ments of lowest mobility (azo pigments $B_{4\cdot3}$, $B_{5\cdot3}$ and $B_{6\cdot3}$) were incompletely separated from those of intermediate mobility (azo pigments $B_{4\cdot2}$, $B_{5\cdot2}$ and $B_{6\cdot2}$) and were therefore not obtained pure. Second, elution of the subfractions from the plates resulted in partially solubilizing the chromatographic support, thus contaminating the preparations with depolymerized material.

Summary. A thin-layer chromatographic system on polyamide was developed that allowed a further fractionation of previously isolated azo pigments from bile. Aldobiouronic, hexuronosylhexuronic and pseudoaldobiouronic acids involved in bilirubin conjugation thus appear to be heterogeneous. Structural elucidation is in progress[4].

Zusammenfassung. Die Aldobiouronid-, Hexuronosylhexuronid- und Pseudoaldobiouronidkonjugate des Bilirubins lassen sich durch Polyamid-Dünnschichtchromatographie in je 3 Subfraktionen unterteilen.

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Evidence of Major Role of the Intestine in Cholesterol Synthesis in the Adult Male Rat

It has recently been shown by means of indirect proof that the digestive tract is the source of 65% of the cholesterol synthesized per day in the rat[1]. Usually the liver has been considered responsible for producing most of the body cholesterol[1]. We felt that it was essential, in order to challenge this traditional theory, to provide a direct proof of the major role played by the intestine.

Material and methods. Our argument runs as follows: acetyl CoA is used by several pathways, some of which are irreversible, such as oxydation and cholesterol synthesis, whereas other pathways, such as the synthesis of fatty acids coupled with their breakdown, are reversible. If we label acetyl CoA at a starting point 0, no labelled acetyl CoA can be produced by breakdown of fatty acids, provided the experimental time is sufficiently short. From the radioactivity standpoint, therefore, reversible systems can be linked to irreversible systems. Hence the distribution of labelled acetyl CoA in each metabolic route where it is used depends on the rate of utilization alone. If m_S is the rate of cholesterol synthesis, m_U the sum of the rates of all types of acetyl CoA utilisation, R_S the radioactivity of the synthesized sterols and R_U the radioactivity of all compounds in all the metabolic routes involved, we obtain

$$\frac{m_S}{m_U} = \frac{R_S}{R_U}$$

The calculation of m_S is valid no matter what variations there may be in the specific radioactivity of the acetyl CoA or in the distribution of radioactivity amongst the various compounds within one and the same metabolic chain.

Employing this method, we used adult male rats weighing 350 g on a control feed[2]. Acetate-1-^{14}C was infused by a catheter placed into the jugular vein some days before[3]. Only rats having a normal food intake during the days preceeding the experiment were used. Specific radioactivity of the expiratory $^{14}CO_2$ was recorded continuously[4]. An equilibrium value was attained from the 3rd h of infusion onwards (Figure 1). 3 groups of 5 rats each were killed at the end of the following periods of infusion respectively: 3.5 h, 6 h, and 10 h. We then determined the radioactivity of free sterols (R_F) and esterified sterols (R_E) from 22 organs or fragments of tissue[5]. These crude radioactivities were corrected to a same specific equilibrium radioactivity of the animal's $^{14}CO_2$ (1 μCi/1% CO_2). From this it was possible to calculate the activities of the free and esterified sterols of the whole rat[2] and their sum (R_S) (Table I). Furthermore, R_U is the difference between the radioactivity of acetate administered and that of the unused labelled acetyl CoA. The latter value can be determined, since we know the pool of acetyl CoA (M) (see below), and its specific radioactivity calculated from that of the $^{14}CO_2$[6].

Finally, to determine the m_U value with precision, we injected 50–100 μCi of 1-^{14}C-acetate into the jugular vein of 8 additional rats and continuously recorded the logarithm of the specific radioactivity of the $^{14}CO_2$ (Figure 2). In a first approximation, the curves can be decomposed into 2 straight lines[6]. The first (steep slope) corresponds to the overall utilization of acetyl-CoA. From this line the fractional turnover rate of acetyl CoA (K = 1.37 h^{-1}) and the size of its pool (M = 3.08 mM) can be determined, and from this the rate of turnover

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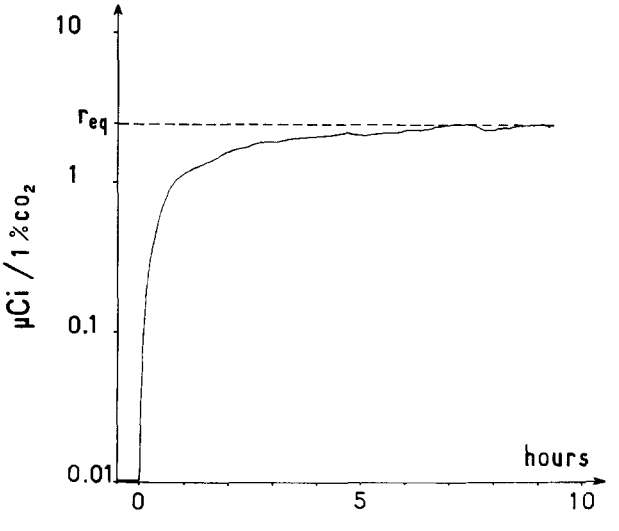

Fig. 1. Logarithm of specific activity (μCi/1% CO_2) of $^{14}CO_2$ expired by a rat as a function of time (h) during an i.v. infusion of 1-^{14}C-acetate. r_{EQ}: specific activity at equilibrium.

Table I. Mass of cholesterol synthesized by rats. Calculation of data and results

Duration of experiment (t)	3.5 h	6 h	10 h
Radioactivity (dpm) of acetate administered (0-t)	$(188 \pm 21.6) \times 10^6$	$(322 \pm 37) \times 10^6$	$(537 \pm 61.6) \times 10^6$
Radioactivity (dpm) of acetate utilised (R_U) (0-t)	$(161 \pm 15.7) \times 10^6$	$(295 \pm 28.8) \times 10^6$	$(510 \pm 49.8) \times 10^6$
Radioactivity (dpm) of whole rat sterols (R_s) (0-t)	$(582 \pm 94) \times 10^3$	$(2806 \pm 446) \times 10^3$	$(3185 \pm 692) \times 10^3$
$R_s/R_U \times 100$	0.36 ± 0.09	0.71 ± 0.18	0.62 ± 0.19
Mass (mg) of cholesterol synthesized by rat (0-t)	1.36 ± 0.52	4.6 ± 1.7	6.7 ± 2.9
Average mass of cholesterol synthesized per h/rat (mg/h) (0-t)	0.39 ± 0.15	0.77 ± 0.28	0.67 ± 0.29

Table II. Radioactivity (dpm $\times 10^{-3}$) of free and esterified sterols (R_F, R_E) contained in the liver, intestine and the whole animal after 3.5 h of 1-^{14}C-acetate infusion. Radioactivity of free and esterified cholesterol of plasmatic origin (R_{PF} and R_{PE}) contained in those organs and the whole animal. Radioactivity of free and esterified sterols synthesized in those organs and remaining in situ (R_{SF}, R_{SE}).

	R_F	R_{PF}	R_{SF}	R_E	R_{PE}	R_{SE}
Liver	87.5 ± 2.6	28.3 ± 2.7	59.2 ± 5.3	19.8 ± 1.9	4.7 ± 2.6	15.1 ± 4.5
Intestine	269 ± 41	1.4 ± 0.3	267.6 ± 41.3	6.9 ± 1.9	0.28 ± 0.11	6.6 ± 2
Whole animal	472.5 ± 38.9	41.1 ± 4.1	431.4 ± 43	109.2 ± 55.4	7.7 ± 3.6	100.5 ± 59

can be deduced: $m_U = KM = 4.2$ mMh^{-1}. The second slope probably reflects the redistribution of activity from fatty acids. Let us remark that the extrapolated specific activity of $^{14}CO_2$ furnished by this pool represents only 1% of the initial specific activity of $^{14}CO_2$.

Results and discussion. The average quantity of cholesterol synthesized per rat per hour during the 3.5 h experiment is lower than the quantities obtained in the 6- and 10-h experiments (Table I). From Figure 2, it is clear that the contribution of recycled ^{14}C-acetyl-CoA for further utilization for cholesterol synthesis is negligeable whatever the sacrifice time of our experiments, as the experimental period is short. The difference observed between the quantities obtained in the 6- and 10- h experiments can most probably be explained by the circadian variation in the rate of cholesterol synthesis which is higher by night than by day[7]. In our work, the 3.5 h experiment was performed by day, whereas the 6- and 10-h experiments were divided roughly equally between day and night. On the basis of preliminary assessment, the average hourly rate obtained in the last 2 sets of experiments would appear to be representative of the average hourly rate for the whole day. We conclude that rats synthesize 16 to 18 mg of cholesterol per day. It should be noted that these figures are identical to those obtained using an isotopic equilibrium method[8].

In respect of the different organs, the method used may only be regarded as strictly reliable provided 2 conditions are fulfilled. First, we should only consider the radioactivity of synthesized molecules remaining in situ. For this purpose we need to know the manner in which labelled cholesterol from the plasma is transferred to the various organs under precisely the same experimental conditions. Preliminary results concerning this problem are known[3]. (Final publication by F. D'HOLLANDER, T. MAGOT and F. CHEVALLIER, in preparation). Radioactivity of free and esterified cholesterol of plasmatic origin (R_{PF} and R_{PE}) contained in each organ is calculated over periods indicated. Subtracting them from the radioactivity of free and esterified sterols (R_F and R_E), we obtain the radioactivity of synthesized sterols remaining in the organs (R_{SF} and R_{SE}). Results pertaining to the liver and the intestine for the 3.5 h experiment can be seen in Table II. Furthermore, the method used may only be regarded as strictly reliable if there is no elimination of radioactive sterols and biliary acids by the considered system (whole rat or individual organ). This elimination by the whole rat is negligible during the experiments and

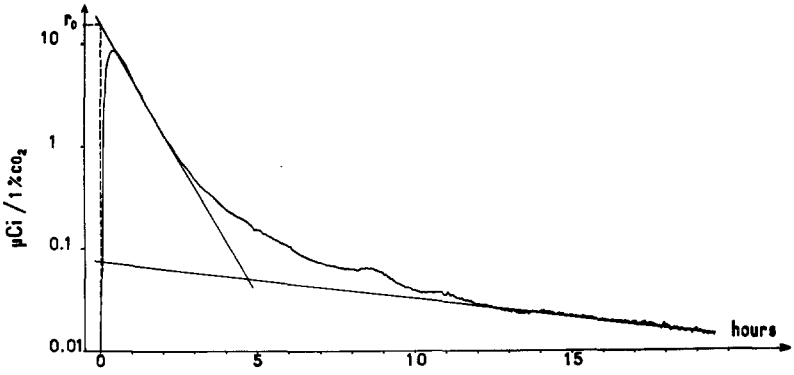

Fig. 2. Logarithm of specific activity (µCi/1% CO_2) of $^{14}CO_2$ expired by a rat as a function of time (h) after an i.v. injection of 1-^{14}C-acetate. r_o: extrapolated specific activity at zero time.

has no appreciable effect upon the calculations shown above. But the elimination by an individual organ must be taken into account. We know that labelled cholesterol synthesized in the organs passes into the plasma and its radioactivity is equal to the sum of R_{PF} and R_{PE} of the whole animal (or R_{SP}) (Table II). A certain adjustment therefore had to be made, so we divided radioactivity R_{SP} in proportion to the synthesized sterols remaining behind in each organ. For intestine, for instance, we have

$$R_{SP}(\text{intestine}) = \frac{R_{SF} + R_{SE}(\text{intestine})}{R_{SF} + R_{SE}(\text{whole rat})} \times R_{SP}(\text{whole rat}).$$

It should be stressed that the shorter the experimental period, the smaller this correction (like the previous one) has to be, i.e. results obtained from the 3.5 h experiments are subject to the smallest margin of error.

Regardless of whether we take free sterol radioactivity (R_{SF}) or the sum of free and esterified sterol radioactivity ($R_{SF} + R_{SE}$), and whether we make the second adjustment (R_{SP}), it becomes apparent that the intestine is the main organ concerned in cholesterol synthesis (Table III). The liver contributes a mere 13.5% to the total. Finally, the part played by the colon and the stomach lies somewhere between 4 and 5% so that it is in the digestive tract that 55–56% of cholesterol synthesis in the rat takes place, a percentage which is close to the one proposed before[1]. So evidence of the major role of intestine in cholesterol synthesis in the adult male rat is furnished. Investigations are carried out for extending this conclusion to female and young male rats, or to various dietary conditions[9].

Table III. Sterols synthesized remaining in situ in free form in the liver and the intestine (m_{SF}). Synthesized sterols remaining in situ but detected in esterified form (m_{SE}). Sterols synthesized and passing into the plasma (m_{SP}). Sterols definitely synthesized ($m_S = m_{SF} + m_{SE} + m_{SP}$) expressed as a percentage of the cholesterol synthesis in the whole rat.

	m_{SF}	m_{SE}	m_{SP}	m_S
Liver	10.2 ± 2.5	2.6 ± 1.2	0.7 ± 0.2	13.5 ± 3.9
Intestine	46 ± 14.5	1.2 ± 0.5	3.5 ± 1.1	50.7 ± 16.1
Whole animal	74.6 ± 19.5	18.7 ± 14	6.7 ± 1.7	100

Summary. By a new in vivo method using 1-^{14}C-acetate, it becomes apparent that the intestine is the main organ concerned in cholesterol synthesis. The liver contributes a mere 13.5% to the total. These results challenge the traditional theory which considers the liver as responsible for producing most of cholesterol synthesized by the rat.

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The Oak Leaf Roller (*Archips semiferanus* Walker) Sex Pheromone Complex: Field and Laboratory Evaluation of Requisite Behavioral Stimuli

The tree defoliating insect *Archips semiferanus* Walker, better known as the oak leaf roller moth (OLR), has caused extensive damage in the forests of the Northeastern United States[1]. Attempts to control this pest began with a comprehensive study of the sexual behavior of the adult moth. In addition to determining that a sex pheromone was present in OLR females[2], over 20 principles[3] were identified in the attractant portion of female abdominal extracts. Previous reports[4] have dealt with chemical analyses and syntheses of the attractants and preliminary investigation of their activity. We wish to report here on the evaluation of these principles by laboratory electroantennogram studies and by field trapping in order to better understand the role of these agents in the sexual message of the oak leaf roller.

A series of 14 carbon monounsaturated acetate isomers which were found in the oak leaf roller female attractant fraction were tested in field traps in Pennsylvania forests. 17 of the 21 isomers tested caught a greater number of male insects than controls (Figure 1). The same set of isomers was tested in the laboratory using the electroantennogram technique; all of the isomers gave better electroantennogram responses than controls (Figure 2). A description of these experiments follow.

Twenty-one Z and E tetradecenyl acetates having double bonds in the 2–5 and 7–13 positions were synthesized, purified by $AgNO_3$ thin layer chromatography and assessed for purity by computerized gas chromatography-mass spectrometry (GC-MS) aided by mass fragmentography[3]. All isomers were found to be better than 97% pure[5]. Field evaluation of the attractancy of these isomers to OLR males was conducted in Moshannon State Forest, Pa. (USA), an area of heavy OLR defoliation, from July 5 to July 16, 1974. 48 vane traps were constructed as previously described[6] and arranged in a square block design with 50 m between each trap. 500 nanograms of each isomer were spotted in a trap beginning July 5 and samples were replenished with the same amount every 2 days until July 16; duplicate traps

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