A) HMT activity in the supernatant and particulate fractions of various regions of the rat brain during development, expressed in nmoles/g tissue/60 min

	2 Days		6 Days		10 Days		Adult	
Brain regions	S	P	S	P	S	P	S	P
Cortex	33.7±27.9	13.7±10.1	68.5±18.1 [c]	19.7±11.0 [b]	71.1±16.8	22.7±12.5	62.1±18.5	19.7±12.2
Hypothalamus	86.8±15.0	62.3±15.3	109.1±19.4 [c]	86.6±19.2 [c]	182.9±19.2 [c]	131.0±17.4 [c]	163.2±17.7 [b]	99.0±18.4
Diencephalon	75.0±18.0	22.9±18.1	84.6±14.0	27.1±15.1	107.1±17.1 [c]	32.0±15.0	89.1±20.4	35.9±14.4
Brain stem	105.3±17.0	22.1±15.3	122.3±15.9 [a]	38.7±19.2 [c]	125.6±19.1	36.6±12.9	110.2±18.7	33.2±12.8
Cerebellum	62.8±14.9	28.9±18.9	63.1±27.6	30.1±13.4	118.2±18.3 [c]	42.6±15.3 [b]	98.5±20.4	39.4±15.4

Each number is the mean value of the 10 animals ± SD.

B) PNMT activity in the supernatant and particulate fractions of the hemispheres and brain stem of the rat brain during development expressed in nmoles/g tissue/60 min

	9 Days		15 Days		21 Days	
Brain regions	S	P	S	P	S	P
Hemispheres	1.31±0.72	2.53±0.96	1.63±0.81	3.88±1.23	1.78±0.75	4.33±1.17
Brain stem	2.10±0.90	4.83±1.08	1.59±0.63	5.82±1.14	1.82±0.66	5.32±0.99

[a] $p < 0.02$; [b] $p < 0.01$; [c] $p < 0.001$
Each number is the mean value of 10 animals ± SD.

Our results suggest that the decline of brain histamine content and the apparent increase of histamine turnover after the 1st week of life, might be the result of enhanced methylation by the increased activity of HMT during this period. This is compatible with the observation of SCHWARTZ et al.[18] that endogenous histamine of rat brain rises sharply after inhibition of histamine methylation. Factors other than metabolism rate, such as modifications in compartmentation[17] and release of histamine, may also contribute to the above developmental changes.

PNMT shows almost a 2-fold increase in specific activity between 17 days of gestation and 14 days after birth. No significant change was observed after the end of the 2nd week, while, as shown by other investigators, tyrosine hydroxylase and dopamine-β-hydroxylase, two other enzymes of the same pathway, show a further increase during the following 2 weeks of life[19, 20].

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Alteration of Cholesterol Synthesis in Rat Liver as Induced by 4-Methyl-5-Hydroxy Valeric Acid

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Summary. The rate of cholesterogenesis in rat liver, measured by the incorporation of labelled acetate or mevalonate into cholesterol, was significantly supressed by the use of 4-methyl-5-hydroxy valeric acid sodium salt. This effect cannot be explained by changes in HMG CoA reductase activity.

It is generally agreed that the rate-limitting step in hepatic cholesterogenesis is the formation of mevalonic acid from HMG CoA (3-hydroxy-3-methylglutaryl CoA)[1]. This reaction, which is catalyzed by the enzyme HMG CoA reductase (EC 1.1.1.34), is altered by dietary cholesterol content[2], hormonal conditions[3] and drugs like cholestyramine[4]. Several compounds, including certain valeric acid derivatives[5], have been tested as hypolipemic agents, some of them having important inhibitory effect on hepatic cholesterogenesis by mechanisms not always involving direct action of the drug upon the regulatory step[6].

This report deals with the effect of 4-methyl-5-hydroxy valeric acid sodium salt (MHVA) on hepatic cholesterol biosynthesis in the rat. MHVA is obtained as a side product in the chemical synthesis of pregnenolone from diosgenin[7].

Materials and methods. Male Wistar rats, 120 to 150 g, maintained on standard laboratory chow supplemented as indicated, were used in all experiments. 4-methyl-5-hydroxy valeric acid was kindly supplied by Dr F. GIRAL (Nat. Univ. Mexico, Sch. Chem.). 1-^{14}C-sodium acetate (62 mCi/ mmole) and DL-2-^{3}H-mevalonic acid lactone (82 mCi/mmole) were obtained from The Radiochemical

Centre. 3-^{14}C-3-hydroxy-3-methylglutaric acid (2.19 mCi/mmole) was obtained from New England Nuclear Corp. All other chemicals were analytical reagents.

Liver slices, approximately 1 mm thick, were placed in 2.5 ml of Krebs Ringer phosphate buffer, pH 7.4, and incubated 2 h at 37 °C with either 5 μCi of sodium acetate or 2 μCi of DL-mevalonic acid lactone. MHVA was dissolved in 0.2 *M* phosphate buffer, pH 7.4. The reaction was stopped by the addition of 30% alcoholic KOH and 5 mg of cholesterol as carrier. For the in vivo experiments, the animals received daily for 4 days, by stomach tube, the MHVA solution in 0.2 *M* phosphate buffer, pH 7.8, or an equivalent volume of buffer solution. The administration was done at approximately the same time of the day. On the 4th day, 1 h after the oral administration, each rat received i.p. either 5 μCi of sodium acetate or 2 μCi of DL-mevalonic acid lactone per 100 g of body weight. The rats were killed 1 h later. The isolation of liver cholesterol was carried out as indicated for the liver slices, the carrier omitted. Total serum cholesterol was measured[8]. Cholesterol from the liver was isolated with digitonin and radioactivity was measured in a liquid scintillation spectrometer[9]. The activity of HMG CoA reductase in the liver microsomes was assayed[10], previously preparing the anhydryde of 3-^{14}C-3-hydroxy-3-methylglutaric acid[11]. Microsomal protein was determined with Folin-phenol reagent[12].

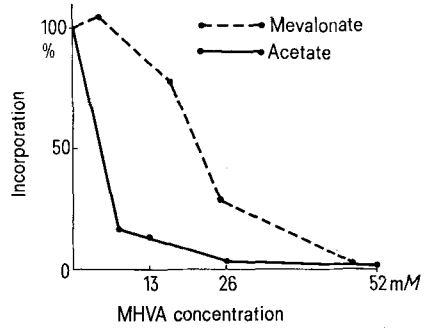

Fig. 1. In vitro incorporation of labelled acetate or mevalonate into cholesterol in liver slices. The results represent the activity in cholesterol from 200 mg liver slices, expressed as the percentage of the control value: 14061 ± 2413 dpm per 200 mg of liver slices and 112121 ± 16758 dpm per 200 mg of liver slices for acetate and mevalonate, respectively. Each point of the curve corresponds to the mean of 3 determinations.

Results. The addition of different concentrations of MHVA produced a decrease in acetate incorporation into cholesterol in liver slices. The effect was dose-dependent (Figure 1). In the case of mevalonate incorporation into cholesterol, MHVA was also inhibitory but at higher concentrations (Figure 1).

MHVA administered parenterally had no effect on acetate incorporation at doses that ranged from 0 to 1 m*M* per 100 g of body weight. On the other hand, oral administration had a critical influence on cholesterol synthesis (Figure 2). The doses-response curve shows that a concentration of 0.32 m*M* per 100 g of body weight produced 20% inhibition of acetate incorporation into cholesterol. The maximum inhibition was obtained with doses from 0.97 to 1.95 m*M* per 100 g of body weight. In the rest of the experiment 1.88 m*M* per 100 g of body weight was employed. This dose inhibits 30% mevalonate incorporation into cholesterol (84 277 ± 5 280 dpm vs 57 253 ± 87 dpm, per g of fresh liver, in the control and MHVA-treated rats, respectively, $p < 0.05$, *t*-test). Serum

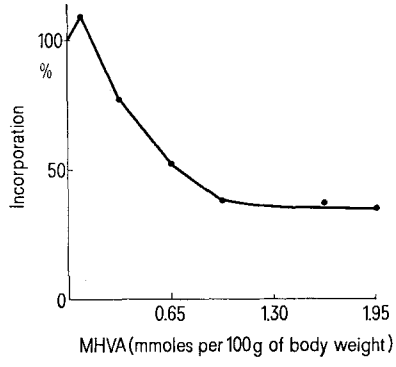

Fig. 2. In vivo incorporation of sodium acetate into cholesterol in the liver of rats treated with MHVA. The results are expressed as the percentage of the control value: 810 ± 121 dpm per mg of cholesterol. Each point corresponds to the mean of 4 rats, which received orally the indicated MHVA dose in phosphate buffer, pH 7.8 for 4 days. At the 4th day, 1 h after the MHVA administration, each rat received i.p. 5 μCi of (1-^{14}C)-sodium acetate. The animals were sacrificed 1 h later. Cholesterol was isolated in the liver as the digitonide.

Table I. *In vivo* incorporation of radiolabeled acetate into cholesterol in rat liver

Group	m*M* g^{-1} h^{-1}	*p*
Control (4)[a]	1600 ± 50[b]	
MHVA (4)	511 ± 87	< 0.001[c]
1% cholesterol (4)	656 ± 241	< 0.01
1% cholesterol + MHVA (4)	386 ± 130	< 0.001
1% diosgenin (4)	4624 ± 338	< 0.001
1% diosgenin + HMVA (4)	2242 ± 432	> 0.10

The animals received the diets indicated for 6 days. Every day for 4 days before their sacrifice 1.88 m*M* per 100 g of body weight of MHVA were administered orally. 1 h before their killing each rat received i.p. 5 μCi per 100 g of body weight of (1-^{14}C)-sodium acetate, Spec. act. 0.166 mCi/m*M*. The results are expressed as nm of acetate incorporated into cholesterol per gram of liver per h. [a] Number of rats of each group is indicated in parenthesis. [b] Standard error of the mean. [c] *p*-value when compared individually with control group, student *t*-test.

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cholesterol in MHVA-treated rats was 84.8 ± 4.1 mg/100 ml vs 88.6 ± 5.8 mg/100 ml in the control group ($p > 0.10$, t-test). The liver cholesterol content in MHVA-treated rats was also similar to that of control rats (265 ± 17 mg/100 g vs 260 ± 4 mg/100 g of fresh liver in the control group). On the contrary, in rats with a 1% cholesterol diet, the hepatic cholesterol content showed an 8% increase. Table I shows that a diet 1% cholesterol plus 0.5% sodium deoxycholate, offered for 48 h, produced 60% inhibition of acetate incorporation into hepatic cholesterol. The administration of MHVA to cholesterol-fed rats produced 77% inhibition. MHVA-treated rats showed 69% inhibition of acetate incorporation into cholesterol. Diosgenin-fed rats showed, as indicate previous reports[9], increased incorporation of acetate into cholesterol. The administration of MHVA to diosgenin-fed rats partly reversed the action of diosgenin. Finally, as MHVA inhibits both acetate and mevalonate incorporation into cholesterol, this effect does not correlate with a direct action on HMG CoA reductase activity. This was confirmed when the enzyme was assayed in liver microsomes. MHVA did not inhibit the activity of HMG CoA reductase (Table II). On the other hand, a 1% diosgenin diet produced a moderate, statistically significant increase in the activity of the enzyme. Also, in agreement with previous reports[13], a high cholesterol diet inhibited the activity of HMG CoA reductase.

Table II. Activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver of MHVA-treated, diosgenin-fed or cholesterol-fed rats

Group	Activity of HMG CoA reductase	
Control (9)[a]	5.47 ± 0.52[b]	
MHVA (4)	5.31 ± 0.28	$p > 0.10$
1% diosgenin (10)	9.48 ± 3.68	$p < 0.005$
1% cholesterol (4)	2.15 ± 0.15	$p < 0.005$

The animals indicated received for 4 days before their sacrifice 1.88 m*M* per 100 g of body weight of MHVA, dissolved in phosphate buffer pH 7.8, or the equivalent volume of phosphate buffer alone. The animals of groups 3 and 4 received the diets indicated 48 h before their sacrifice. Reductase activity is expressed as nm of mevalonate formed per h per mg of microsomal protein. Generally 4 trials were run from each animal. The assay system consisted of 0.8 ml containing 100 m*M* phosphate buffer pH 7.2, 3 m*M* $MgCl_2$, 3 m*M* NADP, 10 m*M* glucose 6 phosphate, 2.5 units glucose 6 phosphate dehydrogenase, 50 m*M* reduced glutathione, 0.2 m*M* (3-^{14}C)-HMG CoA and 0.7–0.9 mg microsomal protein. [a] Number of rats of each group is indicated in parenthesis. [b] Standard error of the mean.

Discussion. Although we cannot ascertain the mechanism of action of MHVA with the data presented in this paper, the results show that this valeric acid derivative inhibits hepatic cholesterol synthesis from acetate or mevalonate without inhibiting HMG CoA reductase activity. On the other hand, diosgenin-fed rats showed a moderate increase in HMG CoA reductase activity compared with the 3-fold increase in acetate incorporation into cholesterol, with no change in mevalonate incorporation into cholesterol[9]. Therefore diosgenin increases cholesterol biosynthesis in the liver at least as a result of increasing the activity of the rate-limiting step in biosynthetic pathway. On the contrary, MHVA inhibits hepatic cholesterol synthesis, but this effect is not a direct action on the activity of HMG CoA reductase. We cannot assure the MHVA action mechanism at the present state of our research, but the effect reported here contributes to the developing of new orally-active agents for human therapy in cholesterol metabolism disorders.

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Effect of Benzimidazole on Nicotinamide Adenine Dinucleotide Phosphate Phosphomonoesterase Activity in Wheat Leaves[1]

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Summary. Nicotinamide adenine dinucleotide phosphate phosphomonoesterase was isolated and partially purified from wheat (*Triticum aestivum* L. var. Selkirk) leaves. The enzyme had K_{NADP} value of 1.4×10^{-4} *M* and a pH optimum of 5.9. *In vitro* activity of this enzyme was unaffected by precursors of NAD (nicotinamide and nicotinic acid) or cytokinins (kinetin and benzimidazole). However, when detached wheat leaves were treated with solutions of these compounds, the precursors lowered the specific activity while the cytokinins enhanced the activity. It is suggested that spatial separation and compartmentation of the enzyme and its substrate NADP account for the similar effect of benzimidazole on both.

Earlier investigations in this laboratory into the effects of benzimidazole on the metabolism of excised leaves presented a varied and complex pattern of its influences[3]. Amongst these are the effect of benzimidazole on structure and integrity of chloroplasts[4] and the metabolism of nicotinamide nucleotides[5]. A marked increase was reported in NADP content and in the NADP/NAD ratio in detached leaves of wheat (*Triticum aestivum* var. Selkirk) treated with solutions of benzimidazole or kinetin[6]. Benzimidazole treated leaves fed with radioactive precursors accumulated radioactivity in NADP > NAD, while leaves floated on water accumulated the radioactivity in NAD > NADP[5,7]. However NAD but not NADP was reported to accelarate the senescence of chloroplasts in plasmolyzed protoplasts of *Elodea* leaves[8]. YOSHIDA's work was extended in this laboratory to detached unplasmolyzed leaves of *Elodea* and wheat. In both cases NAD but not NADP accelerated the senescence (as measured by chlorosis) of detached leaves and benzimidazole overcame this effect[7]. These studies suggest that the senescence of detached wheat leaves and the effect of cytokinins on senescence are directly or indirectly connected to the ratio of the concentrations of NAD and NADP in wheat leaves. NAD kinase (EC 2.7.1.23) which phosphorylates NAD to NADP[9] and NADP-phosphatase (EC 3.1.3.2) which hydrolyzes NADP to NAD[10,11] appear to play important