223. The Stimulation of the Cholesterol Synthesis in Rat Liver by Hydrocortisone

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Summary. A single dose of 1 mg hydrocortisone per rat stimulates the incorporation of labeled acetate into the cholesterol of the liver by a factor of 2.7 measured 18 hours after the administration of the hormone and 2 hours after the tracer dose of labeled acetate.

Information on the influence of glucocorticoids on the cholesterol synthesis is limited. Recently it was observed that hydrocortisone inhibits the β-hydroxy-β-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), the enzyme responsible for the formation of mevalonate in the reaction sequence of the steroid synthesis, and it was concluded that hydrocortisone thus reduces the cholesterol synthesis in rat liver [1] [2].

In previous investigations on the influence of fasting, of cholesterol feeding and of a lipogenic diet, carried out in vivo, no indications could be found, that HMG-CoA reductase plays a key role in regulating the cholesterol synthesis in rat liver, as it is widely assumed [3] [4]. The significance of results obtained on the activity of HMG-CoA reductase for measuring an inhibition or a stimulation of the cholesterol synthesis seemed therefore questionable.

The results reported now on the influence of hydrocortisone on the cholesterol synthesis were obtained with the classical isotope technique, by using labeled acetate as tracer substance in vivo. For getting results as reliable as possible not only the incorporation of the radioactivity into a purified fraction of cholesterol of the liver was measured, but the cholesterol was also isolated in pure form for a direct determination of the 'specific' radioactivity.

Materials and Methods. – Rats with an average weight of 170 g were used and were treated with 1 mg hydrocortisone each by intraperitonal injection (i.p.) dissolved in 0.1 ml ethanol 18 h before sacrificing the animals.

45 μ Ci labeled 1-[^{14}C]-acetate (49 μg) dissolved in 0.1 ml H_2O were administred i.p. per rat of the controls and experimental group consisting of 5 rats each, 2 h before slaughtering.

The pooled livers of each group were homogenized and extracted with a 4-fold volume of methanol/chloroform 1:1 under reflux during $1^1/_2$ h at 70°. The total filtrates were concentrated almost to dryness by vacuum destillation and saponified with a double volume of 15% KOH in methanol/H_2O 1:1 under reflux during 45 min at 85°. After extraction of the unsaponifiable the washed and dried petroleum ether extract was evaporated to dryness and its weight was determined. $^1/_{20}$ of each unsaponifiable fraction was used for TLC. on silica gel with dichloromethane. The total scraped material of the cholesterol zones was extracted with ethyl acetate

and its radioactivity was measured. For isolating the cholesterol, the unsaponifiables were chromatographed on alumina I inactivated with 7% water. The fractions eluted with petroleum ether/ether 95:5 were discarded. The cholesterol was eluted with petroleum ether/ether 8:2. The dried substances were crystallized from ethanol until constant radioactivity was achieved.

Results and Discussion. – Both the incorporation rate of labeled acetate and the specific radioactivity of the isolated cholesterol are enhanced by hydrocortisone. The incorporation rate was increased by a factor of 2.7, the specific radioactivity by a factor of 1.6 (table). The difference of these figures is in agreement with the ob-

Table

	control group (A)	hydrocortisone group (B)	B/A
Total incorporation of radioactivity from labeled acetate into liver cholesterol per rat	61960 dpm	165196 dpm	2,7
Specific radioactivity of liver cholesterol	8239 dpm/mg	13186 dpm/mg	1,6

served augmentation of the unsaponifiable of the liver from 20 mg (control group) to 30 mg (experimental group) indicating a corresponding accumulation of the cholesterol in the liver by hydrocortisone. The radioactive cholesterol synthesized from labeled acetate during the two hour period is thus more diluted by inactive earlier synthesized cholesterol in the experimental than in the control group.

The stimulation of the cholesterol synthesis by hydrocortisone contrasts to the reported inhibition of the HMG-CoA reductase [1] [2] and gives thus additional indications for the invalidity of the generalized assumption of a key role of this enzyme in regulating the cholesterol synthesis in rat liver.

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