Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
Craig, L. C., Gregory, J. D. & Hausmann, W. (1950). *Analyt. Chem.* **22**, 1462.
Crumpler, R., Dent, C. E., Harris, H. & Westall, R. G. (1951). *Nature, Lond.*, **167**, 307.
Dann, M., Marples, E. & Levene, S. Z. (1943). *J. clin. Invest.* **22**, 87.
Datta, S. P. & Harris, H. (1951). *Nature, Lond.*, **168**, 296.
Dent, C. E. (1946). *Lancet*, **2**, 637.
Dent, C. E. (1947). *Biochem. J.* **41**, 240.
Dent, C. E. (1948). *Biochem. J.* **43**, 169.
Dent. C. E. (1952). In *Lectures on the Scientific Basis of Medicine*, vol. 2, p. 213. London: Athlone Press.
Dent. C. E. (1954). *Exp. Med. Surg.* **12**, 229.
Dent, C. E. & Harris, H. (1951). *Ann. Eugen., Lond.*, **16**, 60.
Dent, C. E. & Hodson, R. J. (1954). *Brit. J. Radiol., N.S.*, **27**, 605.
Dent, C. E. & Rose, G. A. (1951). *Quart. J. Med., N.S.* **20**, 203.
Dent, C. E., Senior, B. & Walshe, J. M. (1954). *J. clin. Invest.* **33**, 1216.
Dunn, M. S., Camien, M. N., Shankman, S. & Block, H. (1947). *Arch. Biochem.* **13**, 207.
Eckhardt, R. D. & Davidson, C. S. (1949). *J. biol. Chem.* **177**, 687.
Fanconi, G. (1936). *Jb. Kinderheilk.* **147**, 299.
Fowler, D. I., Harris, H. & Warren, F. L. (1952). *Lancet*, **1**, 544.
Frankl, W. & Dunn, M. S. (1947). *Arch. Biochem.* **13**, 93.
Graf, J. & Hoberman, H. D. (1950). *J. biol. Chem.* **186**, 369.
Gutman, G. E. & Alexander, B. (1947). *J. biol. Chem.* **168**, 527.
Hamilton, P. B. & Van Slyke, D. D. (1943). *J. biol. Chem.* **150**, 231.
Harper, H. A., Kinsell, L. W. & Barton, H. C. (1947). *Science*, **106**, 319.
Harris, H. & Searle, A. G. (1952). *Ann. Eugen., Lond.*, **17**, 165.
Harrison, H. E. & Harrison, H. C. (1954). *Science*, **120**, 606.
Hier, S. W. & Bergeim, O. (1946). *J. biol. Chem.* **163**, 129.
Holzel, A., Komrower, G. M. & Wilson, V. K. (1952). *Brit. med. J.* **1**, 194.
Jervis, G. A. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 83.
Jervis, G. A. (1953). *Proc. Soc. exp. Biol., N.Y.*, **82**, 514.
Jonxis, J. H. P. & Huisman, T. H. J. (1953). *Voeding*, **14**, 400.
Krebs, H. A., Eggleston, L. V. & Hems, R. (1949) *Biochem. J.* **44**, 159.
Lawrie, N. R. (1947). *Biochem. J.* **41**, 41.
Luck, J. M. & Wilcox, A. (1953). *J. biol. Chem.* **205**, 859.
Moore, S. & Stein, W. H. (1951). *J. biol. Chem.* **192**, 663.
Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 893.
Nardi, G. L. (1954). *J. clin. Invest.* **33**, 847.
Neuman, R. E. & Logan, M. A. (1950). *J. biol. Chem.* **184**, 299.
Quick, A. J. (1932). *J. biol. Chem.* **97**, 403.
Reed, G. (1942). *J. biol. Chem.* **142**, 61.
Rosen, H. & Levenson, J. M. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 91.
Schram, E., Moore, S. & Bigwood, E. J. (1954). *Biochem. J.* **57**, 33.
Sheffner, A. L., Kirsner, J. B. & Palmer, W. L. (1948). *J. biol. Chem.* **175**, 107.
Smith, H. W., Goldring, W. & Chasis, H. (1938). *J. clin. Invest.* **17**, 263.
Soupart, P. (1952). *Abstr. 2nd Int. Congr. Biochem., Paris*, p. 374.
Spencer, A. G. & Franglen, G. T. (1952). *Lancet*, **1**, 190.
Steele, B. F., Sauberlich, H. E., Reynolds, M. S. & Baumann, C. A. (1947). *J. Nutr.* **33**, 209.
Stein, W. H. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 705.
Stein, W. H. (1953). *J. biol. Chem.* **201**, 45.
Stein, W. H., Bearn, A. G. & Moore, S. (1954). *J. clin. Invest.* **33**, 410.
Stein, W. H., Paladini, A. C., Hirs, C. H. W. & Moore, S. (1954). *J. Amer. chem. Soc.* **76**, 2848.
Tallan, H. H., Stein, W. H. & Moore, S. (1954). *J. biol. Chem.* **206**, 825.
Voegtlin, C. & Hodge, H. C. (1949). *Pharmacology and Toxicology of Uranium Compounds*. New York: McGraw-Hill.
Westall, R. G. (1955). *Biochem. J.* **60**, 247.
Wilson, V. K., Thomson, M. L. & Dent, C. E. (1953). *Lancet*, **2**, 66.
Woodson, H. W., Hier, S. W., Soloman, J. D. & Bergeim, O. (1948). *J. biol. Chem.* **172**, 613.
Woolf, L. I. (1951). *Great Ormond Street J.* **2**, 77.
Zapp, J. A. & Wilson, D. W. (1938). *J. biol. Chem.* **126**, 19.

The Enzymic Activation of Cholic Acid by Guinea-pig-Liver Microsomes

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(*Received* 28 *July* 1955)

Cholic acid is found in bile conjugated with the two amino acids glycine and taurine. For a long time this process of conjugation has been thought to occur in the liver, although nothing was known about the mechanism by which the peptide-like bond of taurocholic acid is formed. During the study of this problem it was found (Elliott, 1955) that formation of an hydroxamic acid occurs in preparations of guinea-pig-liver microsomes. This is dependent on the presence of cholic acid and of coenzyme A (CoA) and adenosine triphosphate (ATP). The present paper is concerned with this activation of cholic acid.

EXPERIMENTAL

Materials

ATP. This was isolated from rabbit muscle by the method of Needham (1942). The potassium salt was prepared from dibarium ATP by dissolving the latter in the minimum volume of N-HCl, running this solution through

a column of Amberlite IR 100 H and washing out all the ATP with distilled water. The eluate was collected in a vessel cooled in ice water and continuously neutralized with KOH. The pH was finally adjusted to 6·8, the solution concentrated by freeze-drying and the ATP assayed as P_{10} (inorganic phosphate liberated by hydrolysis in N acid for 10 min. at 100°).

Cholic acid. Commercial cholic acid was purified by suspending in boiling ether for 15 min.; the solid was then filtered off and recrystallized from 70% (v/v) aqueous ethanol.

Cysteine. A stock solution of cysteine hydrochloride was stored at −10° and neutralized with KOH immediately before use.

CoA. This was prepared from pig liver by the method described by LePage & Mueller (1949) for TPN preparation up to the stage of precipitating the nucleotides with acetone. The only departure from the method was that all the charcoal suspension was filtered instead of rejecting the considerable portion which floated. CoA was assayed by the method of Stadtman & Kornberg (1953), phosphotransacetylase from *Escherichia coli*, kindly prepared by Dr J. Lascelles, being used. The preparation was found to contain 53·5 Lipmann units/mg. This corresponds to a purity of approximately 13%. The light tan-coloured powder was stored over $CaCl_2$ at −10°. Solutions were neutralized with KOH.

A sample of a 70% pure preparation of CoA was kindly supplied by Dr J. D. Gregory of the Massachusetts General Hospital, Boston.

Hydroxylamine. Commercial samples of NH_2OH,HCl were recrystallized from water. A 4M stock solution was stored at 0° and neutralized immediately before use to pH 7·4 (phenol red) by the addition of an equal volume of KOH solution previously adjusted to the appropriate strength.

Ethyl cholate. This was synthesized by the method of Cortese (1937) from recrystallized cholic acid, and the product recrystallized. (Found: C, 70·8; H, 10·19. Calc. for $C_{26}H_{44}O_5$: C, 71·5; H, 10·16%.)

Cholylhydroxamic acid. A solution of this substance, suitable for use as a marker in chromatography, was prepared from ethyl cholate as follows: 0·14 g. of NH_2OH,HCl and 0·1 g. of metallic Na were separately dissolved in minimum volumes of dry methanol. The solutions were mixed, and to the mixture was added a solution of 0·4 g. of ethyl cholate in 3 ml. of methanol. After about 6 hr. at room temperature in a stoppered flask, the mixture was neutralized to pH 6·8 with methanolic HCl and the NaCl filtered off. The volume was adjusted to 10 ml. with methanol and the solution stored at −10°. A small sample of the solution gave an intense purple colour when treated with a solution of 10% $FeCl_3,6H_2O$ in 0·2N-HCl.

Phosphate buffers were made from solutions of KH_2PO_4 and KOH.

Glyoxaline buffer was prepared by adjusting the pH of solutions of the free base with HCl.

Methods

Estimation of hydroxamic acid. The method of Hill (1947), as described by Kornberg & Pricer (1953), was used. Cholylhydroxamic acid was usually estimated only in the protein-free filtrate, since, as shown by the ethanolic reagent (Hill, 1947), only a small amount was adsorbed by the precipitate. The optical densities of the final solutions were measured in a spectrophotometer at 500 mμ.

A standard curve was obtained as follows. The procedure is that of Peel (1951), slightly changed to suit the method of hydroxamic acid estimation used in the present work. Different volumes of a solution of ethyl cholate in methanol (5 μmoles/ml.) were added to tubes containing 2 ml. of alkaline hydroxylamine (prepared by mixing equal volumes of 2N-NH_2OH,HCl and 2·5N-NaOH). The volume was made up to 2·5 ml. with methanol. After standing for 10–15 min. at room temperature the pH was brought to 7·4 by the addition of 0·2 ml. of 6N-HCl. Then 2·25 ml. of 7% $HClO_4$ was added, followed by 0·5 ml. of $FeCl_3$ reagent. The volumes were adjusted to 6 ml. with water and the optical densities read at 500 mμ. in 1 cm. cells.

The readings were found to be proportional to the amount of ethyl cholate in the range 0–10 μmoles.

Chromatography of hydroxamic acids. Chromatograms were run on Whatman no. 3 paper which had been washed in 2N acetic acid followed by demineralized water until neutral to litmus, and finally distilled water. The two solvents used were (*a*) the di*iso*propyl ether solvent: di*iso*propyl ether–*n*-propanol–acetic acid–water (200:90:30:20, by vol.), and (*b*) the butanol–acetone solvent: equal volumes of acetone and the upper layer of the following mixture; *n*-butanol–acetic acid–water (100:20:80, by vol.). Occasionally the spots streaked badly in the first solvent, but usually slightly elongated spots of good definition were obtained.

Detection of spots. Cholylhydroxamic acid has been detected by spraying with three reagents: (1) 10% $FeCl_3,6H_2O$ in 0·2N-HCl; hydroxamic acids give immediate brown-purple spots. (2) 50% $SbCl_3$ in glacial acetic acid; cholic acid derivatives give rose-pink spots on heating (Siperstein, Harold, Chaikoff & Dauben, 1954). (3) 10% phosphomolybdic acid in ethanol; sterols give blue spots against a green background on heating (Kritchevsky & Kirk, 1952).

Preparation of microsomes. Guinea-pig livers were fractionated in 0·25M sucrose according to the procedure of Schneider (1948). The tissue was homogenized in 4 vol. of sucrose in a Potter & Elvehjem (1936) apparatus. The suspension was centrifuged at 10 000 *g* at 0° for 10 min. to sediment nuclei and mitochondria. The supernatant was then centrifuged at 100 000 *g* at 0° for 30 min., when the microsomes are sedimented. The supernatant was poured off, the tubes were drained in an inverted position for 15 min. and the pellet was suspended in cold distilled water (1 ml./g. of original liver). The suspension was stored at −10°. The preparation was still active after 3 weeks. Longer periods were not tested.

Preparation of pig-liver enzyme. Pig liver was chilled and blended for 40 sec. in 5 vol. of cold 1·15% KCl and the suspension centrifuged at 2000 *g* to sediment the nuclei and mitochondria. The supernatant was adjusted to pH 5·4 by adding 0·1M acetic acid. The resulting precipitate was centrifuged down, resuspended in distilled water and the pH adjusted to 6·8 with KOH. The suspension was stirred into 10 vol. of acetone at −10°, filtered by suction and dried in air. The powder was extracted for 30 min. in 3 vol. of 0·01M-$NaHCO_3$ and dialysed against the same solution overnight at 0°.

RESULTS

Formation of an hydroxamic acid independently of ATP by a pig-liver enzyme fraction. Two different kinds of preparations from mammalian liver have been found to catalyse the synthesis of an hydroxamic acid from cholic acid. The pig-liver enzyme prepared as described above catalysed the rapid formation of a considerable amount of hydroxamic acid in the presence of cholic acid and hydroxylamine but in the absence of ATP and CoA. This contrasts with the reaction catalysed by the microsome preparation, which required both ATP and CoA (see below).

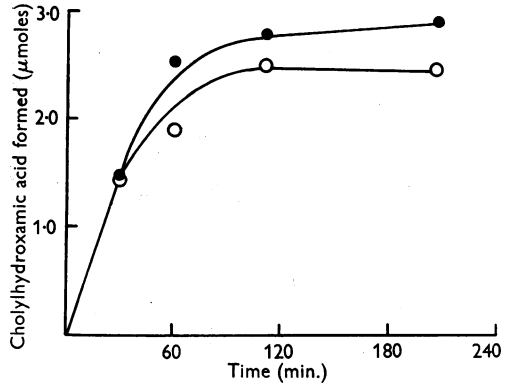

Fig. 1. Synthesis of cholylhydroxamic acid from cholic acid and hydroxylamine in the pig-liver preparation. ○, No ATP; ●, with ATP. Incubations were carried out at 37°. The complete system contained: 0·2 ml. of 0·5M glyoxaline buffer, pH 7·4 (at 37°); 0·3 ml. of 0·04M potassium ATP; 0·1 ml. of 0·1M-$MgSO_4$; 0·5 ml. of 2M-NH_2OH; 0·1 ml. of 0·05M potassium cholate; 0·1 ml. of 0·1M cysteine; pig-liver enzyme, 0·5 ml. The volumes were adjusted to 2·0 ml. with 1·15% KCl.

Fig. 1 shows the course of the reaction catalysed by the pig-liver enzyme; it also shows that the addition of ATP did not affect the initial rate of reaction, although it did increase by 18% the final amount of cholylhydroxamic acid formed. This effect of ATP has not been investigated further, but it would appear to be a secondary effect due perhaps to a slight change of pH or the removal of an inhibitor. The rapid reaction in the absence of ATP suggests that there is no direct participation of ATP. The reaction appears to be similar to that described by Lipmann & Tuttle (1950), in which the formation of hydroxamic acids from fatty acids and hydroxylamine was catalysed by a lipase in the absence of ATP and CoA. This interpretation is supported by the fact that the activity of the system is inhibited by fluoride, as is that of lipase (Lipmann, 1929), and that the pig-liver preparation forms a hydroxamic acid very rapidly from hexanoic acid.

Formation of hydroxamic acid by guinea-pig-liver microsomes. As mentioned above, a hydroxamic acid is synthesized from cholic acid by liver microsomes and the synthesis requires the presence of ATP and CoA (Elliott, 1955). The lipase-catalysed formation of cholylhydroxamic acid is relatively slight in guinea-pig-liver microsomes. As shown in Table 1, this latter reaction is almost completely inhibited by 0·15M-KF, which was therefore added in all subsequent experiments.

Table 1. *Effect of fluoride on the ATP-independent synthesis of cholylhydroxamic acid*

Complete incubation mixtures contained 0·2 ml. of 0·25M phosphate buffer, pH 7·4; 0·5 ml. of 2M-NH_2OH; 0·1 ml. of 0·04M-K cholate; 0·1 ml. of 0·3M-KF; 0·5 ml. of microsome suspension; total vol., 2·0 ml. Incubations at 37° for 1 hr.

Incubation mixture	Hydroxamic acid formed (μmoles)
Complete system	0·3
Same, without fluoride	0·6
Same, without cholic acid and fluoride	0·25

Measurement of activity. Incubations were carried out in centrifuge tubes at 37° for 80 min. The complete system contained the following: 0·2 ml. of 0·25M phosphate buffer, pH 7·4; 0·1 ml. of 0·1M-$MgSO_4$; 100 units of CoA; 0·25 ml. of 0·08M potassium ATP; 0·05 ml. of 0·2M cysteine; 0·1 ml. of 0·04M potassium cholate; 0·5 ml. of 2M-NH_2OH; 0·1 ml. of 0·3M-KF; 0·5 ml. of microsome suspension. The final volume was made up to 2·0 ml. with distilled water where necessary. These standard conditions were used throughout the following experiments, and only variations in the incubation mixtures will be described.

Identification of the hydroxamic acid. The formation of an hydroxamic acid in the microsome system is dependent on the addition of cholic acid, suggesting that cholylhydroxamic acid is the product of the reaction. To identify the hydroxamic acid, it was extracted from 20 ml. of a reaction mixture which had been incubated in an atmosphere of N_2. Control flasks in which ATP and CoA were lacking were simultaneously incubated. After 80 min. at 37° the reaction was stopped by addition of 5 ml. of 2N-HCl. This precipitated the protein and excess of cholic acid, which were then centrifuged off. The supernatants were neutralized with KOH and the solutions concentrated *in vacuo* to about 5 ml. They were then transferred quantitatively to evaporating dishes with a little water and finally

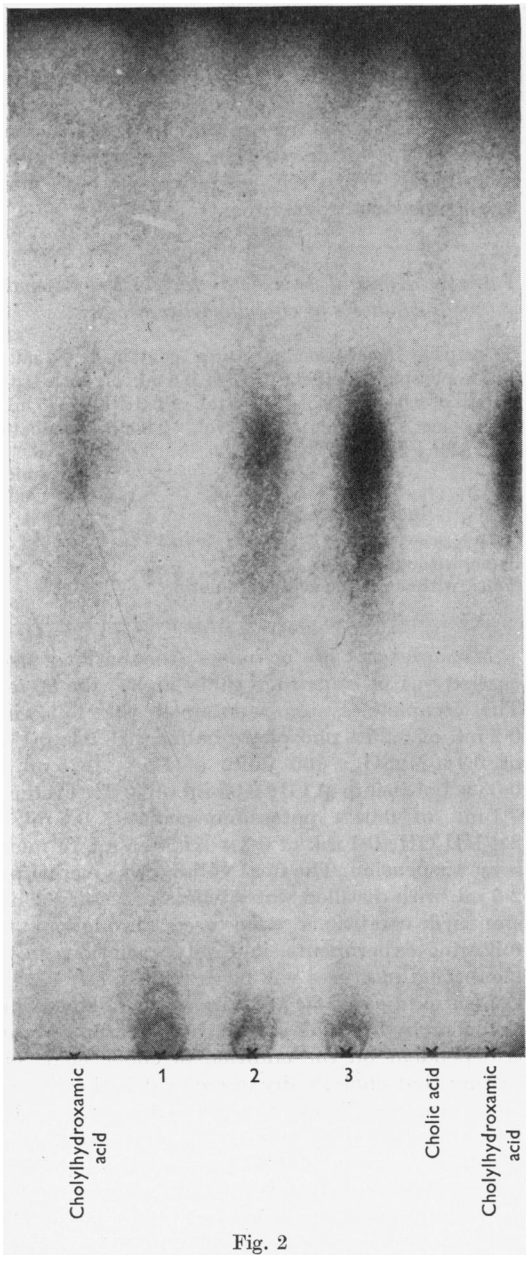

Fig. 2

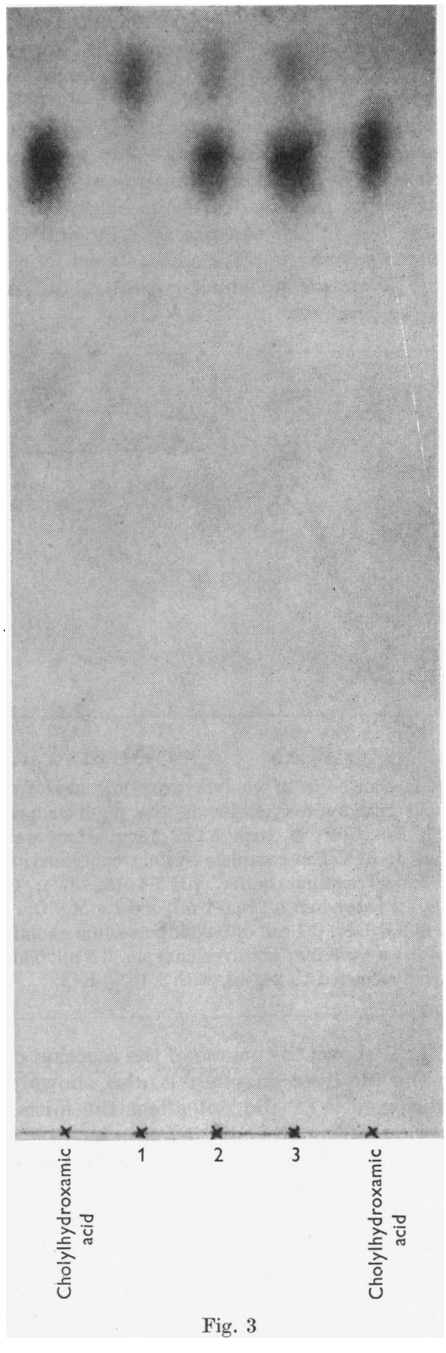

Fig. 3

Figs. 2, 3. Chromatography of enzymically synthesized hydroxamic acid. Chromatograms were sprayed with $FeCl_3$ reagent. Fig. 2 was obtained with the di*iso*propyl ether solvent and Fig. 3 with the butanol–acetone solvent. The marker is cholylhydroxamic acid prepared from ethyl cholate. (1), Control incubation without ATP and CoA; (2), complete incubation system; (3), (2) + cholylhydroxamic acid.

dried in a vacuum desiccator over P_2O_5 and NaOH. The dry solids were moistened with 0·3 ml. of water and extracted three times with 1 ml. lots of ethanol by grinding in small glass mortars. The white precipitates were centrifuged off and the supernatants applied to paper for chromatography. Usually 0·4 ml. was taken when the $FeCl_3$ spray was to be used. For the other more sensitive sprays 0·2 ml. was adequate. Figs. 2 and 3 show photographs of papers run in the di*iso*propyl ether and butanol–acetone solvent systems respectively. In each case the principal hydroxamic acid in the test system (2) corresponds in position with the marker spot of cholylhydroxamic acid synthesized from ethyl cholate. When some of the marker solution was added to the test system the main hydroxamic acid spot was reinforced (3). Cholylhydroxamic acid could not be detected in the control experiment (1). The spots at the solvent front in Fig. 3 have not been identified. It was found that the enzymically synthesized hydroxamic acid in (2) gave, in addition to the reaction with the $FeCl_3$ spray, the same rose-pink colour with $SbCl_3$ as did cholic acid and synthetic cholylhydroxamic acid. It was also found that, with the di*iso*propyl ether solvent, the spots reacted with phosphomolybdic acid to give a blue colour. With the butanol–acetone solvent this spray was not suitable because of the high background colour in the region of the spots.

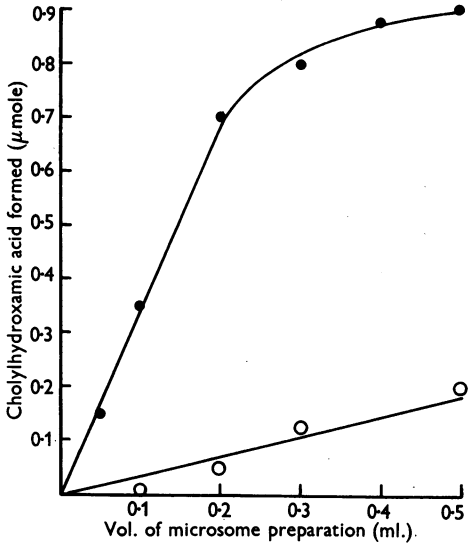

Fig. 4. Effect of volume of microsome preparation on hydroxamic acid synthesis. ●, Complete system; ○, no cholic acid. The values in the upper curve have been corrected for the control values obtained in the absence of cholic acid. In plotting the lower curve the initial blank (0·15 μmole) has been subtracted.

Properties of the cholic acid-activating system

Effect of enzyme concentration. Fig. 4 shows the relationship between hydroxamic acid formation and the volume of microsome suspension added. The curve is linear only at low enzyme concentrations. This is probably due to the fact that the final reaction, that between cholyl-CoA and hydroxylamine, becomes rate-limiting.

Effect of ATP concentration. This is shown in Fig. 5. Half-maximal rates are obtained with an ATP concentration of $1·5 \times 10^{-3}$ M. A concentration of 5×10^{-3} M is required for full activity.

Effect of cholic acid concentration. Fig. 6 shows that the Michaelis constant K_m of the enzyme

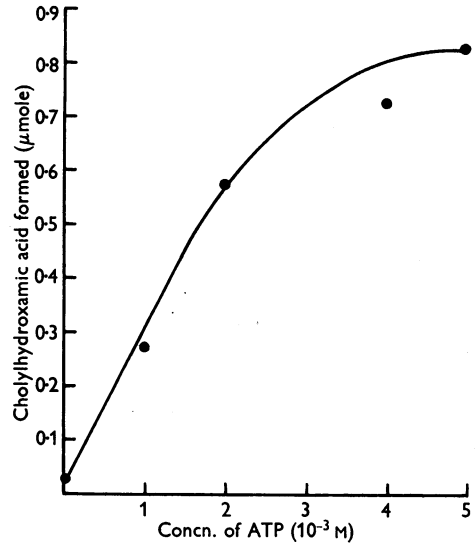

Fig. 5. Effect of ATP concentration on cholylhydroxamic acid synthesis. The curve is corrected for blank values obtained at each ATP concentration in the absence of cholic acid.

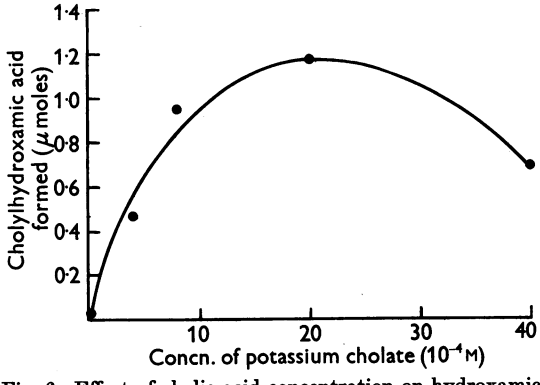

Fig. 6. Effect of cholic acid concentration on hydroxamic acid synthesis. The curve is corrected for blank values obtained at each cholic acid concentration in the absence of ATP.

towards cholic acid is approximately 4×10^{-4}. The optimum concentration is at 2×10^{-3} M. Above this the substrate inhibits, the rate of reaction at 4×10^{-3} M being only 58 % of the optimum rate. The inhibition at higher concentrations of cholic acid may be related to its detergent action.

Effect of CoA concentration. Fig. 7 shows the effect of CoA on the synthesis of hydroxamic acid. The lower curve shows that the system is almost saturated with the pig-liver preparation of CoA at a concentration of 50 units/ml. The upper curve,

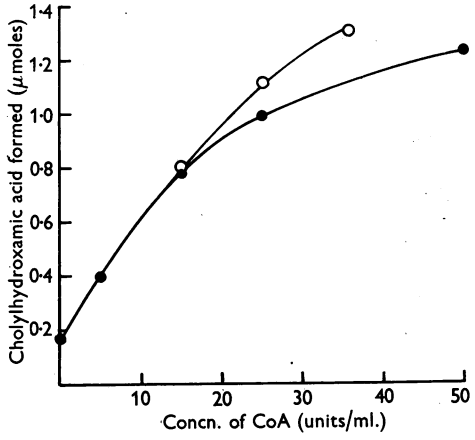

Fig. 7. Effect of CoA concentration on cholylhydroxamic acid synthesis. The curves are corrected for the blank obtained in the presence of 50 units of the 13 % pure CoA but in the absence of ATP. ●, 13 % pure CoA used; ○, 70 % pure CoA used.

which was obtained for a 70 % pure preparation of CoA, shows that the active component in the 13 % pure preparation is in fact CoA. The activity of the two samples is identical at low concentrations on the basis of units of CoA; with a concentration of 30 units/ml. the 70 % pure CoA gave an activity 16 % higher than that given by the crude preparation. This effect may be due to the presence of inhibitory substances in the crude preparation.

Effect of hydroxylamine concentration. This is shown in Fig. 8. It is clear that hydroxylamine concentration is limiting even when it is present at a concentration of 0·5 M.

Activation by metals. The activity of the microsome preparation can be increased by the addition of either Mg^{2+} or Mn^{2+}. Table 2 shows that the requirement for these is not absolute. In the absence of added metal the activity was 53 % of the maximum obtained in the presence of 0·02 M-$MgSO_4$. The activity in the presence of the latter is slightly higher than that in the presence of 0·01 M-$MnSO_4$. Mn^{2+} has no inhibitory action even at high concentrations. Ethylenediaminetetraacetic acid, added as the potassium salt at a concentration of 0·01 M, gave an inhibition of 33 % in the presence of 0·05 M-$MgSO_4$.

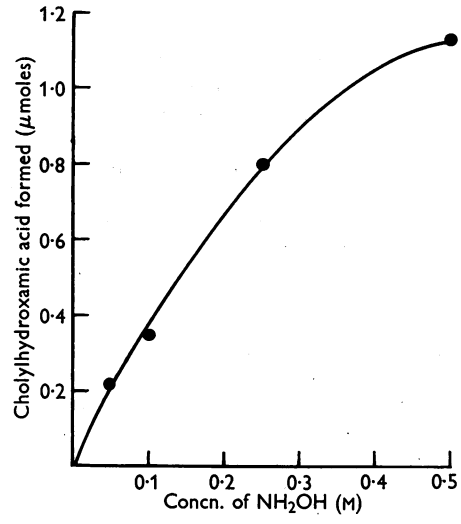

Fig. 8. Effect of hydroxylamine concentration on cholylhydroxamic acid formation. The curve is corrected for the blank obtained with 0·5 M-NH_2OH in the absence of ATP.

Table 2. *Effect of* Mn^{2+} *and* Mg^{2+} *on hydroxamic acid synthesis*

Values are corrected for the blank value in the absence of cholic acid. The metals were added as sulphates.

Metal	(10^{-4} M concn.)	Cholylhydroxamic acid formed (μmole)
None	—	0·45
Mn	5	0·65
Mn	10	0·72
Mn	20	0·80
Mn	40	0·80
Mn	100	0·80
Mg	10	0·72
Mg	20	0·82
Mg	100	0·80
Mg	200	0·85

Table 3. *Hydroxamic acid synthesis by the soluble fraction of guinea-pig liver*

Complete incubation system, as described in text. All values are expressed as cholylhydroxamic acid.

	Hydroxamic acid (μmole)
Complete system	0·5
Same, without cholate	0·57
Same, without ATP	0·3
Same, without cholate and ATP	0·4

Requirement for cysteine. Cysteine is necessary for full activity, presumably to maintain CoA in the reduced form. The requirement is not absolute, since the activity in the absence of cysteine is 45% of that in its presence.

Distribution of the enzyme in the liver fractions. Activity has been conclusively observed only in the microsome fraction. None was present in mitochondria. With the soluble fraction (Table 3) hydroxamic acid is formed from endogenous substrates; this formation is inhibited by cholic acid. The results obtained with this enzyme system do not indicate an activation of cholic acid.

DISCUSSION

The results suggest that the following reactions take place in liver microsomes:

(1) cholic acid + ATP + CoA → cholyl-CoA,

(2) cholyl-CoA + hydroxylamine → cholylhydroxamic acid + CoA.

Reaction 1 is probably the only enzyme-catalysed step in the synthesis of cholylhydroxamic acid. The final step in the reaction, that between cholyl-CoA and hydroxylamine, is most likely non-enzymic in nature and consequently high concentrations of the trapping agent are required to give a reasonably fast rate of reaction. The nature of the breakdown of ATP in reaction 1 has not yet been investigated.

Since this work was completed Siperstein (1955) has reported that CoA stimulates the synthesis of taurocholic acid in rat-liver microsomes plus the soluble fraction of liver.

The next paper (Elliott, 1956) will describe the participation of CoA in taurocholic acid synthesis in guinea-pig-liver microsomes.

SUMMARY

The synthesis of cholylhydroxamic acid by liver preparations has been demonstrated. This has been found to be due to two distinct mechanisms. The first is a simple condensation of cholic acid and hydroxylamine, and is probably lipase-catalysed. The second occurs in the microsome fraction of guinea-pig liver and requires the presence of CoA, ATP, cysteine and Mg^{2+} or Mn^{2+} for full activity. The properties of this cholic acid-activating system are described.

I wish to thank Mr K. M. Jones for useful criticisms and Dr D. C. Torpy for help in the preparation of CoA. I also wish to thank Miss Janet Payne for valuable technical assistance.

REFERENCES

Cortese, F. (1937). *J. Amer. chem. Soc.* **59**, 2532.
Elliott, W. H. (1955). *Biochim. biophys. Acta*, **17**, 440.
Elliott, W. H. (1956). *Biochem. J.* **62**, 433.
Hill, U. T. (1947). *Analyt. Chem.* **19**, 932.
Kornberg, A. & Pricer, W. E. (1953). *J. biol. Chem.* **204**, 329.
Kritchevsky, D. & Kirk, M. R. (1952). *Arch. Biochim. Biophys.* **35**, 346.
LePage, G. A. & Mueller, G. C. (1949). *J. biol. Chem.* **180**, 975.
Lipmann, F. (1929). *Biochem. Z.* **206**, 171.
Lipmann, F. & Tuttle, L. C. (1950). *Biochim. biophys. Acta*, **4**, 301.
Needham, D. M. (1942). *Biochem. J.* **36**, 113.
Peel, J. L. (1951). *Biochem. J.* **49**, 62.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Siperstein, M. D. (1955). *Fed. Proc.* **14**, 282.
Siperstein, M. D., Harold, F. M., Chaikoff, I. L. & Dauben, W. G. (1954). *J. biol. Chem.* **210**, 181.
Stadtman, E. R. & Kornberg, A. (1953). *J. biol. Chem.* **203**, 47.

The Enzymic Synthesis of Taurocholic Acid: A Qualitative Study

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(*Received* 28 *July* 1955)

Cholylhydroxamic acid has been found to be synthesized from cholic acid in the presence of adenosine triphosphate (ATP), hydroxylamine, coenzyme A (CoA) and Mg^{2+} by preparations of guinea-pig-liver microsomes (Elliott, 1955, 1956). It seemed likely that cholyl-CoA would prove to be an intermediate in the synthesis of taurocholic acid from cholic acid and taurine. This has been investigated, and the present publication describes evidence which supports this.

While this work was in progress several reports appeared on the synthesis of conjugated bile acids. Bergström & Gloor (1954) reported the synthesis of taurocholic acid in rat-liver slices and homogenates. More recently, Bremer (1955) has described the production of conjugated bile acids in the