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**Plasma Disappearance of Conjugated and Unconjugated C<sup>14</sup>  
Bilirubin in the Rat with Obstructive Jaundice. (31737)**

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Pure specimens of conjugated bilirubin are not available so that information regarding its metabolism can be obtained only by indirect means. In normal animals a tracer dose of unconjugated C<sup>14</sup> bilirubin will be cleared quickly from the plasma and eliminated in the bile as conjugated C<sup>14</sup> bilirubin(1). If, however, the bile duct is ligated the conjugated C<sup>14</sup> bilirubin will appear in the plasma and then be excreted in the urine. Using the Weber and Schalm technique(2) it has been possible to separate conjugated from unconjugated bilirubin quantitatively and thus to determine their individual rates of disappearance from the plasma.

*Methods. Preparation of C<sup>14</sup> bilirubin.* Radioactive bilirubin was isolated(3) from dog fistula bile collected for 6 hours following an injection of 150  $\mu$ C<sup>14</sup>- $\delta$ -amino levulinic

acid(4). It was recrystallized twice and kept at  $-20^{\circ}$ . Immediately before use the unconjugated C<sup>14</sup> bilirubin was dissolved in 0.1 ml 0.1 N NaOH and 1 ml nonicteric Sprague Dawley rat plasma was added. One ml of this solution was injected and rats 1, 2 and 3 received doses of 0.46  $\mu$ C (S.A. 4,000 dpm/ $\mu$ g), 0.73  $\mu$ C (S.A. 4,900 dpm/ $\mu$ g) and 0.77  $\mu$ C (S.A. 8,100 dpm/ $\mu$ g) respectively.

*Experimental.* Three male Sprague Dawley rats weighing 320, 280 and 300 g, whose bile ducts had been ligated 7-14 days previously, were used. C<sup>14</sup> bilirubin was injected into one of the lateral tail veins while another vein was used for blood sampling. During the first 6 hours the animals were kept in a Cotlove(5) restraining cage and 0.5 ml blood was withdrawn at 2,5,15,30 and 60 minutes and 3 and 6 hours. Further samples were

taken at 24 hourly intervals after the injection. The rats (1,2 and 3) were sacrificed at 72, 96 and 144 hours respectively, when sufficient blood was taken from the aorta for the isolation and crystallization of C<sup>14</sup> bilirubin from the plasma to enable its specific activity to be determined. These analyses showed that 90% or more of the plasma radioactivity was present as C<sup>14</sup> bilirubin so that it seemed justifiable to assume that the changes in total radioactivity in the plasma largely reflected changes in C<sup>14</sup> bilirubin radioactivity.

**Chemical methods.** 0.1 ml plasma was used for the determination of both conjugated and unconjugated bilirubin concentrations by the method of Weber and Schalm(2). To determine the radioactivity of the two pigments, an additional 0.1 ml plasma was added to the Weber and Schalm mixture of lactic acid, ethyl acetate and chloroform. After mixing and centrifugation the 2 layers were separated and quantitatively transferred to counting vials, containing 18 ml of toluene, monoethanolamine and ethylene glycol monomethylether (10:1:7, v/v), as described by Jeffay and Alvarez(6). The vials were counted in a Nuclear Chicago liquid scintillation spectrometer with an efficiency between 40-60%, using C<sup>14</sup> Toluene as a standard. The specific activity (dpm/ $\mu$ g) of both conjugated and unconjugated bilirubin was then calculated, assuming that all the counts in the plasma were present as bilirubin.

**Results.** A high proportion of the injected radioactivity was immediately removed from the plasma so that after 2 minutes only 12.8%, 12.0% and 22.7% of the dose remained in the plasma of rats 1, 2 and 3 respectively. The level of radioactivity in the plasma then decreased gradually so that after 24 hours the retention values were 3.1%, 2.8% and 2.5%. Simultaneously conjugated C<sup>14</sup> bilirubin appeared in the plasma and within 2 minutes it comprised 22% of the total C<sup>14</sup> bilirubin radioactivity. After 30 minutes this value had risen to 70-80% and remained relatively constant for the duration of the experiment (Fig. 1). Similar values for the percentage of endogenous conjugated bilirubin in the plasma were found.

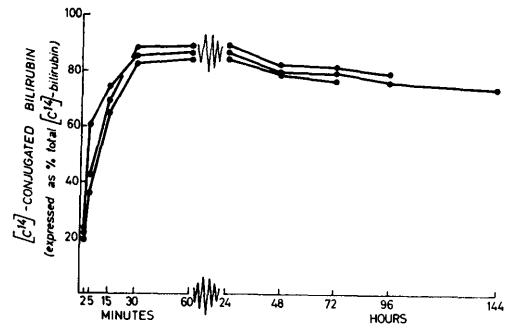


FIG. 1. Percentage of total C<sup>14</sup>-bilirubin present as conjugated C<sup>14</sup> bilirubin in the plasma of 3 bile duct ligated rats after a single injection of unconjugated C<sup>14</sup> bilirubin.

The changes in specific activity of both conjugated and unconjugated C<sup>14</sup> bilirubin during the first hour are illustrated in Fig. 2. During the first 30 minutes the specific activity of the conjugated bilirubin increased while that of the unconjugated bilirubin decreased. Subsequently, there was a period of mixing between the exogenous labelled bilirubin and the endogenous bilirubin body pools and equilibrium was not attained until 24 hours after the injection. After this time, when the plasma concentrations of both endogenous conjugated and unconjugated bilirubin were relatively constant, the specific activity of both C<sup>14</sup> bilirubin fractions declined exponentially (Fig. 3).

Table I gives the results for the calculated half-life and fractional turnover in both C<sup>14</sup> bilirubin fractions. The values in the 3 animals for the half life of C<sup>14</sup> conjugated bilirubin ranged between 30 and 38 hours and were consistently shorter than those of unconjugated C<sup>14</sup> bilirubin which were between 50 and 60 hours.

**Discussion.** The extremely rapid fall in the plasma radioactivity following the injection of unconjugated C<sup>14</sup> bilirubin is probably due to its immediate uptake by the liver since the conjugated C<sup>14</sup> bilirubin, which appeared simultaneously, can only have originated from that organ. This was followed by a period of mixing of both unconjugated and conjugated C<sup>14</sup> bilirubin with their respective endogenous pools. It was not until 24 hours after the injection that a steady state was achieved and the specific activities

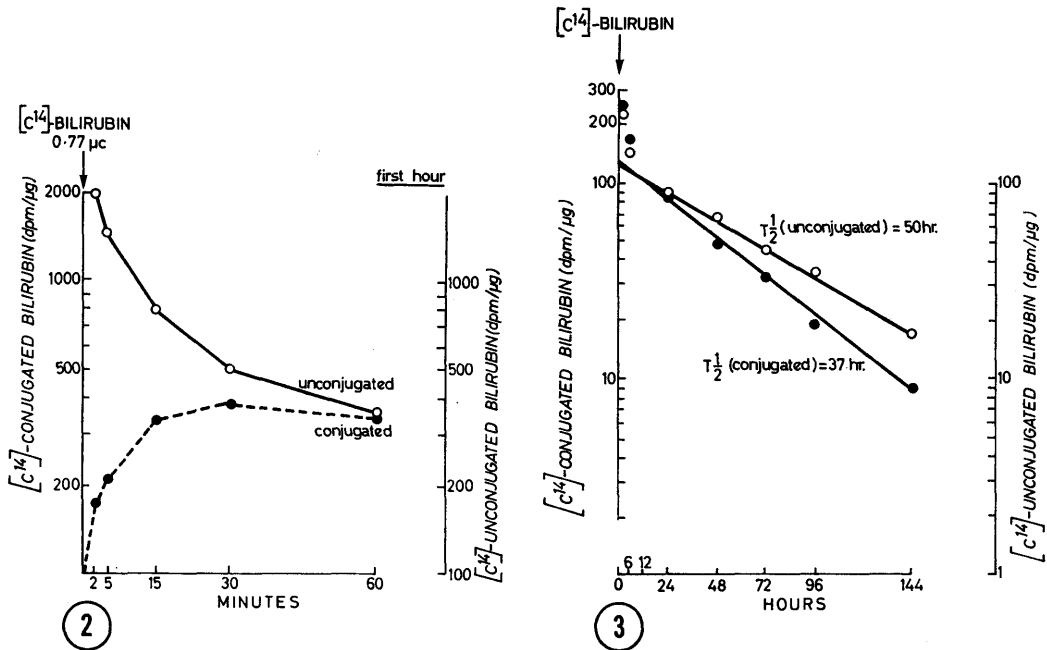


FIG. 2. Changes in specific activities of unconjugated and conjugated C<sup>14</sup> bilirubin in plasma during the first hour after single injection of 0.77 μc unconjugated C<sup>14</sup> bilirubin into a bile duct ligated rat (no. 3).

FIG. 3. Changes in specific activities of unconjugated C<sup>14</sup> bilirubin (o) and conjugated C<sup>14</sup> bilirubin (●) in plasma for 6 days following a single injection of 0.77 μc unconjugated C<sup>14</sup> bilirubin into a bile duct ligated rat (no. 3).

of conjugated and C<sup>14</sup> bilirubin declined exponentially.

The results for the calculated half-life of C<sup>14</sup> unconjugated bilirubin fell within the range of values reported by Schmid and Hammaker for the Gunn rat(7). In the obstructed rat these figures only indicate the true biological half-life of bilirubin, if one assumes that none of the conjugated C<sup>14</sup> bilirubin formed is reconverted to unconjugated C<sup>14</sup> bilirubin; at present there is no definitive information on this matter.

The lower values obtained for the calculated

half-life of conjugated C<sup>14</sup> bilirubin compared with those for unconjugated C<sup>14</sup> bilirubin reflect the losses of conjugated bilirubin by urinary excretion. Although the specific activities of conjugated and unconjugated C<sup>14</sup> bilirubin are of the same order of magnitude at 24 hours the absolute amount of radioactivity present in the form of conjugated bilirubin is considerably greater than that present as unconjugated bilirubin. Therefore, once equilibrium has been established, it can be calculated that not more than 10% of the conjugated C<sup>14</sup> bilirubin present at any time

TABLE I. Half-Life and Turnover of Unconjugated C<sup>14</sup>-Bilirubin in the Obstructed Rat.

C <sup>14</sup> bilirubin	Rat 1 (320 g)		Rat 2 (280 g)		Rat 3 (300 g)	
	Conjugated	Un-conjugated	Conjugated	Un-conjugated	Conjugated	Un-conjugated
Plasma concentration, mg/100 ml at 24 hr	9.8	5.0	4.3	1.3	5.3	.9
Plasma pool, μg	990	505	387	117	49	84
Estimated half-life, hr	38	60	30	50	37	50
Fractional turnover /24 hr	.44	.28	.55	.33	.45	.33

could have been derived from the disappearing unconjugated  $C^{14}$  bilirubin. The actual figure is probably considerably less since it is known that some of the unconjugated  $C^{14}$  bilirubin is metabolised and not converted to conjugated  $C^{14}$  bilirubin. Only a small error is therefore incurred in the calculation of the half-life of conjugated bilirubin in the bile duct ligated rats. Whether the simultaneous retention of conjugated bile salts in this preparation influences the half life of conjugated bilirubin cannot be established without pure specimens of the pigment.

It is not known whether conjugated and unconjugated bilirubin are present in tissues in the same or different proportions as those found in plasma. It has, therefore, not been possible to calculate the size of the total miscible bilirubin pool from the data obtained in this study.

*Summary.* Unconjugated ( $C^{14}$ )-bilirubin was administered intravenously to rats with complete biliary obstruction. The plasma disappearance curves of both the conjugated

and unconjugated ( $C^{14}$ )-bilirubin fractions were determined using a modified Weber and Schalm technique. More than 70% of the ( $C^{14}$ )-bilirubin radioactivity in the plasma was present as conjugated ( $C^{14}$ )-bilirubin after 15-30 minutes of injecting the unconjugated label. The biological half life of unconjugated ( $C^{14}$ )-bilirubin averaged 53 hours while that of the conjugated form 34 hours. This difference could be explained by the clearance of conjugated ( $C^{14}$ )-bilirubin by the kidney.

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### **Effect of Cold Dilute Saline Ingestions on Evaporative Weight Loss of Heat-Exposed Resting Men.\* (31738)**

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When man is exposed to hot environments, any circumstance that reduces his ability to sweat is considered detrimental to his physical well being. One factor that has not been investigated is the effect of heat content of ingested fluids on human temperature regulation. Brief studies(1,2) have been reported on palatability of water at various temperatures but there is a divergence of opinion based on subjective evidence as to what the temperature of ingested fluids should be when man is exposed to warm environments. Environmental studies reflect this condition and when water or other fluids were used to rehydrate heat ex-

posed individuals, temperature of ingested fluids has been ignored, definitely stated or vaguely indicated. Basic to this varied treatment of ingested fluid temperature is the assumption that there is little or no detectable difference in performance following cold or warm water ingestion(3). Such reasoning is based on the small amount of heat used to raise the temperature of ingested cold liquids to that of the body. During a series of heat exposure experiments we obtained data which indicate that this concept is an oversimplification.

*Methods.* Three nude non-acclimatized male medical students were exposed for 12 hours to 43 C D.B. and 29 C W.B. in order to

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