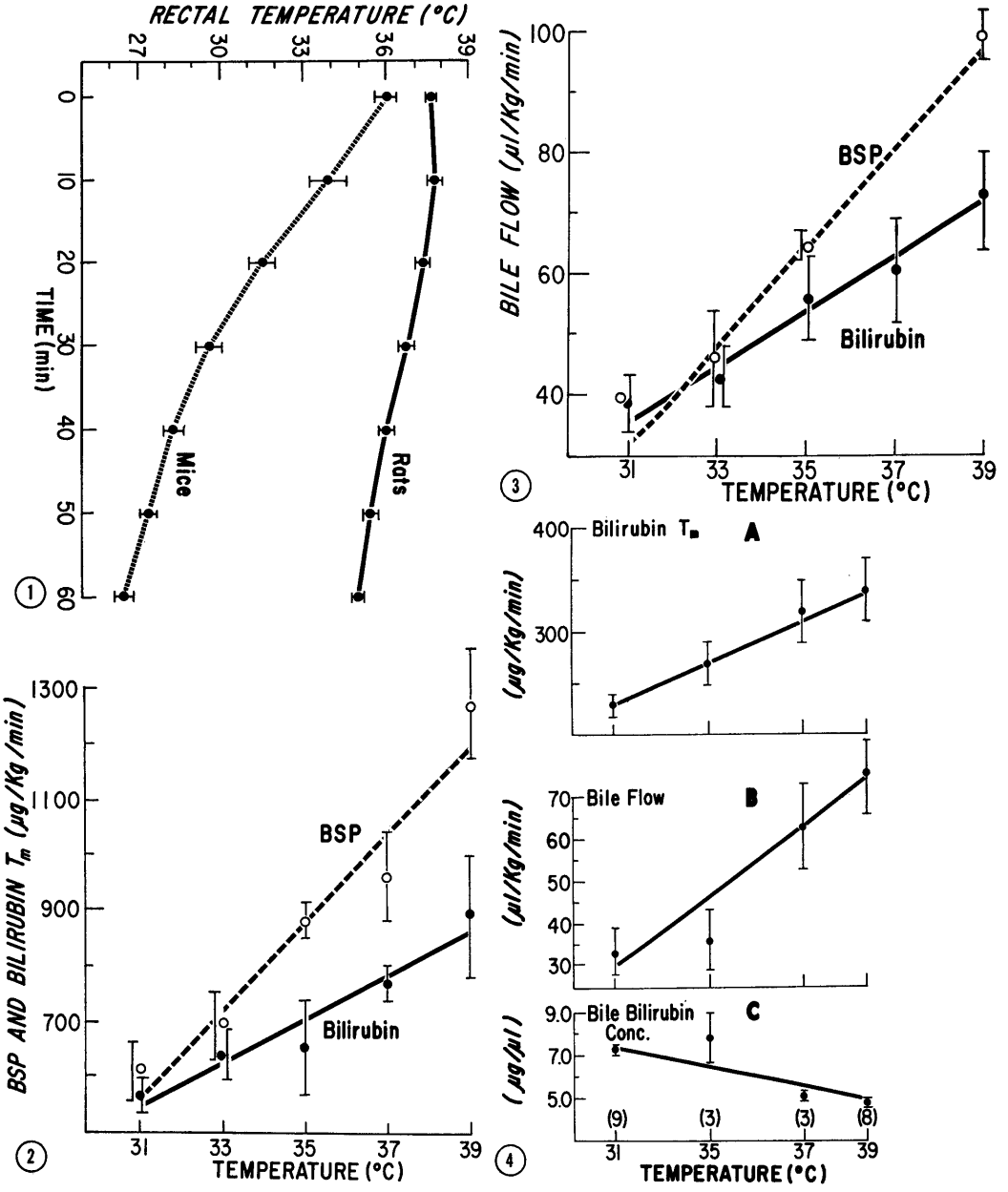

FIG. 1. Effect of pentobarbital-induced anesthesia on rectal temperature in rats and mice. Each point represents mean value ± S.E. for 3 animals. Time represents the period beginning with administration of anesthetic.

FIG. 2. Influence of rectal temperature on Tm of bilirubin and BSP in the rat. Each point represents mean value ± S.E. derived from separate groups of 2 animals each.

FIG. 3. Influence of rectal temperature on bile flow in rats. Each point represents mean value ± S.E. derived from separate groups of 2 animals each.

FIG. 4. Influence of rectal temperature on bilirubin Tm (A), bile flow (B), and bile bilirubin concentration (C) in mice. Each point represents mean value ± S.E. for separate groups of mice. Number of animals used at each rectal temperature shown in parentheses.

rubin and BSP suggest that in the rat temperature has a greater effect on bile flow than on biliary excretion. This differential effect is more evident in the bilirubin studies carried out in mice. Here, while bile flow diminished with hypothermia, the biliary concentration of bilirubin was found to increase significantly. These observations tend to support the contention(1) that the formation of bile is a consequence of at least two separate mechanisms: one controlling the introduction of water and electrolytes; the other controlling the active secretion of specific substances into the bile.

Summary. The influence of temperature on the maximum biliary excretion of bilirubin and sulfobromophthalein was studied in anesthetized rats and mice. Experimental techniques commonly employed in maximum biliary excretion studies were shown to significantly alter normal thermoregulatory mechanisms. A decrease in body temperature significantly decreased the bilirubin transport maximum in both rats and mice. A loss of body temperature in the rat also produced a significant decrease in the BSP transport maximum. In both rats and mice bile flow showed a diminution corresponding with the decrease in rectal temperature. The results emphasize the importance of monitoring body temperature during the course of experiments employing maximum biliary excretion as an endpoint.

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Immunoglobulin Classes of Serum Neutralizing Antibody Formed in Response to Infection with Human A_2 Influenza Virus. (32081)

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Immunity to infection with influenza virus is not always directly related to the level of serum antibody(1-3). Following exposure to influenza virus, some individuals with low serum antibody titers are protected, while others with somewhat higher titers may become ill. Data obtained from volunteer studies suggest that antibody acquired by infection with antigenically related strains of A_2 virus may be less protective than antibody induced by the infecting virus strain(4).

The present work was undertaken to study the sequence of immunoglobulin classes of neutralizing antibodies to human influenza viruses formed in response to experimental infection of adult volunteers with a strain of A_2 virus.

Materials and methods. Serum specimens.