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INHIBITION OF BILIARY SECRETION BY ICTEROGENIN AND RELATED TRITERPENES—I.

EFFECT OF ICTEROGENIN, REHMANNIC ACID AND OLEANOLIC ACETATE ON LIVER MITOCHONDRIA *IN VITRO*

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Abstract—The effect of Icterogenin, Rehmannic acid and oleanolic acetate on respiration, mitochondrial structure and oxidative phosphorylation in rabbit and rat liver mitochondria was studied.

All three substances at a concentration 1×10^{-4} M and 5×10^{-5} M inhibit the respiration of NAD-linked enzymes. Only Icterogenin in the concentration 1×10^{-5} M increased the respiration rate. The succinoxidase activity was inhibited by Rehmannic acid and oleanolic acetate. Icterogenin at the concentrations 1×10^{-4} M and 5×10^{-5} M did not cause any definite changes in respiration with succinate as substrate, but at the concentration 1×10^{-5} M the respiration was always increased.

The substances were found to increase the mitochondrial membrane permeability and Icterogenin had a swelling effect of the large amplitude type.

The oxidative phosphorylation was uncoupled by Icterogenin and Rehmannic acid and inhibited by oleanolic acetate. Rehmannic acid at a concentration 5×10^{-5} M almost completely abolished the phosphate uptake when glutamate was used as substrate. The effect of Icterogenin was less. The respiration with glutamate as substrate was inhibited 50 per cent by the former and to about 25 per cent by the latter. With succinate as substrate, both Rehmannic acid and Icterogenin were potent uncouplers, abolishing the phosphate uptake, the respiration being inhibited by the former and increased by the latter.

The connection between chemical structure and effect on oxidative phosphorylation is discussed.

THE ISOLATION and crystallization of pure Icterogenin from the South African plant Lippia Rehmanni was reported in 1937 by Rimington, Quin and Roets.[1] Icterogenin, a pentacyclic triterpene acid, is known to cause biliary retention, photosensitization and jaundice in sheep without gross visible damage to the liver cells.[2] The light sensitivity is thought to depend on accumulation in the serum of a porphyrin phylloerythrin, normally eliminated in the bile.[3,4] Other effects of Icterogenin reported are decreased ruminal movements and stasis in the large intestines in the sheep.[4] *In vitro* it has been found to inhibit the contractions of the isolated small intestines of rabbits and sheep. Low concentrations allowed recovery by substitution of fresh incubation fluid. In higher concentrations the inhibition was permanent, the muscle remaining fully relaxed. Perfusion experiments with rabbit hearts caused decrease in amplitude and

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inhibition of the heart beat. Further, Icterogenin possesses strong hemolytic properties *in vitro*.[1]

The typical picture, decline in bile flow and bilirubin content per unit of time and the negligible effect on the liver revealed by light microscopy examination, is also shown by the triterpenes 22β-angeloyloxyoleanolic acid and 22β-angeloyloxy-24-hydroxyoleanolic acid isolated from Lippia Rehmanni.[5,6] The partially synthetic compounds 22β-angeloyloxyhedragolic acid and 22β-angeloyloxy-24-oxo-oleanolic acid produced similar symptoms.[7] Considering the structural formulae of these compounds the activity was found to be based upon the presence of a 22β-angeloyl side chain and a β-equatorial hydroxyl group at either C 3 or C 24 on the triterpene molecule. Increase in icterogenic activity is associated with shift of the hydroxyl from C24 to C3.[7]

Among other triterpenes tested, especially the sodium salt of Rehmannic acid, 22β-angeloyloxyhedragonic acid[8] and the α-axial epimer of 22β-angeloyloxyoleanolic acid[7] were found to depress the bile flow to a marked extent, without any effect on the bile bilirubin content. A similar but weak action was caused by several triterpenes tested e.g. Oleanolic acid. The mechanism by which Icterogenin and substances with a similar action interfere with biliary secretion is not known. Evidence has been provided that interference with secretion occurs both with bilirubin and bromsulphalein normally conjugated and with coproporphyrin and phylloerythrin apparently secreted in the free form. The inhibition cannot therefore be solely due to interference with the conjugating mechanism.[9] Not only is the secretion inhibited but prevention of uptake by the liver of injected coproporphyrin during the height of the Icterogenin effect has been reported.[10]

Evidence that biliary secretion is dependent on the metabolic activity of the liver cells has been provided,[11–14] and that secretion is partly if not mostly an active transfer[15,13] utilizing energy. Such processes are known to be driven by breakdown of energy-rich phosphate bonds, such as those of adenosine triphosphate (ATP). The energy is released from ATP by the action of adenosinetriphosphatase (ATPase). Some ATP is generated during glycolysis but the major source is the oxidative phosphorylation. In view of this it was considered possible that Icterogenin and substances with a similar action may in some way affect the energy mechanism required for transport and therefore inhibit biliary secretion. The localization of the main energy production to the mitochondria as the seat of oxidative phosphorylation was the reason for directing the study to these particles.

MATERIALS AND METHODS

Mitochondria from rabbit and rat livers were obtained by differential centrifugation of the liver homogenate in 0·25 M sucrose.

Male rabbits fasted for 24 hr, weighing about 2 kg. were killed by cervical dislocation, the liver rapidly removed and immersed in ice-cold 0·25 M sucrose. The homogenization was performed for 2 min in a glass homogenizer with a nylon pestle, clearance 7/1000 in., to give a 10% homogenate. The supernatant obtained for separation of mitochondria was obtained by centrifuging at 1000 *g* in an MSE Major centrifuge at 0° for 10 min. Sedimentation of mitochondria was performed in an MSE refrigerated centrifuge at 10,000 *g* for 10 min, resuspension being carried out once and recentrifugation being performed at 13,000 *g* for 10 min. The mitochondria were

finally suspended in 0·25 M sucrose to give about 0·7 mg N/0·5 ml. Nitrogen determinations were carried out by Micro-Kjeldahl.

Rats of Wistar strain were used throughout the investigation and the mitochondrial preparation was carried out as above, but the first centrifugation was performed at 600 *g* for 10 min.

Respiration was determined according to Warburg's direct method.[16] The standard medium used had a final concentration of adenosinetriphosphate (ATP) 1·7 mM, $MgSO_4$ 6·7 mM, KCl 25 mM, sodium orthophosphate buffer pH 7·4, 16·7 mM, cytochrome C (Sigma type II) 0·14 mM, substrate 10 mM except for succinate 33 mM and octanoate 16 mM. L-malate as primer for fatty acids and pyruvate was added to a final concentration of 1 mM. The volume was 3 ml plus 0·2 ml NaOH in the centre well, gas phase air, and temperature 37°.

Oxidative phosphorylation was assayed using the same standard medium with the addition of hexokinase (sigma practical) 400 Kunitz units and a final concentration of glucose 30 mM.

Mitochondrial respiratory control was determined by omitting hexokinase and glucose from the medium. Fresh mitochondria had a control ratio between 3–4 indicative of coupling i.e. inhibited respiration in absence of a phosphate acceptor. This inhibition is released in presence of a phosphate acceptor, an uncoupler or structural disorganization of the mitochondria.

Mitochondrial membrane permeability was tested by the method of Christie and Judah.[17]

Mitochondrial swelling was measured according to Jeffrey and Smith.[18]

The triterpenes were kindly provided by Professor C. Rimington.

Icterogenin (Fig. 1) and Rehmannic acid (Fig. 2) were crystallized from ethanol. Oleanolic acetate was prepared by the method of Halsall, Jones and Swayne[19] from oleanolic acid (Fig. 3) and crystallized from ethanol. The substances were dissolved in twice distilled ethanol, pH adjusted to 7·4 and the additions used were 50 μl.

Crystalline sodium glutamate was a gift from Dr. K. R. Rees.

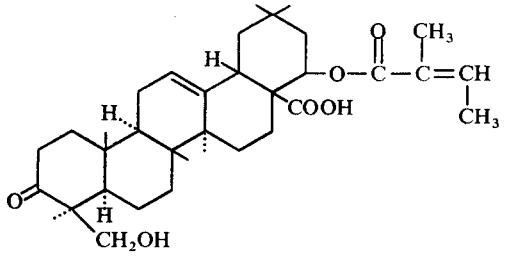

FIG. 1.

Crystalline sodium pyruvate was prepared according to Robertson,[20] sodium succinate according to Gallagher[21] and *l*-malic acid recrystallized according to the method in Colowick and Kaplan.[22]

To the chilled Warburg flasks containing the standard medium the substrates and the triterpenes, 0·5 ml of the mitochondrial suspension was added and the flasks were preincubated in the cold for 15 min. The reason for using preincubation in *in vitro* experiments was to display latent capacities in the mitochondria. In respiration

experiments the oxygen uptake was measured for 40 min after a 10 min thermoequilibration and the results expressed as QO_2 N = μl O_2 taken up per mg mitochondrial N/hr.

In oxidative phosphorylation measurements the hexokinase and glucose were present during a 7 min thermoequilibration and the oxygen uptake was measured for 15–20 min.

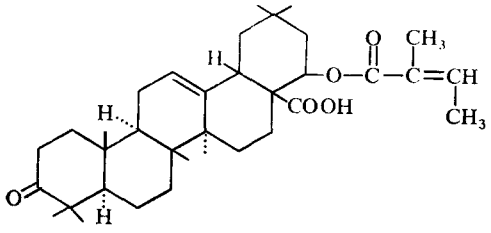

FIG. 2.

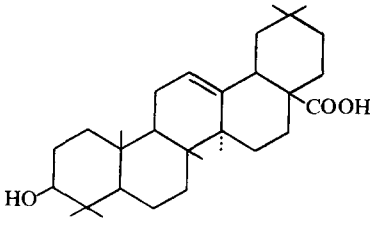

FIG. 3.

The reaction was stopped by adding 2 ml of cold 20% trichloroacetic acid. In order to measure net phosphate uptake identical control flasks were used, in which the reaction was stopped at the end of thermoequilibration. The flasks were kept at 0° until the inorganic phosphorus was measured according to Fiske and Subbarow.[23] The difference in inorganic phosphate content between the experimental and control flasks was considered as net phosphate uptake.

The experiments were performed with mitochondria from 2–3 different animals, all experiments showed the same qualitative tendencies. One illustrative experiment is presented.

RESULTS

The following compounds were tested for their effect on the mitochondria; Icterogenin having the structure required for decline in bile flow and bilirubin content, Rehmannic acid having the angeloyloxy side chain, but lacking the β equatorial hydroxyl group, causing only depression of the bile flow and oleanolic acetate lacking the angeloyloxy side chain but having a β equatorial hydroxyl group esterified with acetic acid, believed to affect the bile flow to about the same extent as oleanolic acid.

Icterogenin in a final concentration 1×10^{-4} M inhibited respiration *in vitro* by liver mitochondria from normal rabbits with NAD-linked enzymes (Table 1) whereas the concentration 1×10^{-5} M caused a slight increase in respiration rate. As opposed to the NAD-linked enzymes, the succinoxidase system showed slight or no inhibition or even an increased respiration rate at the concentration 1×10^{-4} M and 5×10^{-5} M, whereas the concentration 1×10^{-5} M always caused a remarkable increase in respiration rate. The glutamate and succinate experiments were performed with

mitochondria 5-hr-old wherefore some permeability changes might have occurred. The preincubation used might in fact have served to bring about good succinoxidase activity. However, Icterogenin added to fresh mitochondria caused a greater increase in succinoxidase activity. When swollen mitochondria were tested the succinoxidase activity was inhibited with all three concentrations of Icterogenin. Similar effects by Icterogenin and Rehmannic acid upon respiration and oxidative phosphorylation have been found when using an oxygen electrode assembly at room temperature instead of the Warburg manometer (T. A. J. Heikel and N-E. Saris, to be published).

TABLE 1. EFFECT OF ICTEROGENIN ON THE RESPIRATION OF MITOCHONDRIA FROM RABBIT LIVER

System and level of substrates used as described in Methods. $QO_2 = \mu l\ O_2$/mg N/hr. The figures in brackets indicate the value in per cent of the controls. Mitochondrial N approx. 0·7 mg; for succinate approx. 0·35 mg.

Substrate	Substance tested		QO_2
Pyruvate		nil	174
		ethanol	227 (100)
	1×10^{-5} M	Icterogenin	281 (124)
	5×10^{-5} M		66 (29)
	1×10^{-4} M		5 (2)
Citrate		nil	249
		ethanol	266 (100)
	1×10^{-5} M	Icterogenin	280 (105)
	5×10^{-5} M		98 (37)
	1×10^{-4} M		20 (8)
Glutamate*		nil	212
		ethanol	228 (100)
	1×10^{-5} M	Icterogenin	236 (102)
	5×10^{-5} M		51 (22)
	1×10^{-4} M		34 (15)
Succinate*		nil	448
		ethanol	514 (100)
	1×10^{-5} M	Icterogenin	807 (157)
	5×10^{-5} M		443 (86)
	1×10^{-4} M		426 (83)
Succinate*		nil	404
		ethanol	375 (100)
	1×10^{-5} M	Icterogenin	708 (189)
	5×10^{-5} M		665 (177)
	1×10^{-4} M		552 (147)

* Mitochondria 5-hr-old.

Ethanol used in the concentration necessary to dissolve the triterpenes was in general not inhibitory, rather a slight increase in respiration rate was observed presumably due to damage of the mitochondrial membrane.

Rehmannic acid tested under the same conditions caused, like Icterogenin, inhibition of NAD-linked citrate respiration (Table 2), but no rise in respiration rate was found at a concentration 1×10^{-5} M. Other NAD-linked enzymes behaved similarly. Contrary to experience with Icterogenin the succinoxidase was clearly inhibited.

The experiments with oleanolic acetate resemble those with Rehmannic acid (Table 2). The inhibition of citrate oxidation and other NAD-linked enzymes was less

than that recorded in the experiments with Rehmannic acid but the effect on succinoxidase was of the same order; no rise in respiration rate was observed.

The inhibition of respiration is dependent on the concentration of the materials used as well as the amount of mitochondria present (Table 3). Further it is influenced by the preincubation as in preliminary experiments without preincubation in the cold of the triterpenes with the mitochondria, inhibition was about 20 per cent less than with preincubation. In these experiments the respiration was limited by endogenous acceptors of phosphate and the increase produced by Icterogenin could be due to uncoupling.

TABLE 2. EFFECT OF REHMANNIC ACID AND OLEANOLIC ACETATE ON THE RESPIRATION OF MITOCHONDRIA FROM RABBIT LIVER

Conditions as in Table 1.

Substrate	Substance tested		QO_2
Citrate		nil	249
		ethanol	266 (100)
	1×10^{-5} M	Rehmannic acid	263 (99)
	5×10^{-4} M		93 (35)
	1×10^{-4} M		48 (18)
	1×10^{-5} M	Oleanolic acetate	267 (100)
	5×10^{-5} M		200 (75)
	1×10^{-4} M		104 (39)
Succinate*		nil	448
		ethanol	514 (100)
	1×10^{-5} M	Rehmannic acid	362 (70)
	5×10^{-5} M		79 (15)
	1×10^{-4} M		48 (9)
	1×10^{-5} M	Oleanolic acetate	429 (83)
	5×10^{-5} M		40 (8)
	1×10^{-4} M		29 (6)

* Mitochondria 5 hr old.

TABLE 3. EFFECT OF CONCENTRATION OF RAT LIVER MITOCHONDRIA ON THE INHIBITION OF RESPIRATION CAUSED BY ICTEROGENIN

Conditions as described in Methods. Malate was used as substrate. Icterogenin in a final concentration of $4{\cdot}1 \times 10^{-5}$ M.

Substance tested	QO_2 m = 0·8 mg N	QO_2 m = 0·4 mg N
ethanol	188 (100)	180 (100)
$4{\cdot}1 \times 10^{-5}$ M Icterogenin	96 (51)	15 (8)

Since the inhibition of mitochondrial respiration with NAD-linked enzymes by the triterpenes and the increase in succinoxidase activity caused by Icterogenin could be due to an effect on the mitochondrial membrane, this possibility was further investigated. The fact that intact mitochondria catalyse neither the reduction of external NAD nor the oxidation of external $NADH_2$[24] provided a method for investigation of this problem. With l-malate as substrate and mitochondria in 0·25 M sucrose as enzyme system it was clearly shown that Icterogenin in the concentrations 5×10^{-5} M and

1×10^{-5} M greatly accelerates the rate of increase of extinction at 340 mμ (Fig. 4) Ethanol used to dissolve the Icterogenin did not influence the rate. Icterogenin itself was without effect on the extinction at 340 mμ. The effect of Rehmannic acid and oleanolic acetate is shown in Figs. 5 and 6.

In an experiment where the mitochondrial membrane barrier had been removed by using mitochondria suspended in water the rate of l-malate oxidation was accelerated and addition of Icterogenin did not noticeably increase this rate. This experiment was repeated with frozen and thawed mitochondria which gave the same result.

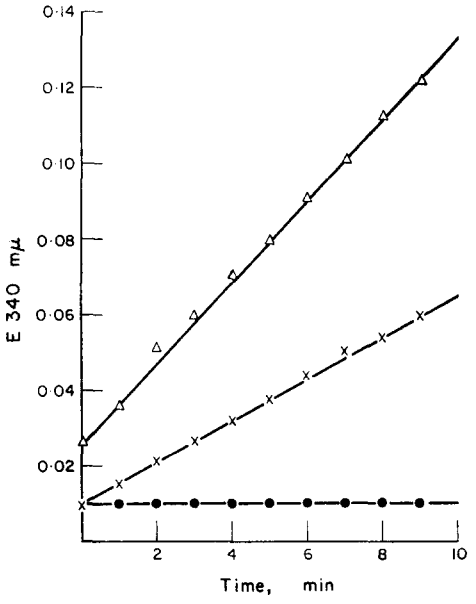

FIG. 4. Reduction of NAD by rat liver mitochondria in 0·25 M sucrose. Conditions as described in Methods. 50 μl mitochondria corresponding to 3·6 μg N. NAD in a final concentration of 1 mM. Gas phase air, temperature 20°.
—— Complete system + ethanol; ●—● Complete system, Icterogenin 1×10^{-5}, no *l*-malate; ×—× Complete system Icterogenin 1×10^{-5} M; △—△ Complete system Icterogenin 1×10^{-5} M

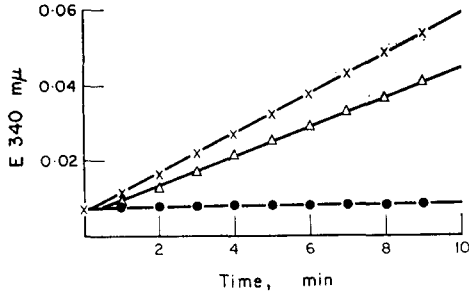

FIG. 5. Reduction of NAD by rat liver mitochondria in 0·25 M sucrose. Conditions as in Fig. 4. 50 μl mitochondria corresponding to 2·16 μg N.
—— Complete system + ethanol; ●—● Complete system, Icterogenin 1×10^{-5} M, no *l*-malate; ×—× Complete system Icterogenin 1×10^{-5} M; △—△ complete system Rehmannic acid 1×10^{-5} M.

The membrane experiments give evidence for that the triterpenes eject internal bound NAD, allowing externally added NAD to occupy the site where it can be reduced. It is, therefore, likely that addition of NAD to some extent might have stimulated the inhibited respiration of NAD-linked enzymes. This possibility has not been tested. respiration with fresh mitochondria with pyruvate and glutamate as substrates was not affected by the addition of NAD to a final concentration of 1 mM.

As the triterpenes used were found to have an effect on the mitochondrial membrane, their effect on swelling was tested. Only Icterogenin was found to have a clear effect indicative of swelling of the large amplitude type (Fig. 7).

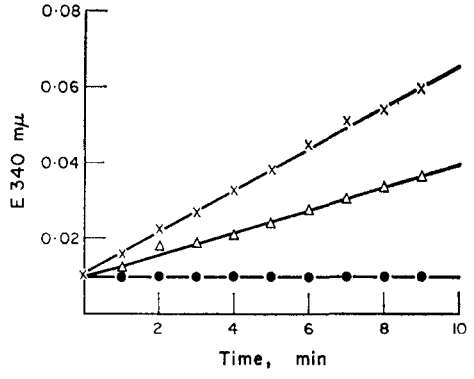

FIG. 6. Reduction of NAD by rat liver mitochondria in 0·25 M sucrose. Conditions as in Fig. 4. —— Complete system + ethanol; ●—● complete system, Icterogenin 1 × 10^{-5} M, no *l*-malate; ×—× complete system, Icterogenin 1 × 10^{-5} M; △—△ complete system, oleanolic acetate 1 × 10^{-5} M.

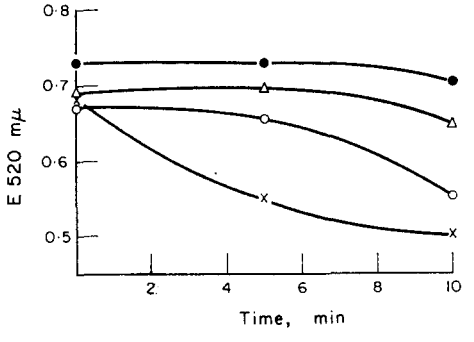

FIG. 7. Effect of Icterogenin, Rehmannic acid and oleanolic acetate on o.d. of rat liver mitochondria. Conditions as described in Methods. Mitochondria 50 μl corresponding to 0·063 mg N. ○—○ Blank containing ethanol; ×—× Icterogenin 1 × 10^{-4} M; ●—● Rehmannic acid 1 × 10^{-4} M; △—△ Oleanolic acetate 1 × 10^{-4} M.

It has thus been shown that the triterpenes tested have an effect on the mitochondrial membrane and this makes an effect on oxidative phosphorylation likely. When glutamate was used as substrate Icterogenin in the concentration 5 × 10^{-5} M depressed the phosphate uptake to about 50 per cent. With half the quantity of mitochondria phosphate uptake was virtually abolished by the above concentration of Icterogenin.

The effect of lower concentrations was less. The respiration was slightly inhibited or even increased (Table 4). Rehmannic acid at the concentration 5×10^{-5} M abolished almost completely the phosphate uptake, the respiration being inhibited to about 50 per cent. In the concentration 1×10^{-6} M only a small increase in respiration was observed (Table 4).

Oleanolic acetate at a concentration 5×10^{-5} M depressed both oxygen and phosphate uptake, the latter more markedly, lowering the P/O to 1·7.

Oxidative phosphorylation with succinate as substrate revealed that both Icterogenin and Rehmannic acid at a concentration 5×10^{-5} M nearly abolished the phosphate uptake. The respiration in presence of Icterogenin was increased, that with Rehmannic acid was inhibited to about 50 per cent.

Again, oleanolic acetate caused but a slight depression of the P/O ratio, the inhibition of the phosphate uptake being slightly higher than that of the respiration (Table 4).

The stronger effect on oxidative phosphorylation by Rehmannic acid compared to that of Icterogenin occurred both when glutamate and succinate were used as substrates and this excludes a substrate-level phosphorylation connected with glutamate as the reason for the difference. Both Rehmannic acid and Icterogenin uncouple all three phosphorylation sites, the extent of uncoupling depends on the concentration of triterpene and on the mitochondrial concentration.

DISCUSSION

Evidence has been provided that Icterogenin and Rehmannic acid uncouple oxidative phosphorylation. Further it has been shown that Rehmannic acid, especially, and to a slight extent Icterogenin in higher concentrations have a depressing effect on the respiration connected with phosphorylation, these substances thus acting as inhibitory uncouplers, i.e. depressing the phosphorylation more than respiration.[25] Oleanolic acetate was found only to inhibit the oxidative phosphorylation. Evidence is also afforded that the triterpenes affect the mitochondrial membrane permeability and that Icterogenin exerts a definite swelling effect of the large amplitude type.

Considering the swelling effect of Icterogenin a structural disorganization of the mitochondria seems likely. Disorganization of the mitochondrial structure would cause hydrolytic cleavage of an energy-rich intermediate, resulting in uncoupling of oxidative phosphorylation and activation of ATPase activity which in this case is Mg^{2+} dependent.[26] The disorganization brought about by Icterogenin will mask any possible uncoupling effect of the substance itself. In comparison with the effects produced by Rehmannic acid (uncoupling in the absence of swelling), such a possibility seems likely. Rehmannic acid is a potent uncoupler of oxidative phosphorylation, more so than is Icterogenin. The differences in effect on oxidative phosphorylation shown by the triterpenes tested are likely to be due to structural differences. The absence of an uncoupling effect by oleanolic acetate is likely to be due to lack in this molecule of the unsaturated angeloyloxy side chain, which seems to be of great importance. It appears furthermore that substitution of a methyl group for a hydroxymethyl group increases the effect on oxidative phosphorylation as is evidenced by Rehmannic acid. All three substances are lipid soluble and the lipid solubility of most uncouplers has been pointed out and they have been shown to act in the lipid phase of mitochondria.[27]

While it has been proposed that dinitrophenol, the classical uncoupler of oxidative phosphorylation, causes hydrolysis of an energy-rich intermediate not containing phosphate, a phosphate intermediate has been assumed to be sensitive to ageing.[26] Since the action of Icterogenin in causing instant hemolysis resembles that of surface active compounds provoking ageing it could probably be visualized that Icterogenin promotes hydrolysis of an energy-rich phosphate intermediate. Whether the hydrolysis

TABLE 4. EFFECT OF ICTEROGENIN, REHMANNIC ACID AND OLEANOLIC ACETATE ON OXIDATIVE PHOSPHORYLATION IN RAT AND RABBIT LIVER MITOCHONDRIA

1. Effect of Icterogenin on oxidative phosphorylation in rat liver mitochondria.
Systems as described in Methods. Glutamate was used as substrate. Mitochondria corresponding to 0·7 mg N. The figures in brackets indicate the value in per cent of the controls.

Substance tested		μ atoms O	μmole P	P/O
	ethanol	9·9 (100)	21·4 (100)	2·2
1×10^{-6} M	Icterogenin	11·1 (112)	20·8 (97)	1·9
$2{\cdot}5 \times 10^{-6}$ M		10·7 (108)	20·0 (93)	1·9
1×10^{-5} M		10·2 (103)	16·4 (77)	1·6
5×10^{-5} M		8·8 (89)	10·0 (47)	1·1

2. Effect of Icterogenin, Rehmannic acid and oleanolic acetate on oxidative phosphorylation in rabbit and rat liver mitochondria.
Conditions as above.

Substance tested		μ atoms O	μmole P	P/O
	ethanol	8·9 (100)	19·4 (100)	2·2
1×10^{-6} M	Icterogenin	9·3 (104)	19·2 (99)	2·1
5×10^{-5} M		6·0 (67)	5·9 (30)	1·0
	ethanol	9·0 (100)	21·6 (100)	2·4
1×10^{-6} M	Rhemannic acid	10·4 (116)	21·7 (100)	2·1
5×10^{-5} M		4·4 (49)	1·6 (7)	0·4
	ethanol	13·2 (100)	27·0 (100)	2·0
5×10^{-5} M	Oleanolic acetate	9·7 (74)	16·4 (61)	1·7

* P/O value with fresh mitochondria 1·9–2·3.

3. Effect of Icterogenin, Rehmannic acid and oleanolic acetate on oxidative phosphorylation in rabbit and rat liver mitochondria.
Conditions as above. Succinate was used as substrate (mitochondrial N approx. 0·35 mg)

Substance tested		μ atoms O	μmole P	P/O
	ethanol	14·5 (100)	18·2 (100)	1·3
1×10^{-6} M	Icterogenin	15·2 (105)	17·5 (96)	1·2
5×10^{-5} M		16·2 (112)	2·7 (15)	0·2
	ethanol	8·6 (100)	9·2 (100)	1·1
1×10^{-6} M	Rehmannic acid	9·5 (110)	6·4 (70)	0·7
5×10^{-5} M		4·4 (51)	0·0 (0)	0·0
	ethanol	8·1 (100)	11·7 (100)	1·4
1×10^{-6} M	Oleanolic acetate	8·1 (100)	10·8 (92)	1·3
5×10^{-5} M		4·0 (49)	4·0 (34)	1·0

* P/O value with fresh mitochondria 1·2–1·5.

of a phosphate energy-rich intermediate or one not containing phosphate is promoted, in either case uncoupling and activation of ATPase activity occurs.

In the first stage of action of the triterpenes the oxidative phosphorylation becomes uncoupled due to hydrolysis of an energy-rich intermediate. Higher concentrations or prolonged treatment inhibit the respiration connected with phosphorylation. The inhibition of NAD-linked enzymes could probably depend on ejection of internal bound NAD. The pyridine nucleotide dependent dehydrogenases would secondarily be inhibited by lack of their electron acceptors. However, the inhibition of succinate oxidation especially, by Rehmannic acid, requires an explanation. The possible formation of stable uncoupler-enzyme complexes, as has been suggested by Chance and Hollunger[28] for some uncouplers, may provide an explanation for the blocking of respiration, as the energy-rich intermediate has to be split before respiration proceeds.[26]

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