We should not rule out, however, the possibility that PGE_1 produces its effect by changing the acetate pool within the liver cell. If more unlabelled acetate becomes available in the hepatocytes, the rate of synthesis of fatty acid and sterols (as measured by the in vitro incorporation of ^{14}C-acetate) may be underestimated.

It is difficult to predict whether the effect of PGE_1 which we observed in vitro also occurs in vivo, as the amount of PGE_1 used in this study may be far above the concentration of prostaglandins which is present in the liver in normal conditions.

In the majority of tissues, the concentrations of both free and bound prostaglandins are very low; but there is evidence that nutriotional[10] and hormonal[8] stimuli can increase the concentration of PGE_1 by stimulating a rapid rate of de novo synthesis. Furthermore it should be taken into account that probably only a small proportion of PGE_1 present in the incubation system is taken up by the liver, so that the intracellular concentration of PGE_1 may be within physiological levels. It is also important to stress that the concentration of PGE_1 which inhibits in vitro fatty acids and sterol synthesis in the liver is much lower than that which is known to inhibit norepinephrine-induced lipolysis in adipose tissue[14].

The mechanism of action of PGE_1 in the mammalian tissue is still a matter of controversy. However, a great deal of evidence has now been accumulated which indicates that many of the pharmacological effects of PGE_1 are present in those systems where cAMP formation can occur. PGE_1 may act through an inhibition of cAMP accumulation, as in adipose tissue[14–15], or, more frequently through an increase of the intracellular level of cAMP, as has been demonstrated in some tissues, such as thyroid[16] heart[17] and kidney[18].

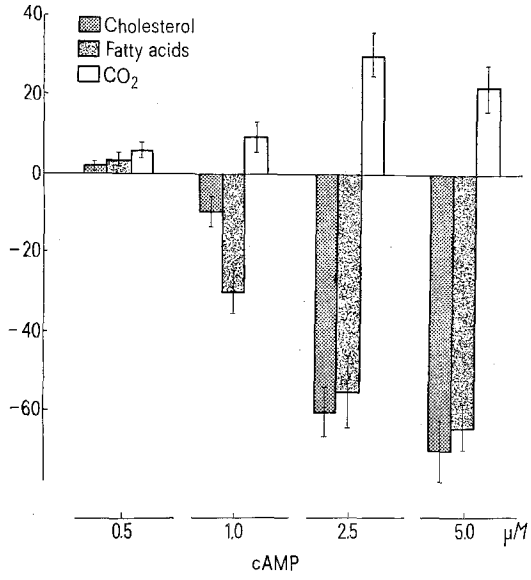

Fig. 2. Effect of increasing amounts of cyclic 3′, 5′-AMP on the incorporation of acetate-2-C^{14} into cholesterol (DPS), fatty acids and metabolic CO_2.

For these reasons we also studied the in vitro effect of cAMP on the synthesis of cholesterol and fatty acids by liver slices. The addition of cAMP to the incubation system was followed by a significant suppression of the rate of incorporation of acetate in both fatty acids and digitonin precipitable sterols. CO_2 production was found to be higher after the addition of cAMP as compared to the controls. These data are in accord with the results recently found by BRICKER and LEVY[19]. These results indicate that PGE_1 and cAMP have a similar effect on hepatic synthesis of fatty acids and sterols, although they may act at different biochemical levels. This close association would suggest that either PGE_1 could exert its effect on hepatic synthesis of lipids through a stimulation of cAMP formation, or that accumulation of cAMP in the liver cells leads to a release of PGE_1 which in turn inhibits lipid synthesis from acetate.

Whichever mechanism is involved, we feel that this observation provides a tool for a further understanding of the role essential fatty acids on lipid metabolism in mammalian liver. It may be reasonable to assume that whenever hepatic concentration of these precursors increases because of a high exogenous intake, the production of prostaglandins may also be increased.

Riassunto. La prostaglandina E_1 (PGE_1) ha un duplice effetto sulla sintesi epatica del colesterolo: a basse concentrazioni determina un incremento della incorporazione di acetato in colesterolo, mentre a concentrazini superiori a 50 nmoli essa determina una riduzione di questo paramentro.

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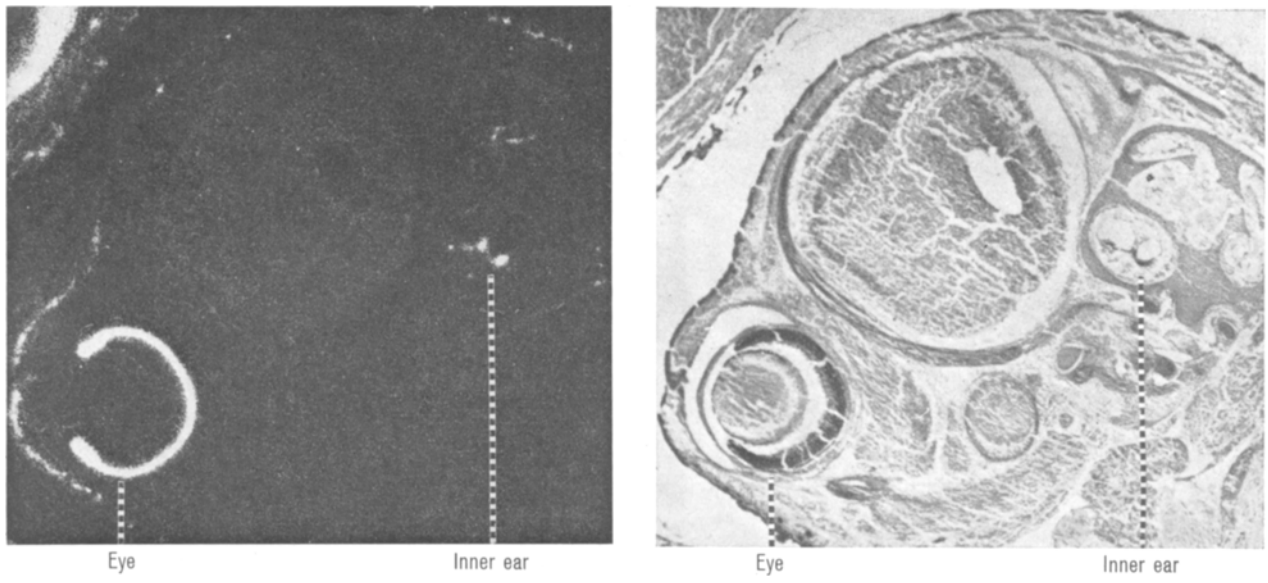
27 June 1973.

[14] R. W. BUTCHER and E. C. BAIRD, J. biol. Chem. *243*, 1713 (1968).
[15] E. W. HORTON, Physiol. Rev. *49*, 122 (1969).
[16] T. KANEKO, U. ZOR and J. B. FIELD, Science *163*, 1062 (1969).
[17] B. E. SOBEL and A. K. ROBISON, Circulation *40*, suppl. 3, 189 (1969).
[18] N. P. BECK, J. B. FIELD and B. DAVIS, Clin. Res. *18*, 494 (1970).
[19] L. A. BRIKER and G. S. LEVEY, J. biol. Chem. *247*, 4914 (1972).

Mechanism of Drug-Induced Chronic Otic Lesions. Role of Drug Accumulation on the Melanin of the Inner Ear

Although drugs such as salicylic acid may cause a hearing reduction of short duration, most of the injuries to the inner ear are of a chronic and serious nature[1]. They are caused by many compounds, but two main groups can be distinguished: 1. the streptomycin group of antibiotics, and 2. quinine and its many synthetic successors (especially chloroquine). The injuries caused by all these drugs have many characteristics in common. Both hearing and balance difficulties may appear. Functional disturbances correspond to evident histopathologic changes. High dosage and long-term therapy are usually involved. The damage has a marked tendency to be irreversible. Furthermore, symptoms will often appear only after a long latent period – several months after the medication has been discontinued.

[1] P. A. SHEFFIELD and J. S. TURNER JR., Sth. med. J. Bgham. Ala. *64*, 359 (1971).



Detail of an autoradiogram (upper) with the corresponding section (lower) of a pregnant pigmented mouse 24 h after an i.v. injection of ^{14}C-chloroquine. There is high accumulation in the fetal eye and the inner ear. Hematoxylin-eosin.

These characteristics, especially the latent period, make it seem very likely that a strong mechanism of accumulation and retention of these drugs in the inner ear is present, and current investigations in our laboratory are producing an increasing amount of evidence in favour of such a hypothesis.

It is the melanin in the inner ear which is responsible for the drug accumulation (Figure). Melanin is mainly present in the stria vascularis in the cochlea and in the planum semilunatum in the ampullae – sites where the endolymph is considered to be produced. In our autoradiographic distribution studies in pigmented mice[2,3], melanin has been shown to accumulate and retain many substances of a polycyclic nature. This accumulation was not seen in albino animals. The drug affinity for melanin seems to be unique: no other tissue accumulates and retains foreign compounds in a similar manner. A high retention was found even 1 year after administration of a single dose of ^{14}C-chloroquine. The retention of many drugs can be expected to last for several years in humans.

In vitro investigations made in our laboratory (using beef eye melanin according to Potts), showed that kanamycin had the highest melanin affinity (89%), followed by chloroquine (85)%, quinine (68%), dihydrostreptomycin (65%), streptomycin (60%) and viomycin (46%). Salicylic acid and the tuberculostatics, isoniazid and paraaminosalicylic acid showed no melanin affinity.

In addition to the inner ear, melanin is found in the eye, skin, nuclei of the brain stem (substantia nigra and locus coeruleus), and in some other tissues. The inner ear, therefore, seems to be only one of several organs, which can be affected according to the same general mechanism. It is now well established that the affinity of chloroquine and the phenothiazines for melanin is the primary factor determining their ocular toxicity. Some drugs (e.g. chloroquine and quinine) can simultaneously cause damage both to the eye and to the inner ear in a very similar pattern[1–5]. In both organs melanin is critically situated in structures which form the fluid nourishing the receptor cells. The damage to the receptor cells in both these organs is very likely secondary to a damage in the melanin-containing cells of the fluid-forming structures.

In preliminary histopathologic investigations in young guinea-pigs treated with kanamycin, serious damage was found in the stria of the pigmented animals, but not in the albinos. The animals received 200 mg/kg body wt. for 3 weeks and were then examined after an additional period of 2 months. Special features concerning the pigment morphology were observed. While normally melanin granules are evenly distributed in the strial cells[6], most of the granules in the kanamycin-treated animals were aggregated, a phenomenon which is often seen in drug-induced melanin disorders[3]. Furthermore, pycnotic nuclei, vacuolization throughout the stria, and disruption of the epithelial cells were observed.

Previous literature on the drug induced histopathology of the inner ear also yields abundant evidence for early damage to the stria, although these authors were not aware of the drug accumulation in the melanin. Thus, histochemical disturbances[7,8] and histopathologic alterations[9–11] in the stria have been observed to precede, or accompany, lesions of the organ of Corti. It has also been suggested that ototoxic antibiotics cause hair cell

[2] N. G. Lindquist and S. Ullberg, Acta pharmac. Copenh. *31*, Suppl. 2 (1972).
[3] N. G. Lindquist, Acta radiol. Stockh. Suppl. 325 (1973).
[4] H. M. Dekking, Ophthalmologica *118*, 743 (1949).
[5] M. G. Betten, R. C. Bilchik and M. E. Smith, Am. J. Ophthal. *72*, 720 (1971).
[6] H. Engström, F. S. Sjöstrand and H. Spoendlin, Pract. oto-rhinolaryng. *17*, 69 (1955).
[7] F. Nakamura, Ann. Otol. St. Louis *66*, 1080 (1957).
[8] K. Müsebeck and W. Schätzle, Arch. Ohr.-Nas. Kehlk.-Heilk. *181*, 41 (1962).
[9] C. Beck and P. Krahl, Arch. Ohr.-Nas.-Kehlk.-Heilk. *179*, 594 (1962).
[10] F. Ostyn and J. Tyberghein, Acta oto-laryng., Stockh. Suppl. 234 (1968).
[11] L. Rüedi, K. Graf and B. Tschirren, Schweiz. med. Wschr. *83*, 951 (1953).

degeneration due to changes of the endolymph, secondary to atrophy of the stria[12].

Zusammenfassung. Nachweis einer Melanin-Affinität ototoxischer Medikamente im Zusammenhang mit histopathologischen Änderungen in der Stria pigmentierter Tiere, nicht aber bei Albinos, durch Kanamycin.

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Department of Toxicology, University of Uppsala, Biomedical Center, Box 573, S-75123 Uppsala (Sweden), 25 May 1973.

[12] L. G. JOHNSSON and J. E. HAWKINS jr., Laryngoscope, St. Louis *82*, 1105 (1972).

[13] This work was supported by grants from the Swedish Board for Technical Development and the Swedish Medical Research Council (No. B 73-14 X-28 76-04 A).

Duodenal Ulcer in Rats by 3-Allyl-5-isobutyl-2-thiohydantoin and its Related Compound

Although many drugs and chemicals can cause gastric ulcer in animals, few compounds are known consistently to induce duodenal ulcer. Infusion of gastrin, either alone or in combination with other secretagogues such as histamin and carbachol, can produce duodenal ulcer in various animal species[1-3]. SZABO et al.[4] reported production of solitary, often perforating, duodenal ulcers in female rats by injections of propionitrile. While studying the acute toxicity of 3-allyl-5-isobutyl-2-thiohydantoin, an anti-convulsant[5], we incidentally found that a single administration of this compound could consistently induce duodenal ulcer in rats.

Materials and methods. Male Wistar rats weighing around 120 g were kept in mesh-bottomed metalic cages to avoid coprophagia. The test compound, 3-allyl-5-isobutyl-2-thiohydantoin, was suspended in 10% aqueous solution of gum acacia such that 1.0 ml contained the necessary amount for a single administration, and given to rats after overnight fasting. Fasting was continued for 24 h after medication. Experiments were conducted in the following series:

In the first experiment 6 groups of 5 rats each received an oral or s.c. administration of the compound in the dose levels shown in Table I and were killed 24 h after administration by exsanguination from the carotid artery under ether anesthesia. The stomach and duodenum were excised, opened along the greater curvature, spread on a rubber board and examined with a binocular microscope. Another 10 rats served as control and were killed 24 h after oral or subcutaneous administration of 1.0 ml of the suspension vehicle.

In the second experiment 10 rats received subphrenic vagotomy[6] under pentobarbital anesthesia, and another 10 rats were submitted to sham operation. 10 days after the operation, they were given a s.c. injection of either 175 mg/kg of the compound or 1.0 ml of the suspension vehicle and killed 24 h after injection. The stomach and duodenum were examined in the way mentioned above.

In the third experiment, the 11 compounds shown in Table III were tested for the ulcerogenic effect. They were suspended in 10% aqueous solution of gum acacia and given to rats orally or s.c. in the dose levels shown in Table III. The stomach and duodenum were examined 24 h after administration.

Results and discussion. Ulceration of the duodenum occurred in 21 out of 30 rats given 3-allyl-5-isobutyl-2-thiohydantoin, while in control rats the stomach and duodenum had a normal appearance. The ulcers were elongated, often in pairs and located on the anti-mesenteric aspect of the duodenum about 6 to 10 mm caudal from the pylorus. Severity of the duodenal lesion was apparently dose-dependent (Table I). Perforating ulcer with consequent peritonitis occurred in 4 out of 20 rats given 250 mg/kg or more of the compound. Besides, in thiohydantoin-treated rats the stomach was distended with accumulation of acidic fluid (pH 1 to 2), and small

Table I. Occurrence of duodenal ulcer in rats following a single administration of 3-allyl-5-isobutyl-2-thiohydantoin

Route of administration	Dose (mg/kg)	No. of rats	Grades of duodenal ulcer[a] —	+	++	+++
Subcutaneous	0	5	5	0	0	0
	125	5	4	1	0	0
	250	5	1	1	2	1
	500	5	0	0	1	4 (1)[b]
	0	5	5	0	0	0
Oral	125	5	4	1	0	0
	250	5	0	0	1	4 (1)
	500	5	0	0	1	4 (2)

[a] — no ulcer; + small shallow ulcer of up to 2 mm in diameter; ++ elongated or linear ulcer of 3 to 6 mm in length; +++ elongated or linear ulcer of 6 mm or longer in length. [b] Numerals in parentheses indicate the number of rats with perforating ulcer.

Table II. Inhibitory effect of vagotomy on occurrence of duodenal ulcer in rats by 3-allyl-5-isobutyl-2-thiohydantoin

Treatment	Dose (mg/kg)	No. of rats	Grades of duodenal ulcer[a] —	+	++	+++
Vagotomy	0	5	5	0	0	0
Vagotomy	175	5	5	0	0	0
Sham operation	0	5	5	0	0	0
Sham operation	175	5	0	2	2	1

[a] See the note of Table I.

[1] W. G. GOBBEL JR. and R. BENTON ADKINS, Am. J. Surg. *113*, 183 (1967).
[2] S. EMAS and M. I. GROSSMAN, Gastroenterology *52*, 959 (1967).
[3] A. ROBERT, T. J. STOUT and J. E. DALE, Gastroenterology *59*, 95 (1970).
[4] S. SZABO and H. SELYE, Arch. Path. *93*, 390 (1972).
[5] R. M. GESLER, C. E. LINTS and E. A. SWINYARD, Toxic. appl. Pharmac. *3*, 107 (1961).
[6] H. SHAY, S. A. KOMARV and M. GRUENSTEIN, Arch. Surg. *59*, 210 (1949).