slightly. Ethanolamine in the presence of added betaine is quite able to overcome the action of 2-amino-2-methylpropanol but in the presence of added methionine is unable to do so. Even though the inhibitory actions of 3AP and 2A2MP are quite similar in the rat, the latter substance appears to be the stronger inhibitor. An action difficult to explain is the decrease of liver fat when the level of 2A2MP is increased in the diet. Such is not observed when 3AP is increased.

Radioactive isotope studies(5) indicate that 2A2MP inhibits the incorporation of ethanolamine and dimethylethanolamine into rat liver phospholipids. Choline incorporation on the other hand is only slightly inhibited. Additional observations(6) indicate that 2A2MP itself is incorporated into liver phospholipid. It is suggested that ethanolamine and its N-methyl derivatives must first be incorporated into phospholipid before they are methylated to choline(7,8,9). It is postulated that 2A2MP and 3AP increase the severity of choline deficiency by inhibiting the incorporation of ethanolamine into phospholipid thus preventing its methylation to choline.

If it is demonstrated that 3AP is incorporated into phospholipid and is also an inhibitor of natural amino alcohol incorporation into phospholipid and thus methylation to choline (lecithin), it with 2A2MP should be of value in the study of phospholipid synthesis and metabolism. Further, they should be of value in studies involving the role of phospholipids in other biological mechanisms or systems.

Summary. 3-Aminopropanol, like 2-amino-2-methylpropanol, increased the incidence of hemorrhagic kidneys and the level of liver fat of weanling male rats consuming a low-choline diet. Choline, dimethylethanolamine and monomethylethanolamine prevented the antilipotropic action of 3-aminopropanol. In the presence of 3AP betaine and methionine prevented the occurrence of kidney hemorrhages but only partially reduced the liver fat. Ethanolamine had little influence on the occurrence of hemorrhagic kidneys and only partially reduced the liver fat. Betaine and ethanolamine together in the diet were quite effective, whereas, methionine and ethanolamine together were only moderately effective. The inhibitory action of 3-aminopropanol was not as great as that of 2-amino-2-methylpropanol.

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Plasma Bilirubin and Bromsulfophthalein Clearance During Simulated High Altitude in Unanesthetized Rats.* (29867)

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Recently Berendsohns(1) has reinvestigated the problem of bilirubinemia in residents of high altitude. There appeared to be a relationship to the degree of polycythemia. Though some tendency to parallel the bilirubin

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TABLE I. Effect of 28,000 ft Simulated Altitude on Plasma Bilirubin and BSP Levels 32 min After Injection in Unanesthetized Rats.

No. of rats	Body wt (g)	Blood Hb (g/100 ml)	Plasma Hb (g/100 ml)	Treatment	Plasma concentration (mg/100 ml)
13	334	14.5	—	4 mg/100 g bilirubin	29.3
13	325	15.0	—	4 mg/100 g bilirubin + altitude	37.4*
17	268	14.7	.3	1 mg/100 g BSP	.15
14	273	15.7	.3	1 mg/100 g BSP + altitude	.23†

* p: .014 for difference from control.
† p: <.001 for difference from control.

binemia was seen, retention of bromsulfophthalein (BSP) was normal. The bilirubinemia could be explained as due to deficient liver mechanisms consequent to either the increased bile pigment load or to the effect of hypoxia or both. In the present study we were interested in the effect of simulated high altitude upon plasma clearance of both exogenous bilirubin and BSP in the unanesthetized rat.

Materials and methods. The day before the experiment the vena cavae of rats under ether anesthesia was cannulated with polyethylene tubing according to the method of Popovic and Popovic(2). Without anesthesia or fasting on the next day the dose of bilirubin or of BSP was rapidly injected into the permanent cannula simultaneously in a control and in an experimental rat and a stopwatch started. The rat to be subjected to anoxic hypoxia was quickly transferred to a decompression chamber, and ascent to the simulated altitude started. Within 9 minutes after the injection 28,000 ft (P_B 246.8 mm Hg) was reached and maintained until 30 minutes after injection. Recompression required about one-half minute. The blood sample was taken as rapidly as possible with completion occurring within 1½ minutes on the average.

In the case of the bilirubin series a dose of 4 mg/100 g body weight was given in an isotonic sodium carbonate-sodium chloride solution freshly prepared as 4 mg/ml from reagent grade bilirubin (Mann Research Laboratories). The BSP dose of 1 mg/100 g body weight was given in isotonic saline made up with sulfobromophthalein sodium (Hynson, Westcott & Dunning, Inc.) in a concentration of 1 mg/ml.

Plasma bilirubin was determined in duplicate 1 ml samples by the method of Malloy and Evelyn(3) using a Fisher photoelectric colorimeter standardized against reagent grade bilirubin. Plasma BSP concentration was determined in duplicate 1 ml samples with the acetone method of Henry *et al*(4) with readings at 585 mμ on the Beckman spectrophotometer. Blood and plasma hemoglobin were determined by the cyanmethemoglobin method(5).

An attempt was made to study the effect of acclimatization through daily 8-hour exposures of rats to a simulated altitude of 24,000 ft. After a month of the discontinuous hypoxia, clearance studies were carried out with bilirubin as above. However, due to the increased viscosity of the polycythemic blood, hemolysis was so great in the blood samples that the bilirubin method was considered unreliable.

Results. Table I shows that within about 32 minutes after injection of either bilirubin or BSP the plasma concentration of both was higher in altitude exposed rats than in controls maintained outside the decompression chamber. In respect to bilirubin the difference is 8.1 mg/100 ml which is 28% higher than the control level, and is significant with a p value of 0.014. The terminal BSP con-

centration was 0.08 mg/100 ml higher than the control, which is 53% greater, and significant at less than 0.001.

Two rats whose hemoglobins on the day of the experiment were below 10.9 g/100 ml were excluded from the series. Furthermore, one control rat had a BSP value greater than 3 standard deviations from the mean and was excluded.

Discussion. It has been found in dogs and in man that plotting the log of plasma concentration of BSP against time after a single intravenous injection produces a curve with 2 slopes, an early steep and a later flatter slope. On the basis of liver uptake and bile secretion studies(6) it has been determined that the early slope defines liver uptake primarily, while the later one is dominated by the rate of BSP secretion into bile. In the dog the later slope emerges well before 30 minutes after the intravenous injection.

Similar relationships following intravenous injection of bilirubin have not been quantitated. However, the curves of Arias(7) and of Grodsky *et al*(8) following such injections into anesthetized rats are not incompatible with such a possibility.

On the basis of such considerations the 30-minute plasma levels of bilirubin and of BSP in the above experiments were chosen to indicate excretory function of the liver as affected by anoxic hypoxia.

Since Brauer(9) found that oxygen consumption in anesthetized rats decreases with the oxygen saturation of the blood entering the liver, the reduced clearance of bilirubin and BSP in these experiments is not strange. The exact details of the mechanisms for liver uptake, conjugation and concentration in the bile are not completely known. However, conjugation is known to require oxidative energy(10), and no doubt the transport from plasma against a concentration gradient of a hundred-fold or more into the bile must require it also(11).

Though the results presented here do not measure excretory function of the liver directly, they are unbiased by the presence of the complication of anesthesia. The fact that both bilirubin and BSP clearance appeared to suffer as a result of the hypoxia has some implications for the study of Berendsohns who found BSP clearance unaffected in his residents at high altitude. It might be argued that evidence of frank hypoxic liver derangement was not seen and that increased bile pigment load, the other variable here, was of major importance in producing the bilirubinemia of chronic anoxic hypoxia.

Summary. The simulated altitude of 28,000 ft in a decompression chamber was found to increase the 32-minute plasma levels of injected bilirubin and BSP by about 28 and 53% respectively in the unanesthetized rat in which vena cava polyethylene cannulas had been implanted. The implications of these findings for the bilirubinemia of chronic anoxic hypoxia have been discussed.

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