From the data presented in this investigation, it is obvious that ASA significantly inhibits the metabolism of methanol in vivo, and provides protection against methanol poisoning in mice. Should the view of methanol oxidation in vivo (by catalase-H_2O_2 system) be accepted, it is suggested that it is the depletion of the peroxide pool rather than the reduction in catalase activity which causes inhibition of methanol oxidation. This may be achieved by inhibiting enzyme systems involved in H_2O_2-delivering reactions, e.g. xanthineoxidase and monoamineoxidase (AEBI et al.[14]). The suggested mechanism of inhibition receives support from the inhibition of C^{14}-formate metabolism in vivo by ASA, since formate is known to possess a specific requirement for catalase-H_2O_2 complex (KEILIN and HARTEE[15] and AEBI and HASSAN[16]). C^{14}-formate was administered intraperitoneally and the measurement of the expired $C^{14}O_2$ served as a test system. It was found that a dose of 0.5 g ASA per kg per mouse inhibited formate oxidation to almost the same extent as that of methanol.

It is not unlikely that the antipyretic activity of ASA is related in a way to the depletion of H_2O_2 sources rather than to reduction in catalatic decomposition of the peroxide by catalase[8].

From this work, the following may be concluded: (1) ASA significantly inhibits methanol and formate oxidation in vivo. (2) ASA does not inhibit catalase in vivo, but it does in vitro. (3) ASA reduces the toxic effects of methanol poisoning in mice. (4) The mechanism of inhibition of methanol oxidation by ASA is believed to be due to reduction of the peroxide pool rather than the inhibition of catalase.

Zusammenfassung. Acetylsalicylsäure hemmt die Oxydation von Methanol und Formiat in vivo erheblich, die Katalase nur in vitro, und vermindert den toxischen Effekt der Methanolvergiftung. Der Mechanismus der Methanoloxydationshemmung durch Acetylsalicylsäure könnte eher auf die Verminderung des Peroxyd-Pooles – als die Katalasehemmung – zurückzuführen sein.

A. HASSAN, M. I. ELGHAMRY, and F. M. ABDEL-HAMID

Department of Biology, Atomic Energy Establishment, Cairo and Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt (U.A.R.), July 8, 1965.

[14] H. AEBI, J. QUITT, and A. HASSAN, Helv. physiol. Acta *20*, 148 (1962).
[15] D. KEILIN and E. F. HARTEE, Biochem. J. *60*, 310 (1955).
[16] H. AEBI and A. HASSAN, Helv. chim. Acta *43*, 544 (1960).

Ouabain-Sensitive Fatty Acid Release from Isolated Fat Cells[1]

Although the control of free fatty acid (FFA) mobilization has been extensively studied in many laboratories[2], the detailed mechanism by which stored triglycerides are released from fat cells as fatty acids and glycerol is as yet incompletely understood. In particular, it is not known whether FFA released from triglycerides simply diffuse through the cell membrane, whether they are actively transported, or indeed whether they are released within or close to the cell membrane itself. The recent finding[3] that FFA release induced by lipolytic hormones such as ACTH and epinephrine is inhibited by ouabain suggests that the release mechanism may involve an active transport step, since it has previously been shown that this agent can be used to differentiate between active and passive transport processes[4]. This preliminary report establishes the ouabain-sensitive nature of the FFA releasing system in isolated fat cells.

Material and methods. Male albino rats weighing 130 to 170 g and fed ad libitum were used throughout. Free adipose cells were prepared from epididymal fat pads using the technique of RODBELL[5] and incubated in bicarbonate buffer. FFA were measured using the method of DOLE and MEINERTZ[6].

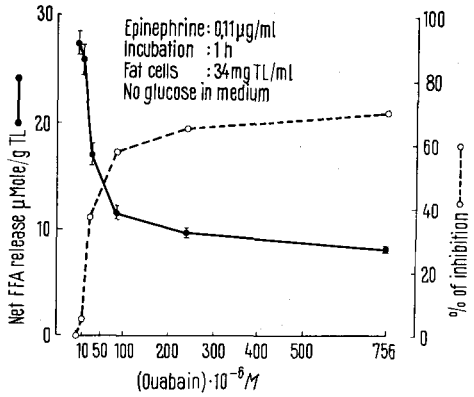
Results and discussion. As illustrated in the Figure, the addition of ouabain to the incubation medium was associated with a decrease in the net change in FFA concentration during incubation in the presence of epinephrine. At an epinephrine concentration of 0.11 μg/ml, the minimum effective dose of ouabain was $9 \cdot 10^{-6} M$. The inhibition reached maximum at an ouabain concentration around $2.5 \cdot 10^{-4} M$. At this concentration the net FFA increase observed during incubation in the absence of ouabain was inhibited to the extent of between 60 and 70%. The concentration range of this dose response curve for ouabain was comparable to that which obtains in the studies of ouabain effects on the active transport of sodium and potassium.

Although ouabain increased the uptake and metabolism of glucose by fat cells, it is noteworthy that the inhibition of FFA release demonstrated in the Figure occurred in the total absence of glucose. Furthermore, it was found that ouabain did not increase the uptake and esterification of labelled FFA added to the incubation medium. These observations are interpreted to mean that the ouabain-induced inhibition of FFA release was not the result of an increase in the re-esterification process. Accordingly, the metabolic effect of the agent must be related either to the transport of FFA across the cell membrane, or to lipolysis, or both.

The inhibitory effect of ouabain on FFA release was not limited to epinephrine-stimulated lipolysis, but applied equally to the lipolysis induced by the presence of

[1] This work has been supported by the Fonds National Suisse de la Recherche Scientifique, Grant No. 3244, Bern (Switzerland) and by a grant-in-aid from the Fondation Emil Barell pour le Développement des Recherches Médico-Scientifiques, Basel (Switzerland).
[2] A. E. RENOLD and G. F. CAHILL Ed., *Handbook of Physiology*, Section 5: *Adipose Tissue* (American physiol. Society, Washington 1965).
[3] S. J. Ho, R. J. Ho, and H. C. MENG, unpublished data.
[4] T. Z. CSÁKY, Ann. Rev. Physiology *27*, 415 (1965).
[5] M. RODBELL, J. biol. Chem. *239*, 375 (1964).
[6] V. P. DOLE and H. MEINERTZ, J. biol. Chem. *235*, 2595 (1960).

ACTH or glucagon, as illustrated in the Table. This Table also shows that, even in the absence of ouabain, the lipolytic activity of ACTH, epinephrine, or glucagon was markedly decreased by the omission of potassium from the incubation medium. These observations suggest that the inhibitory effect of ouabain on FFA release is a general phenomenon and, furthermore, that it is mimicked by removal of potassium from the medium. It would seem likely, therefore, that the mechanism of FFA release from isolated fat cells is linked to the Na^+ and K^+ related steps of active transport.



Dose response curve of ouabain on inhibition of net FFA change stimulated by epinephrine.

Comparison of the inhibitory effect of ouabain and of K^+-free medium on FFA mobilization from isolated fat cells. Mean of 4 ± S.E., expressed as µmoles/g TL/h. No glucose in medium

Additions	K^+ in buffer	ACTH (2 mU/ml)	Epinephrine (0.1 µgm/ml)	Glucagon (1 µgm/ml)
Control	+	9.7 (4.0)	3.75	0.46 (0.15)
Hormones	+	55.3 (6.2)	37.50 (9.1)	18.40 (1.3)
Hormones + ouabain	+	13.2 (5.2)[a]	6.82 (4.3)[b]	11.80 (2.3)[b]
Control	−	5.2 (2.7)	1.16	−
Hormones	−	10.1 (2.4)	3.16 (2.5)	11.10 (1.4)

[a] Ouabain $5.5 \cdot 10^{-4} M$. [b] Ouabain $2.4 \cdot 10^{-4} M$.

It remains to be seen whether the site of the inhibitory effect of ouabain on FFA release is the chain of reactions leading to the activation of a hormone-sensitive lipase or a still hypothetical active FFA transport through the cell membrane, or both. As we have shown in very recent experiments that ouabain also significantly inhibits the FFA release induced in fat cells by N^6C'-2-dibutyril-cyclic-3′,5′-AMP[7] as well as epinephrine-stimulated glycogenolysis, the most likely present hypothesis is that of an inhibitory ouabain effect on both adenyl cyclase and on membrane ATP-ase. The former enzyme is related to the activation of the hormone-sensitive lipase[8] while the latter[9] is well known to be linked to active transport systems in membranes[10].

Résumé. La mobilisation des acides gras libres (FFA) induite par les hormones lipolytiques dans les cellules adipeuses isolées est inhibée par l'adjonction d'ouabaïne. Le transport des FFA hors de la cellule semble lié en partie à la pompe Na^+-K^+. Une hypothèse sur le mode d'action de l'ouabaïne est discutée.

R.-J. Ho, B. Jeanrenaud, and A. E. Renold

Institut de Biochimie Clinique, Université de Genève (Switzerland), September 14, 1965.

[7] N^6C'-2-dibutyryl-cyclic-3′-5′-AMP was obtained through the courtesy of Professor Th. Posternak, Ecole de Chimie, Université de Genève, (Switzerland).

[8] R. W. Butcher, R. J. Ho, H. C. Meng, and E. W. Sutherland, abstracts of 6th International Congress of Biochemistry (New York 1964), p. 173.

[9] J. C. Skou, Physiol. Rev. *45*, 596 (1965).

[10] The authors are indebted to Miss A. Eisold and Miss E. Keller for their skilful technical help.

The Occurrence of 4-Ethyl- and 2,5-Dimethylazulene in Cracking Column Products

Some time ago we received small samples of intensely blue coloured distillates (Table I), products from a cracking column at Grangemouth(Scotland), from which we isolated three azulenes (see Table I). Dilution of the distillate fractions with petroleum ether (b.p. 40–60°), extraction with ice-cold phosphoric acid, and regeneration of the acid-soluble material gave blue oils comprising about 0.5% of the original distillate fraction. Chromatography on alumina (petroleum ether-benzene 9:1) showed that each was homogeneous. The compounds, indicated by I, II and III, were characterized by their visible spectra[1] in hexane (Table II) and by their crystalline trinitrobenzene (TNB) complexes.

Elemental analysis of the TNB-complex of III, brown needles (from ethanol) m.p. 141–142°, showed that III was a $C_{12}H_{12}$ hydrocarbon, i.e. an ethyl- or dimethylazulene; found, 58.4% C; 3.9% H; 11.8% N ($C_{18}H_{15}N_3O_6$ requires 58.5% C; 4.1% H; 11.4% N).

The fine structure in the visible spectrum of compound III (Table II) had an average hypsochromic displacement of 17 nm with respect to the corresponding maxima for azulene. This indicated, according to the Plattner rules on the effect of alkyl substitution on the visible spectra of azulenes[2], as only possible structures for III 2-ethyl-[3],

[1] Measured on a Unicam SP 500 spectrophotometer.

[2] See E. Heilbronner, Tetrahedron *19*, supplement 2, 289 (1963), and refs. 18 and 19 contained therein.

[3] Pl. A. Plattner and A. Fürst, Helv. Chim. Acta *28*, 1636 (1945).