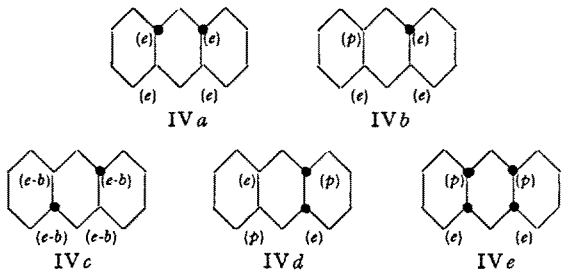
tho(p) bonds, the ring involved may be forced into the boat conformation.

According to the above rules the six perhydrophenanthrenes have the following order of stability: III*e* [4(*e*)] > III*b* and III*d* [3(*e*), 1(*p*)] > III*c* [2(*e*), 2(*p*)] > III*a* [2(*e*), 2 meta(*p*)]; and III*f* [4(*e*–*b*)] < III*b* and III*d*.



Similar analysis of the perhydroanthracenes leads to the following order of stability: IV*a* [4(*e*)] > IV*b* [3(*e*), 1(*p*)] > IV*d* [2(*e*), 2(*p*)] > IV*e* [2(*e*), 2 meta(*p*)]; and IV*c* [4(*e*–*b*)] < IV*b*. Therefore that isomer, m. p. 90°, which is formed from IV*e* or IV*b* by the action of aluminium chloride[1] probably corresponds to IV*a*, and the form IV*c* which was suggested by Cook, McGinnis and Mitchell[1] as a possible, although less probable, alternative because it also contains two trans fused ring systems, can be excluded. The boat-boat-boat conformation of IV*e* (m. p. 61°) suggested by these authors might indeed be less stable than the chair-chair-chair form in spite of the 2 meta (*p*) substituents required by the latter.

Zusammenfassung

Ausgehend von neueren Anschauungen über die Stabilität verschiedener Konstellationen (conformations) monozyklischer Ringverbindungen wurden die Verhältnisse bei perhydrierten polyzyklischen Verbindungen diskutiert. Daraus konnten gewisse Regeln über die relative Stabilität der einzelnen Stereoisomeren abgeleitet werden.

[1] J. W. Cook, N. A. McGinnis, and S. Mitchell, J. Chem. Soc. *1944*, 286.

Effect of Urethane on the Incorporation of C^{14} into Animal Tissue

By G. Hevesy, R. Ruyssen, and M. L. Beeckmans[1]

From previous experiments it is known that administration of urethane enhances the incorporation of C^{14} into most tissue fractions of the mouse after injection of labeled acetate[2]. The present paper deals with the effect of urethane on the incorporation of C^{14} into phosphatides, cholesterol, and other tissue fractions of various organs of the mouse.

Experimental

In each experiment two groups of 10 to 15 adult mice of nearly equal weight (20 g) were used. Into all animals was injected intraperitoneally 0·2 ml of sodium chloride solution containing about 0·2 mg sodium acetate labeled in the carboxyl group[3].

After five minutes the urethane group was injected intraperitoneally with 0·15 ml of a 20% aqueous urethane solution, while the control group received 0·15 ml of a physiological salt solution. The animals were killed at different times.

The same organs from each group were combined and frozen in solid CO_2. A small fraction was directly dried at 70°C and measured as total tissue.

The ground organs were extracted with a boiling mixture of ether-alcohol $^1/_3$ for 3 hours. The residue was washed repeatedly with water, then treated twice for 10 minutes at 90°C with a 5% aqueous solution of trichloroacetic acid, the proteins being thoroughly rinsed with water after each treatment; they were then dried by washing with alcohol and ether.

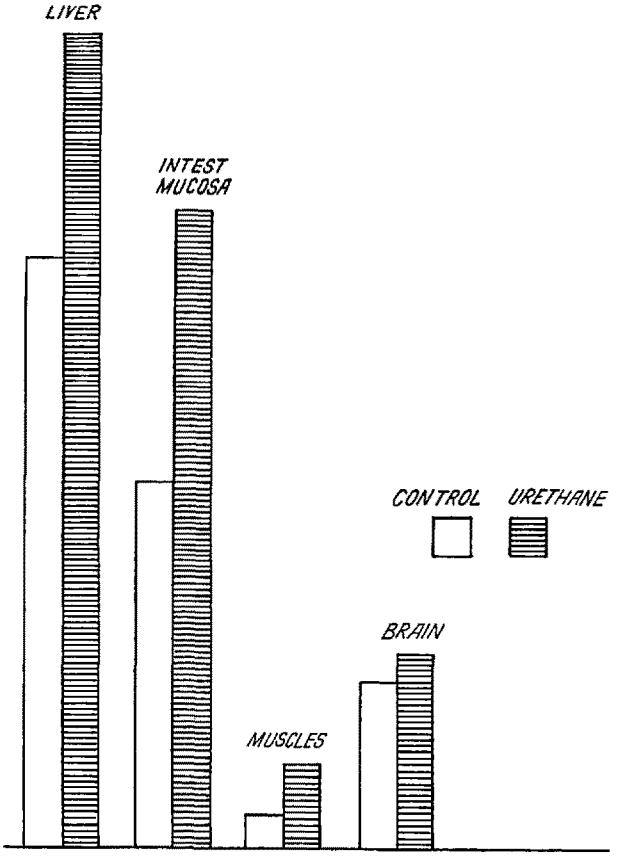


Fig. 1. – Effect of urethane on the incorporation of C^{14} into tissue phosphatides. Mice killed 100 minutes after injection of $NaCH_3C^{14}OO$.

The ether-alcohol solution was evaporated and the residue extracted with petroleum-ether. After evaporation of the petroleum-ether the total fats remained. They were purified from urea and other impurities according to the procedure proposed by Folch and Van Slyke[1]. This procedure involves the loss of some of the phosphatides.

In order to obtain the phosphatides, the total fats were dissolved in petroleum-ether and precipitated with twice the amount of cold acetone and 5 drops of an alcoholic solution of 4·5% $MgCl_2$, precipitation being completed when the preparation had stood for 2 hours in the refrigerator. The phosphatides were centrifuged. The precipitate, dissolved in petroleum-ether, was precipitated again in the same way. After centrifugation the precipitate was dissolved in petroleum-ether and the $MgCl_2$ removed by repeatedly washing with distilled water. After evaporation of the petroleum-ether the phosphatides remained.

[1] J. Folch and D. D. van Slyke, Proc. Soc. Exp. Biol. and Med. *41*, 514 (1939).

[1] Institute for Research in Organic Chemistry, University of Stockholm, and Pharmaceutical Institute, University of Gent.

[2] G. Hevesy, Nature *164*, 1007 (1949).

[3] We are much indebted to Dr. Loos for preparing the labeled acetate.

The supernatants from the precipitation of the phosphatides were combined and evaporated. The residue was saponified by boiling for 8 hours with 10 ml of an aqueous solution of 40% KOH and 20 cm^3 alcohol. After saponification the solution was extracted several times with petroleum-ether. The petroleum-ether solution, concentrated to a small volume, was treated with twice its volume of a 0·5% solution of digitonin in 80% alcohol. The quantity of digitonin added was about 5 × the amount of cholesterol present. The precipitated cholesterol digitonin was washed once with 80% alcohol to eliminate the excess of digitonin, with a mixture of aceton-ether (1:2) and finally with ether, and dried at 37°C. The radioactivity of the dry tissue, fat and protein samples was measured without converting the samples into barium carbonate.

Results and Discussion

Administration of urethane to fed mice (which obtained, however, no food after injection of labeled acetate) increases markedly the incorporation of C^{14} into the phosphatides of the liver, intestinal mucosa, muscles, and brain. The most pronounced effect is shown by the muscle phosphatides, as appears from figure 1. A much less pronounced effect is exhibited by the raw protein fractions (cf. figure 2). Hardly any difference can be observed between the C^{14} uptake of the liver proteins of the urethanized and that of the control mice. Incorporation of C^{14} into the liver cholesterol was found to be increased by 67% and into the intestinal cholesterol by as much as 290%. The brain proteins of the urethanized animal show a slightly lower C^{14} content than those of the controls. The mice were killed 100 minutes after injection of the labeled acetate, which took place 5 minutes before the administration of urethane.

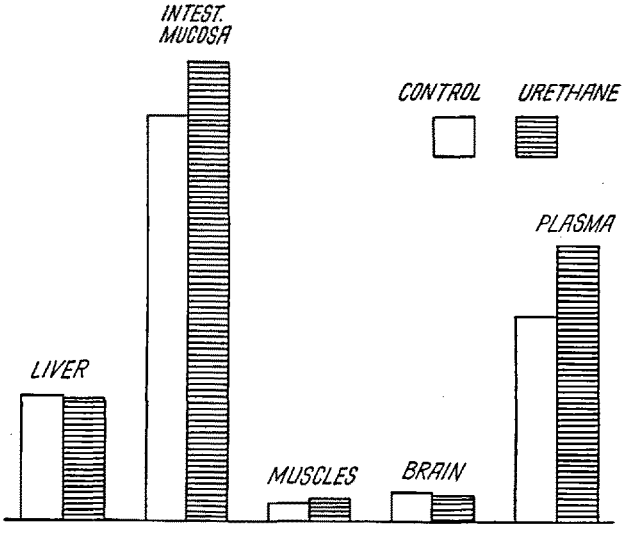


Fig. 2. – Effect of urethane in the incorporation of C^{14} into tissue proteins. Mice killed 100 minutes after injection of $NaCH_3C^{14}OO$.

In another set of experiments the effect of urethane on the incorporation of C^{14} into the total tissue, the total fats, the raw proteins was investigated. The mice were killed 110 minutes after injection of the labeled acetate. In all fractions investigated, urethane administration enhanced the incorporation of C^{14}, though the effect is markedly less pronounced in most of the protein fractions (see figures 3, 4, and 5).

In a third set of experiments, in which the animals were killed $4^1/_2$ hours after injection of acetate, administration of urethane increased the incorporation of C^{14} into all fractions (see figures 6,7,and 8). The increase was markedly less pronounced in protein than in the fatty fractions of the liver and intestinal mucosa.

When producing labeled cholesterol and numerous other labeled compounds by biosynthesis, administration of urethane may thus result in an appreciable increase in the specific activity of the product obtained.

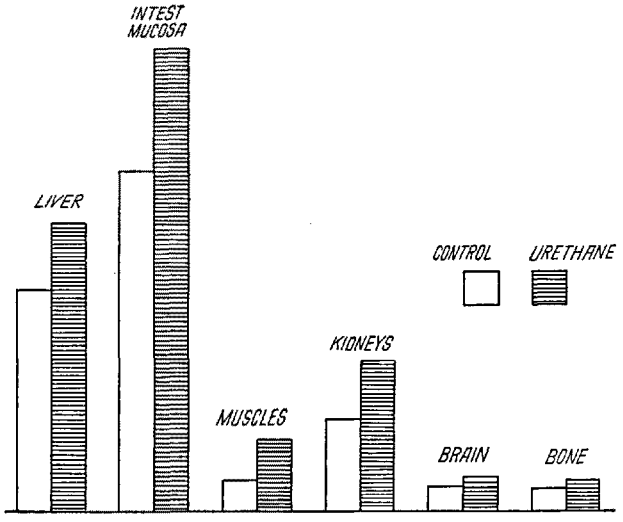


Fig. 3. – Effect of urethane on the incorporation of C^{14} into dry tissue. Mice killed 110 minutes after injection of $NaCH_3C^{14}OO$.

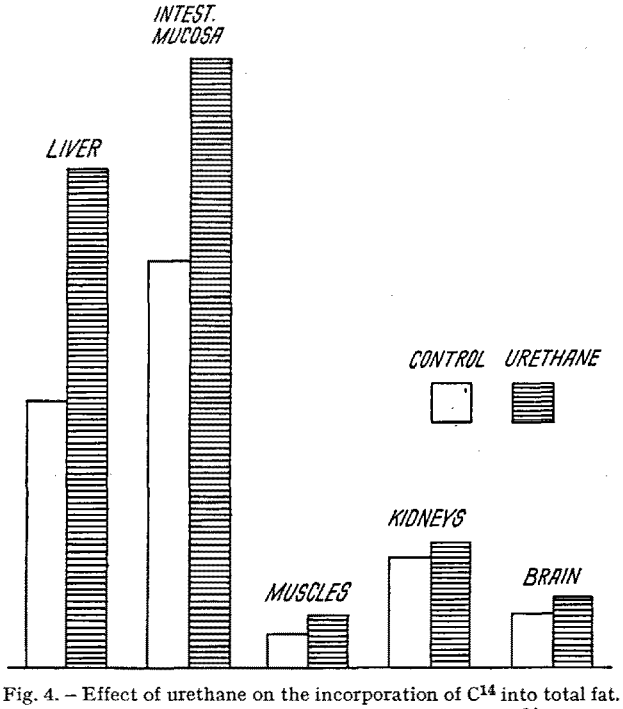


Fig. 4. – Effect of urethane on the incorporation of C^{14} into total fat. Mice killed 110 minutes after injection of $NaCH_3C^{14}OO$.

In another experiment the mice were killed 15 minutes after injection of acetate, respectively 20 minutes after injection of urethane. In this experiment, administration of urethane resulted in a decreased C^{14} uptake by the urethane-treated mice in the total fats of the organs investigated, except in the case of the intestinal mucosa. This result agrees with our expectation. In the early stage of the experiment a decreased metabolic rate leads to a decreased incorporation of C^{14}, while later, when the speci-

fic activity of the C^{14} of the tissue fat decreases (some of the labeled molecules being now renewed in a less active medium) the opposite takes place.

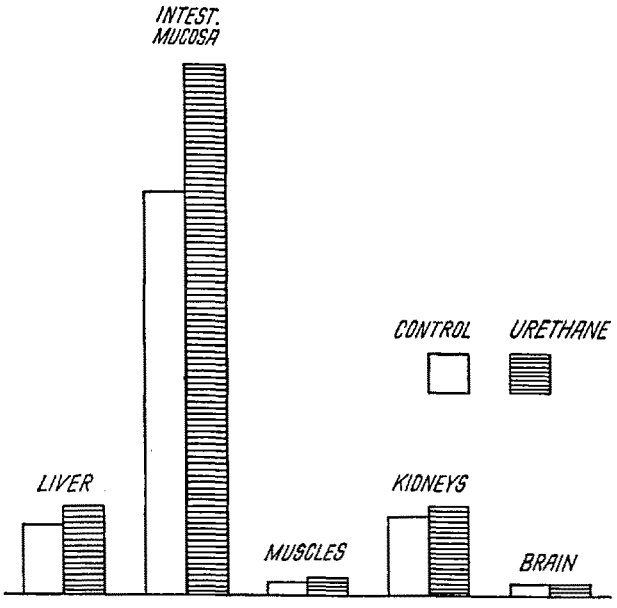


Fig. 5. – Effect of urethane on the incorporation of C^{14} into tissue proteins. Mice killed 110 minutes after injection of $NaCH_3C^{14}OO$.

A decreased rate of incorporation of C^{14} was also found in the case of the slower metabolising compound in the earliest stage of the experiment, while an increased rate was observed in a later phase when the specific activity of

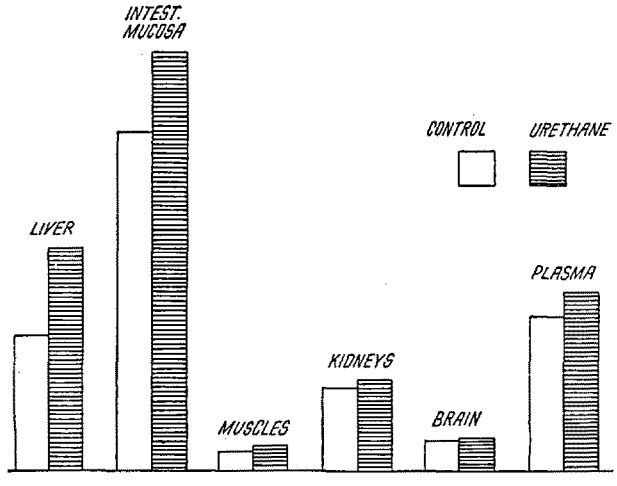


Fig. 6. – Effect of urethane on the incorporation of C^{14} into dry tissue. Mice killed 4·5 hours after injection of $NaCH_3C^{14}OO$.

the exhaled CO_2 was compared after injection of labeled acetate and succinate[1], and furthermore in the study of the effect of dinitro compounds on fat metabolism[2]. Succinate is metabolised at a slower rate than acetate; 2·3 dinitrophenol and similar compounds, when properly dosed, increase the metabolic rate.

As seen in table II, the C^{14} content of the protein fraction, is, however, not diminished in the urethane-injected mouse in the 15 minutes experiment.

[1] R. G. Gould, F. M. Sinex, I. N. Rosenberg, A. K. Solomon, and A. B. Hastings, J. Biol. Chem. *177*, 295 (1949).

[2] M. L. Beeckmans, H. Casier, and G. Hevesy, Arch. Int. Pharmacodyn. Thérap *86*, 33 (1951).

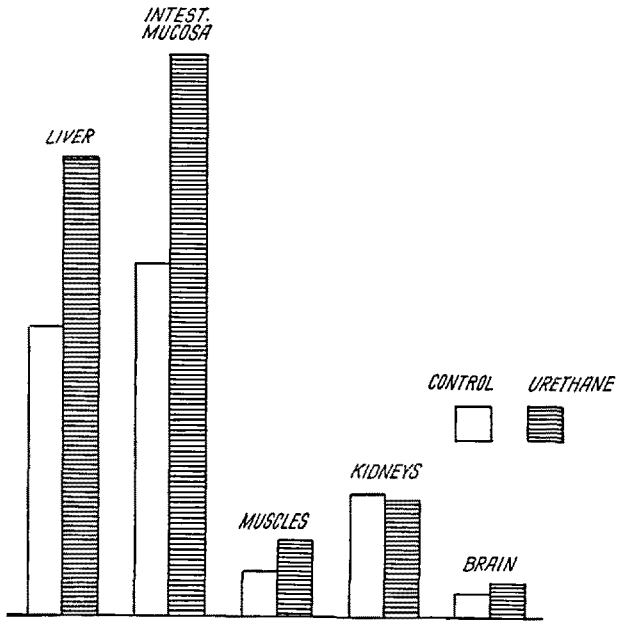


Fig. 7. – Effect of urethane on the incorporation of C^{14} into total fat. Mice killed 4·5 hours after injection of $NaCH_3C^{14}OO$.

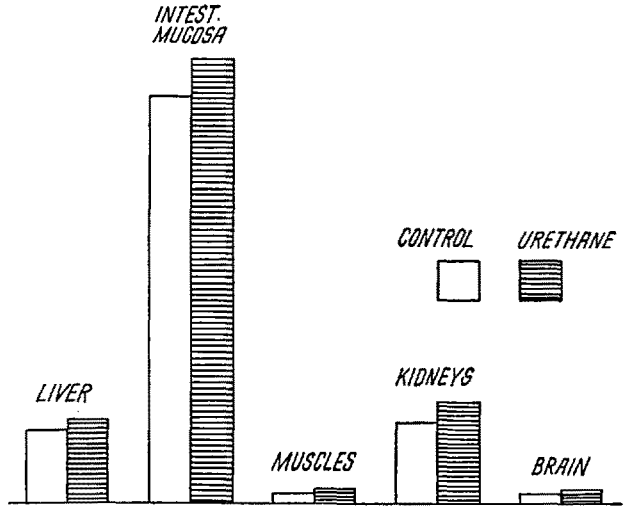


Fig. 8. – Effect of urethane on the incorporation of C^{14} into tissue proteins. Mice killed 4·5 hours after injection of $NaCH_3C^{14}OO$.

Table I

Effect of Urethane on the Incorporation of C^{14} into Total Fats of Organs. Mice killed 15 minutes after intraperitoneal injection of $NaCH_3C^{14}OO$

Organ	Relative C^{14} content of the organ fat of controls.	Percentage change in C^{14} incorporation due to urethane administration
Liver	204 (1005)	– 9·9
Intestinal mucosa	100 (493)	+13·5
Brain	9·1 (45)	– 6·8
Muscles	8·4 (42)	–17·5

50 mg of total fats of intestinal mucosa contain 0·29% of the 172,000 counts of C^{14} injected into a mouse weighing 18 g. The figures in parenthesis indicate the number of counts per minute.

Table II
Effect of Urethane on the Incorporation of C^{14} into the Raw Proteins of Organs

Organ	Relative C^{14} content of the organs of controls	Percentage change in C^{14} incorporation due to urethane administration
Liver	37·4 (195)	+11
Intestinalmucosa	100 (522)	+25
Brain	28 (12·3)	+ 7·3
Muscles	11·7 (27·9)	+ 4·3

50 mg of intestinal mucosa proteins contain 0·33% of the 172,000 counts injected into a mouse weighing 18 g. The intestine is the only organ investigated in which the protein fraction takes up more C^{14} than does the fat fraction. Brain fats take up almost 4 times more C^{14} than do brain proteins.

We investigated also the effect of urethane on the incorporation of C^{14} into tissue fractions of mice which were starved 19–24 hours prior to the administration of labeled acetate. In these experiments the effect of urethane was both less pronounced and less uniform.

Different explanations can be put forward for the enhanced C^{14} uptake by tissue fractions under the action of urethane. Let us, for example, consider the incorporation of C^{14} into the liver fats, the C^{14} content of which is in our experiments largely due to the presence of labeled fatty acids.

About $^1/_4$ of the liver fatty acids was found[1] to be formed from acetate, $^3/_4$ being built up from other precursors. Should urethane interfere with the formation of fatty acid from the last mentioned inactive or slightly active precursors, the fatty acids in the urethane treated mouse would be built up mainly from active acetate and correspondingly would contain more C^{14} than the fatty acids of the controls.

Oxygen consumption is diminished by urethane administration. In the guinea pig 70–130 minutes after injection of 1·4 g urethane per kg body weight, a dose similar to ours, oxygen consumption was found to be reduced by 7·1 ± 1·6%[2] only. Metabolic changes produced by urethane administration are indicated more markedly by following the rate of incorporation of C^{14} into fatty acids than by measurements of oxygen-consumption.

In the grasshoper embryo 60% of the total respiration was found to be sensitive to urethane[3].

Another possible explanation of the enhanced incorporation of C^{14} into the urethane-treated animal is the following. As shown by BLOCH and RITTENBERG[4] administered labeled acetate is strongly diluted by endogenous (in experiments of short duration almost inactive) acetate. They found that in 300 g rat 35 mM acetate is produced per day. We can thus expect the daily production of endogenous acetate by a 20 g mouse to be 2 mM or somewhat more in view of the more rapid metabolism in the mouse. The injected active acetate is thus strongly diluted by inactive endogenous acetate in the body fluids, and similar considerations may apply to the degradation products of acetate.

Should the administration of urethane result in the reduction of the catabolic rate of fatty acids or of proteins and carbohydrates, the catabolism of which leads to the formation of acetate as well, the radioactive acetate would become less diluted by endogenous acetate in the urethanized mouse than in the controls, and the specific activity of the acetate would be higher in the first mentioned animal. If the rate of formation of fatty acids is not influenced, or influenced to a minor extent only, by the presence of urethane, the formation of the same amount of fatty acids in the urethanized animal will lead to a larger C^{14} content. This will be due to the higher activity level of the precursor in the urethane injected mouse.

Under physiological conditions the amount of fatty acid formed corresponds approximately to the amount simultaneously catabolized. This is not necessarily the case in the mouse injected with urethane.

Considering fat metabolism of the carcass only, PIHL and assoc.[1] arrive at the result that a 300 g rat, having a fatty acid content of 20 g, which is turned over with a half time of 18 days, produces 22 mM of acetic acid per day. Thus from this source alone about $^2/_3$ of the daily fatty acid production of 35 mM is already covered. Should the presence of urethane reduce carcass fatty acid metabolism by half of its normal value without influencing the rate of formation of fatty acids, this would lead to an increase of 0·13 percent only in the fatty acid content of the carcass in the course of the experiment taking 100 minutes. Simultaneously the specific activity of the acetate of the urethane-injected mouse would increase to about twice the value found in normal animals, which in turn would lead to a larger incorporation of C^{14} into the fatty acids of the urethane injected mouse.

In experiments of short duration, not only catabolism of fatty acids, but also of proteins and carbohydrates, leads to the formation of practically inactive acetic acid, and an interference with these processes would also cause a reduced dilution of the labeled acetate by inactive endogenous acetate and thus also a higher acetate activity level.

A dilution process of the above mentioned type can, however, not be the only explanation of the difference found in the C^{14} content of the fractions of urethane treated and control mice, as the C^{14} content of proteins is much less influenced than that of the fatty components. Urethane, which is known largely to attack dehydrogenases, presumably interferes with different metabolic steps. By making use of RITTENBERG's and BLOCH's[2] acetylphenylaminobutyric acid method it should be possible to decide whether the C^{14} level of the body acetate of the mouse is influenced by administration of urethane, and to what extent.

After the lapse of 100 minutes the C^{14} content of cholesterol in the intestinal mucosa was found to be about seven times as active as that of the liver. A similar result was recently obtained by POPJAK and BEEKMANS[3], who found, in experiments taking 20 hours or more, cholesterol extracted from the small intestine to contain three to five times more C^{14} than cholesterol in the liver.

In the liver, however, a few minutes after the beginning of the experiment we find the specific activity of cholesterol to be much lower than the corresponding value of the total fatty acids. In experiments of long duration the specific activity of cholesterol was found to be markedly higher than that of the fatty acids[4]. This difference is presumably due to the presence of a rapidly renewed small fatty acid fraction in the liver[5] which is detectable only in experiments of short duration.

The authors wish to acknowledge the effective assistance of Miss DE ELLIOTT, Miss HOECK and Mrs. VAN DE PUTTE.

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