lipidemic response of HMG could involve a shift in lipoprotein spectrum[14,15]. Recently we have shown that HMG has no effect on orotic acid fatty liver in rats[24]. Since Triton is known physically to alter VLDL, and HMG is capable of counteracting Triton-induced hyperlipidemia, the possibility of HMG exerting its hypolipidemic effect through inhibition of lipoproteins synthesis appears more plausible. Furthermore, the method used detects compounds which inhibit lipid biosynthesis or its catabolism[10], the hypolipidemic activity of HMG may be mediated through its effect on lipid metabolism. FOGELMAN et al.[25] have very recently shown that normally 12% of mevalonic acid is catabolized through a shunt pathway in mammalian system involving *trans*-3-methylglutaconyl CoA. A derailment in the operation of this pathway might explain the hypercholesterolemic condition in rats and man. In view of earlier reports on hypolipidemic activity of HMG[11–18] in animals and man, it is tempting to suggest, therefore, that HMG may in some way correct the derailed pathway.

[1] Acknowledgments. We are grateful to Dr. M. SALEEMUDDIN for encouragement, Dr. P. E. SCHURR, Upjohn Co. USA for generous gift of Triton W. R 1339 and Lady Tata Memorial Trust (India) for financial assistance to one of us (SYKY).

[2] M. FRIEDMAN and S. O. BYERS, J. exp. Med. *97*, 117 (1953).
[3] I. D. FRANZ and B. T. HINKELMAN, J. exp. Med. *101*, 225 (1955).
[4] R. L. HIRSCH and A. KELLNER, J. exp. Med. *104*, 1 (1956).
[5] M. C. SCHOTZ, A. SCANU and I. H. PAGE, Am. J. Physiol. *188*, 399 (1957).
[6] M. FRIEDMAN and S. O. BYERS, Am. J. Physiol. *190*, 439 (1957).
[7] A. SCANU, P. ORIENTE, J. M. SZAJEWSKI, L. J. MCCORMACK and I. H. PAGE, J. exp. Med. *114*, 279 (1961).
[8] S. O. BYERS, M. FRIEDMAN and T. SUGIYAMA, Am. J. Physiol. *204*, 1100 (1963).
[9] E. P. JOROLAN and B. W. JANICKI, Progr. Biochem. Pharmac. *2*, 308 (1967).
[10] P. E. SCHURR, J. R. SCHULTZ and T. M. PARKINSON, Lipids *7*, *68* (1972).
[11] Z. H. BEG and M. SIDDIQI, Experientia *23*, 380 (1967).
[12] Z. H. BEG and M. SIDDIQI, Experientia *24*, 791 (1968).
[13] S. Y. K. YOUSUFZAI and M. SIDDIQI, Experientia *32*, 1033 (1976).
[14] P. J. LUPIEN, M. TREMBLAY and Z. H. BEG, Atherosclerosis *18*, 407 (1973).
[15] A. N. K. YUSUFI and M. SIDDIQI, Atherosclerosis *20*, 517 (1974).
[16] S. Y. K. YOUSUFZAI and M. SIDDIQI, submitted to Lipids
[17] S. Y. K. YOUSUFZAI, M. SIDDIQI and A. K. ABDULLAH, Lipid in press.
[18] P. J. LUPIEN, D. BRUN and M. MOORJANI, Lancet, *2*, 101, 1256 (1973).
[19] R. ALTSCHUL, in *Niacin in Vascular Disorders and Hyperlipemia* (Ed. R. ALTSCHUL; Thomas, Springfield 1964), vol. 3, p. 3.
[20] P. HILL and D. DVORNIK, Can. J. Biochem. *44*, 1043 (1969).
[21] W. R. BLOOR, K. F. PELKAN and D. M. ALLEN, J. biol. Chem. *52*, 191 (1922).
[22] E. VAN HANDEL and D. B. ZILVERSMIT, J. Lab. clin. Med. *50*, 152 (1957).
[23] G. V. MARINETTI, J. Lipid Res. *3*, 1 (1962).
[24] S. Y. K. YOUSUFZAI and M. SIDDIQI, submitted to Lipids.
[25] A. M. FOGELMAN, J. EDMOND and G. POPJAK, J. biol. Chem. *250*, 1771 (1975).

Resistance of Cold-Exposed Rats to Aflatoxin

T. B. G. TILAK, V. NAGARAJAN and P. G. TULPULE

National Institute of Nutrition, Indian Council of Medical Research, Jamai-Osmania, Hyderabad–500007 (A. P., India), 6 November 1975.

Summary. Male rats kept at a temperature of 4°–5 °C were refractory to a lethal dose of aflatoxin compared to animals at 20°–21°C which exhibited a high mortality and marked liver damage. It is suggested that this decreased susceptibility is mediated through a stimulated microsomal drug-metabolizing system in cold environment.

The harmful effects of aflatoxin have been found to be modified by a variety of conditions including protein deficiency[1], vitamin A-deficiency[2], hypophysectomy[3] and pregnancy[4]. A critical review of these conditions had led to the suggestion that the increased susceptibility of rats in these conditions is due to a defective drug-metabolizing system in the liver[5]. The influence of environmental factors such as cold exposure, which modify the hepatic microsomal drug-metabolizing system have been studied in other hepatotoxins[6]. However, no such studies appear to have been investigated in aflatoxicosis.

Materials and methods. 12 male albino rats weighing approximately 120 g were divided into 2 groups. One group was kept at room temperature (20–21 °C) while the other group was placed in an adequately ventilated cold room having a temperature of 4–5 °C. All the animals received the colony stock diet and water ad libitum. At the end of 72 h, all the animals received i.p. a pure preparation of aflatoxin containing predominantly B_1 in a dose of 8 mg/kg body weight. The experiment was terminated by sacrificing all animals in both groups which were alive 72 h after administration of the toxin. The livers of the dead as well as the sacrificed animals were fixed in formalin and processed for microscopic examination in the usual manner.

Results and discussion. Between 40 and 48 h following the administration of the toxin, 5 of the 6 animals at room temperature died. Livers of these animals exhibited extensive necrosis of liver cells in the periportal and midzones. The rats exposed to low temperature, however, were apparently unaffected. Livers of these animals showed no parenchymal damage. There was, however, bile-duct and ductular proliferation with prominent portal tracts.

On the basis of the mortality and the histology of livers, it is clear that the susceptibility of rats to aflatoxin is decreased by exposure to cold. Exposure to lower temperature has been found to result in an increase in the microsomal drug-metabolizing enzymes[7]. Earlier, we had

[1] T. V. MADHAVAN and C. GOPALAN, Arch. Path. *80*, 123 (1965).
[2] G. S. REDDY, T. B. G. TILAK and D. KRISHNAMURTHI, Fd. Cosmet. Toxic. *11*, 467 (1973).
[3] C. M. GOODALL, Newzealand med. J. *67*, 32 (1968).
[4] W. H. BUTLER and J. S. WIGGLESWORTH., Br. J. exp. Path. *47*, 242 (1966).
[5] T. B. G. TILAK, V. NAGARAJAN and P. G. TULPULE, Experientia *31*, 953 (1975).
[6] M. W. RADOMSKI and T. ORME, J. Nutr. *92*, 19 (1967).
[7] G. C. FULLER, W. F. BOUSQUET and T. S. MIYA, Toxic. appl. Pharmac. *23*, 10 (1972).

suggested that the drug-metabolizing enzymes are rate-limiting in the detoxification of aflatoxin[5]. The acute toxicity is inversely related to the total activity of drug-metabolizing system. The present experiment lends support to this suggestion.

A variety of compounds have been tested in cold environment in animals[6]. A typical experiment of immediate relevance is the reported increased susceptibility of mouse liver to carbon tetrachloride (CCl_4) toxicity under cold environment[8]. The behaviour of CCl_4 is found to be exactly opposite to that of aflatoxin in a given situation[5]. It is also believed that CCl_4 requires activation before being toxic[9,10]. This metabolic basis appears to be responsible for the increased susceptibility of mouse liver to CCl_4 under cold environment. Investigations of this nature thus help to explain the mechanism of action of a compound under study.

[8] S. E. I. ADAMS and E. THORPE, Br. J. exp. Path. *51*, 394 (1970).
[9] R. O. RECHNAGEL and A. K. GHASHAL, Lab. Invest. *15*, 132 (1966).
[10] T. F. SLATER, Nature, Lond. *209*, 36 (1966).

Interference with Histamine and Imidazole Acetic Acid Metabolism by Salicylates: a Possible Contribution to Salicylate Analgesic Activity?

M. A. BEAVEN, ZDENKA HORAKOVA and H. R. KEISER

Pulmonary Branch and Hypertension-Endocrine Branch, National Heart and Lung Institute, National Institutes of Health, Building 10, Room 5N107, Bethesda (Maryland 20014, USA), 9 March 1976.

Summary. In man, rats and mice, the urinary excretion of the histamine and L-histidine metabolite, imidazole acetic acid, is increased and that of the conjugated metabolite, ribosylimidazole acetic acid, decreased by small doses of salicylates. In contrast to salicylates, other non-salicylate anti-inflammatory drugs, indomethacin, phenylbutazone, phenacetin and acetaminophen do not influence the excretion of the urinary metabolites of histamine and L-histidine. Since imidazole acetic acid is reported to have analgesic and narcotic activity, there is the inference that the analgesic properties of salicylate might be due in part to interference in imidazole acetic acid metabolism.

In an earlier study, we reported that in man and rat administration of salicylates results in a reduction in the urinary excretion of the histidine and histamine metabolite, ribosylimidazole acetic acid[1]. This reduction is accompanied by a corresponding increase in the excretion of free imidazole acetic acid. Additional studies in vitro have indicated that salicylates inhibit (50% inhibition at 0.2 m*M*) imidazoleacetate phosphoribosyl transferase, the enzyme responsible for the ribosylation of imidazoleacetate in vivo[2]. Since it has been reported that imidazole acetic acid has analgesic and narcotic activity in mouse[3,4], accumulation of imidazole acetic acid during salicylate therapy might contribute to the analgesic action of these drugs. As part of a continuing study to explore this possibility, this report compares the effect of salicylates on imidazole acetic acid metabolism in mouse with that in rat and man.

Materials and methods. Histamine (2-ring-^{14}C) was purchased from Amersham/Searle (Illinois). The ^{3}H-imidazole acetic acid was prepared from unlabeled material by catalytic exchange with tritium gas (New England Nuclear Corp.) and was purified by thin layer chromatography[1].

Human subjects (18–54 years of age) included 2 normal volunteers and 9 patients with mild hypertension or Raynaud's disease who were receiving no drugs except aspirin. Aspirin was administered 4 times daily for 1 week before the injection of ^{14}C-labeled histamine. In control studies, aspirin treatment was suspended for 1 week before injection of ^{14}C-histamine. All subjects were fully informed of the nature of the experiments and were free to discontinue their participation in the study if they wished. Animals were kept in glass metabolic cages (1 rat per cage, 5 mice per cage) with free access to food and water. Food was withdrawn during the period of urine collection. Aspirin and other drugs were administered orally by stomach tube in a single or repeated (q.i.d.) doses.

^{14}C-Histamine (5 μCi/kg in man or 25 μCi/kg in rat and mouse) and ^{3}H-imidazole acetic acid (20 μCi/kg) were given i.v. 2 h after the administration of drug. Urine was then collected for 6 h periods and frozen. Aliquots (10 μl) of urine were assayed for labeled histamine metabolites by thin layer chromatography on chromatograms of cellulose powder on flexible 20 × 5 cm plastic sheets in butanol:ethanol:ammonia (80:10:30 parts by volume) solvent for 90 min and by isotope dilution-derivative techniques[1,2]. As discussed elsewhere[1], there is close agreement in values obtained by the two procedures.

Results. In urine of all species, 3 major peaks of radioactivity – ribosylimidazole acetic acid, unconjugated acids (imidazole and methylimidazole acetic acid), and methylhistamine – were identified on thin layer chromatograms after the injection of ^{14}C-histamine (Figure). 2 major peaks – ribosylimidazole acetic acid and imidazole acetic acid – were identified after the injection of ^{3}H-imidazole acetic acid (not shown in the Figure). Upon treatment with aspirin, the ribosylimidazole acetic acid peak disappeared in all species (Figure) and reappeared once aspirin treatment was stopped.

In humans, after treatment with 4 × 900 mg or 4 × 600 mg aspirin daily, the excretion of ^{14}C-ribosylimidazole acetic acid decreased from 18% to 4% (percent of injected label). The output of unconjugated acids increased by a corresponding amount. The excretion of histamine and methylhistamine was unchanged (Table). In all subjects, ribosylimidazole acetic acid reappeared in urine when aspirin was stopped.

In rats, the ribosyl derivative of imidazole acetic acid accounted for the major part of the label in urine (Figure and Table). Treatment with as little as 25 mg/kg aspirin reduced urine excretion of the ribosyl conjugate by 50–60%; higher doses of aspirin produced an even greater reduction[1]. As in humans, the excretion of unconjugated acid increased by a corresponding amount while that of methylhistamine and histamine was unchanged (Table).