conjugates of their corresponding monohydroxybenzo[a]-pyrene derivatives. This was confirmed by the results of enzymic incubations of the suspected sulphate conjugates of 9- and 7-hydroxybenzo[a]pyrene with sulphatase, when no unchanged conjugates were detected and both compounds yielded products with similar chromatographic and fluorescent properties to the corresponding monohydroxybenzo[a]pyrene derivatives. However, when the suspected sulphate conjugates were incubated with ketodase, unchanged conjugates but not monohydroxybenzo[a]pyrenes were detected. In a recent study when benzo[a]pyrene was cultured with human, hamster or rat lung, benzo[a]-pyren-3-yl hydrogen sulphate was identified as a major ethyl acetate-soluble metabolite by comparison of its fluorescence excitation and emission spectra with those of the synthetic metabolite [8].

Thus monohydroxybenzo[a]pyrenes, one of the major groups of metabolites formed metabolically from benzo-[a]pyrene, are converted into their corresponding ethyl acetate-soluble sulphate esters. These sulphate conjugates, with a mol. wt of approx. 349, which is probably below the threshold for biliary excretion in man [13], would not be expected to be excreted in the bile. The lipophilicity of these conjugates as determined by their solubility in ethyl acetate would also suggest that they will not be readily excreted in the urine. Thus the sulphate conjugates of monohydroxybenzo[a]pyrene derivatives may be retained in man and may require further metabolism before being excreted. If these sulphate esters are further metabolised then the conjugation with sulphate may alter the reactivity of the ring thus changing the orientation of any further metabolism. The increased hydrophilicity of these sulphate conjugates relative to benzo[a]pyrene or their monohydroxybenzo[a]pyrene precursors may also alter the distribution of benzo[a]pyrene related material. The biological importance of the sulphate conjugates of monohydroxybenzo[a]pyrenes and their metabolic products remain to be elucidated.

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Inhibitory effect of xanthines on the incorporation of [^{14}C]valine into proteins and on the secretion of albumin in isolated parenchymal rat liver cells

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Since the observation by Butcher and Sutherland[1] that phosphodiesterases are inhibited by xanthines, these substances have often been added to tissue preparations in order to maintain an increased level of cyclic AMP produced by administration of glucagon or epinephrine. The concentration generally used is 1–5 × 10^{-3} mole l^{-1}. During studies on the role of cyclic AMP in liver protein metabolism we have observed a strongly inhibitory effect of xanthines both on the incorporation of [^{14}C]valine into liver proteins and on the secretion of albumin.

MATERIALS AND METHODS

Female Wistar rats fed *ad lib.* and weighing about 200 g were used. Cells were prepared as described earlier [2]. Aminophylline (theophylline ethylendiamine), caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) were obtained from Sigma, St. Louis, U.S.A. L-[U-^{14}C] valine was from Radiochemical Centre, Amersham, England. Other materials, incubation medium, and incubation were as described [3]. All cell suspensions were preincubated for 20 min. Then the incubations were started by adding [^{14}C]valine (0.25 μC) and xanthine or solvent. The incubation time was 60 min. The incorporation of [^{14}C] valine into liver cell protein (i.e. incorporation into proteins in the cell suspension minus incorporation into proteins in the medium), proteins in the medium and albumin in the medium and also the immunologically measured increase in albumin concentration in the medium were determined as described earlier [2, 3].

RESULTS AND DISCUSSION

Aminophylline, caffeine and theophylline inhibited the incorporation of [^{14}C]valine into liver cell protein with

Table 1. Effects of xanthines on incorporation of [^{14}C]valine into proteins and on albumin secretion in isolated parenchymal rat liver cells

	Incorporation into liver cell protein (cpm × 10^{-3}/ml cells)	Incorporation into total proteins in the medium (cpm × 10^{-3}/ml cells)	Incorporation into secreted albumin (cpm × 10^{-3}/ml cells)	Albumin secretion (μg albumin/ml cells)
Control	881 ± 15	280 ± 27	96 ± 4	458 ± 54
Aminophylline (5×10^{-3}M)	369 ± 5	44 ± 6	18 ± 1	189 ± 22
Difference	516 ± 19	236 ± 21	77 ± 4	267 ± 33
	P < 0.01	P < 0.01	P < 0.01	P < 0.02
Caffeine (5×10^{-3}M)	511 ± 17	105 ± 6	36 ± 4	257 ± 34
Difference	370 ± 28	176 ± 21	60 ± 1	201 ± 27
	P < 0.01	P < 0.02	P < 0.001	P < 0.02
Theophylline (5×10^{-3}M)	658 ± 18	143 ± 15	46 ± 3	277 ± 35
Difference	224 ± 34	137 ± 13	50 ± 1	181 ± 20
	P < 0.05	P < 0.01	P < 0.001	P < 0.02

Parenchymal rat liver cells were preincubated for 20 min. Incubations were started by adding 0.25 μC [^{14}C]valine and xanthine (sufficient to give a final concentration as indicated) or solvent. The incubation time was 60 min. Figures are given as means ± S.E.M. of 3 experiments.

59 per cent, 42 per cent, and 25 per cent, respectively (Table 1). Also the incorporation into total proteins in the medium and into albumin was inhibited by xanthines, the inhibition being 84 per cent and 80 per cent, 63 per cent and 63 per cent, and 49 per cent and 52 per cent, respectively (Table 1). Albumin secretion was also inhibited (58 per cent, 44 per cent, and 40 per cent, respectively), but to a lesser degree than the incorporation into total proteins in the medium and into albumin (Table 1). The lesser inhibition of the secretion of albumin is probably due to the fact that the cells contain considerably amounts of preformed, unlabelled albumin. The inhibition caused by the administration of xanthines will probably not change the secretion of preformed albumin. Of the xanthines tested aminophylline seems to be the most powerful inhibitor followed by caffeine and theophylline. We have shown earlier [3] that cyclic AMP inhibits the same protein parameters as measured in this communication. It is therefore tempting to explain the inhibitory effect of xanthines presented here by an inhibition of phosphodiesterase followed by an increase in the level of cyclic AMP. However, this is not likely. Firstly theophylline alone either raises the level of cyclic AMP very little [4] or not at all [5]. Secondly the inhibition produced by exogenous or endogenous glucagon-stimulated cyclic AMP [3] is smaller than that produced by xanthines. It has been shown earlier that xanthines are not specific in their action [6, 7].

Our findings are in agreement with other *in vitro* observations that theophylline can diminish the incorporation of amino acids into proteins in muscle tissue [8, 9] and in adrenal cortex [10]. Meanwhile, using cell-free systems, aminophylline and theophylline have been shown to stimulate amino acid incorporation into proteins by rat liver and brain microsomal and ribosomal systems [6]. These xanthines have also been shown to enhance polypeptide synthesis in mammalian systems [11]. The reason for the difference between cell systems and cell-free systems is not known. An inhibitory effect of xanthines on amino acid transport over the liver cell membrane could be the reason for this difference. Meanwhile, present evidence argues against this explanation, as theophylline has an increasing effect on the amino acid uptake by jejunal mucosa [12]. Further an increasing effect of the xanthines on the amino acid transport across the cell membrane would not inhibit the immunologically measured albumin secretion.

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