# Effect of acetone on VLDL secretion by the isolated rat liver

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*Summary.* High acetone levels occur in uncontrolled diabetes and after isopropanol administration to rats. In both conditions, very low density lipoproteins (VLDL) secretion is depressed. Acetone, however, failed to affect the VLDL secretion rate by the isolated perfused rat liver, suggesting that this metabolite is not involved in impaired VLDL production in diabetes and after isopropanol administration.

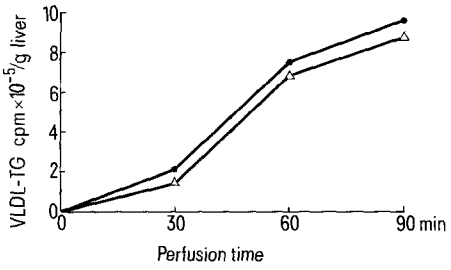
Isopropanol administration to rats depresses the VLDL secretion rate and induces and accumulation of triacylglycerol in the liver[1]. Most of isopropanol is metabolized in the hepatocyte by alcohol dehydrogenase (EC 1.1.1.1.) which oxidizes it to acetone by reducing NAD to NADH[2]. Fatty liver is probably accelerated by high influx of plasma fatty acids (NEFA) whose levels are also increased by isopropanol. The metabolic effects of isopropanol administration (decreased VLDL secretion, liver steatosis, high acetone production and increase of plasma NEFA levels) are also found in uncontrolled human and experimental diabetes[3-5]. Since acetone overproduction and high acetone plasma levels are a common feature in both types of steatosis, the present experiments were designed in order to evaluate a possible direct effect of acetone on the VLDL secretion by the isolated rat liver. The results indicate that acetone has a poor, if any, influence on lipoprotein production.

*Materials and methods.* Wistar female rats, 180–200 g b.wt, were used as liver donors. They were fasted and given a 10% glucose solution 18 h before the experiments. The perfusion method and apparatus have been described previously[6]. The perfusate was composed of rat blood obtained from fasted rats and diluted with Krebs-Henseleit buffer pH 7.4 containing 0.58 mM human albumin (Cohn's fraction V). Albumin-bound fatty acid concentration in the perfusate was adjusted to 300 μM. Disappearance of the NEFA from the perfusion medium gives their uptake rate by the liver[7]. NEFA and acetone concentrations of the perfusate were determined according to Dole's[8] and Greenberg and Lester's[9] methods respectively. Perfusate aliquots were sampled 30, 60 and 90 min after the addition of $^{14}$C-palmitic acid (57 mCi/mmole) and $^{3}$H-leucine (208 mCi/mmole). When present, acetone was added 30 min before the addition of the radioactive precursors and its concentration was kept constant (15.2±1.4 mM) during the perfusion by continuous infusion of buffer-diluted acetone solution. Acetone concentration was adjusted to values generally encountered in diabetic ketoacidosis[5]. After centrifugation, the perfusion medium was mixed with nonradioactive rat plasma (1/1; v/v) and VLDL were recovered after ultracentrifugation at 105,000×g during 20 h at 4 °C. Incorporation of $^{14}$C-palmitic acid into triacylglycerol was determined by liquid scintillation counting after TLC of the lipid extract[10]. Incorporation of $^{3}$H-leucine into VLDL apoproteins was measured after precipitation with 10% trichloroacetic acid of VLDL aliquots and filtration on fiberglass filters (Whatman GF/C) which were dried, transferred to vials containing 5 ml of xylene based scintillation mixture (Lipoluma, Lumac) and counted in a β-spectrometer (Intertechnique, France).

Effect of acetone on NEFA concentration in the perfusate

| Perfusion time (min) | NEFA (μM) | |
|---|---|---|
| | 1 | 2 |
| 0 | 327±17 | 317±30* |
| 30 | 218±21 | 196±25* |
| 60 | 139±18 | 132±16* |
| 90 | 120±13 | 98±11* |

1: Standard perfusate. 2: Perfusate supplemented with acetone (15.2±1.4 mM). Values are the mean±SEM from 6 perfusions per group. Perfusions were performed as described in Materials and methods. The 2 experimental groups were compared according to the Mann-Whitney's U-Text. * not significant.




Effect of acetone on VLDL secretion by the perfused rat liver. VLDL were isolated by ultracentrifugation from aliquots of the perfusion medium 30, 60 and 90 min after addition of albumin bound $^{14}$C-palmitic acid. Standard perfusate: ●—●; perfusate supplemented with acetone: △—△; VLDL-TG: triacylglycerol of VLDL (d < 1.006). Mean of 6 perfusions per group.

*Results and discussion.* As the VLDL secretion rate is closely related to the NEFA influx into the hepatocyte[11], it was necessary to verify that acetone did not change the liver NEFA uptake. NEFA clearance (table) is the same whether acetone is added to the perfusion medium or not. Thus, we can assume that the specific radioactivity of the precursor for VLDL triacylglycerol is the same in the 2 experimental groups. As shown on the figure, addition of acetone to the perfusion medium does not impair significantly the VLDL triacylglycerol secretion rate by the liver. Incorporation of $^{3}$H-leucine into VLDL apoproteins (standard perfusate: 426±87 cpm/min/g liver; perfusate supplemented with acetone: 449±40 cpm/min/g liver; mean±SEM from 6 perfusions per group) confirms this lack of effect on lipoprotein production. These results agree with our previously published results of acetone administration in vivo to rats[12].

Whereas in the control experiments, bile secreted during the perfusion is devoid of acetone, in the bile secreted during the acetone experiments the acetone concentration (30 mM) was 100% higher than in the corresponding perfusion medium (15 mM). These data demonstrate an effective uptake and biliary secretion of acetone by the liver and rule out that the lack of effect of acetone on VLDL secretion results from an inability of the organ to take it up under our experimental conditions.

In conclusion, acetone, at concentrations in the range of those found in diabetic ketoacidosis or after isopropanol administration, does not affect lipoprotein production by the isolated rat liver. Thus, liver steatosis in these 2 experimental conditions results probably from an increased fatty acid inflow and from a defective oxidation associated or not with a decreased availability of the apoproteins necessary for lipid export by the hepatocyte as previously shown by Heimberg[3].

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## Mutagenic activity of sodium bisulphite in barley

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*Summary.* Sodium bisulphite (7 and 10 mM) treatments administered at 30 °C for 6 h have been found to be strongly mutagenic in barley.

Sodium bisulphite is commonly used as bacterial inhibitor in wine, fermented beverages, fruit juices and as a preservative in dried fruits. In recent years, considerable evidence has been gathered to show that bisulphite (sulphur dioxide), even at low concentration, is a strong environmental pollutant. A strong statistical correlation has also been established between the $SO_2$ pollution level and several human diseases, including various types of cancer[1]. Bisulphite has also been reported to be mutagenic in some microbial systems[2-5].

The present communication reports the mutagenic activity of bisulphite in a higher plant system. Solutions of sodium bisulphite made in distilled water were freshly prepared to final concentration of 7 and 10 mM. Caryopsis of barley (*Hordeum vulgare* var. NP-114) were used as test system. For each treatment, repeated thrice, 50 seeds were taken in conical flasks and treated with 25 ml of the chemical. The treatments were given at 30 °C for 6 h. At the end of the treatment, seeds were given a quick wash with tap water and planted in glass petridishes on moist filter papers for germination and measuring seedlings injury, which was recorded on 10th day of planting. Another batch of similarly treated seeds was sown in the experimental nursery to raise $M_1$-generation. Spikes of $M_1$-plants harvested at maturity were sown in nursery for mutation analysis and $M_2$-seedlings were raised for scoring chlorophyll deficient mutations.

The data presented in the table show that sulphite produces significant reduction in seedling height, resulting in upto 40% of seedling injury. $M_2$-generation was grown on $M_1$-spike basis and the frequency of $M_1$-spikes segregating for chlorophyll-deficient mutations and $M_2$-mutant seedlings was worked out. The frequency of $M_1$ spikes segregating for mutations ranged from 17 to 24.1%, whereas the frequency of $M_2$-mutant seedlings ranged between 0.8 and 2.3%. The types of mutations recorded included Albina, Viridis and Alboviridis. The last class being the most prominent.

The data presented show that sulphite is a strong mutagen in barley and could be equally efficient in other higher plants. At the chemical level, it is a very reactive substance and undergoes various types of reaction with the cellular apparatus, which may include deamination of cytosine, transamination, cross-linking of proteins and nucleic acids and free radical reactions leading to production of mutations[1,6-9].

Effect of sodium bisulphite on seedling injury and chlorophyll mutations in barley

| Treatment | Seedlings height (cm) | $M_1$-spikes tested | Mutation/100 $M_1$-spikes | $M_2$-seedlings tested | Mutations/100 $M_2$-seedlings |
|---|---|---|---|---|---|
| Control | 10.2±0.3 | 163 | – | 2044 | – |
| Sodium bisulphite | | | | | |
| 7 mM | 6.8±0.6 | | 17.0 | 2133 | 0.8 |
| 10 mM | 6.1±0.3 | | 24.1 | 3409 | 2.3 |

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