half of the amount synthesized on the twenty-eighth day of pregnancy.

3. Synthesis of both saturated and unsaturated fatty acids in the foetal liver and extrahepatic tissues (embryonal adipose tissue) has been demonstrated. The fatty acids in the foetal liver contained much less deuterium than those in the extrahepatic tissues; this difference might be due to dilution of fatty acids in the foetal liver by maternal substances. However, when ^{14}C -labelled acetate was given, the fatty acids in the foetal liver contained more ^{14}C than in the foetal carcass. The discrepancy in the results obtained with deuterium and ^{14}C suggests that the fat precursors in the foetal liver and extrahepatic tissues are not identical.

4. The foetal placenta synthesizes all lipids studied, but it also absorbs these from the maternal circulation. The maternal placenta appears metabolically inert, at least towards the end of pregnancy.

5. A method, based on principles developed by Goldwater & Stetten (1947), is presented for the calculation, in isotope experiments, of the rate of

accumulation, synthesis and degradation of a substance in a growing system.

6. Since there was no apparent destruction of fat in the foetus, it is inferred that the primary source of energy in the foetus must be carbohydrate and that growth is characterized not so much by increased rate of synthesis as by reduced rate or absence of degradation.

7. The synthesis of cholesterol and fatty acids in the non-lactating mammae of pregnant rabbits has been shown. Evidence for the specific utilization of acetate for the formation of $\text{C}_4\text{--C}_8$ acids in the mammae is also presented (see also Popják *et al.* 1949).

We are indebted to Mr D. O'Connor for the mathematical formulation presented in the discussion, to Mr D. Hart for his skilful technical assistance and to Mr F. Crisp for the artificial insemination of the rabbits. The ^{14}C used in this investigation was obtained from the U.S. Atomic Energy Commission; the labelled acetate was synthesized from $\text{Ba}^{14}\text{CO}_3$ at the Radiochemical Centre, Amersham, Bucks.

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APPENDIX 1

Water Bath suitable for the Maintenance of Temperatures to within $\pm 1.5 \times 10^{-3}^\circ$. Micropipette for the Accurate Delivery of Small Volumes

By G. POPJÁK

Water baths suitable for the determination of deuterium in water samples by the falling-drop method are not available commercially in this country. While such baths have probably been constructed in many laboratories, descriptions are not readily available. The author has been requested from several quarters to describe the bath used in the preceding investigation and which can be constructed by very simple means. No originality is claimed.

Dimensions and materials of bath: $30 \times 30 \times 50$ (height) cm.; galvanized-copper sheet. The bath was made with plate-glass windows at the front and back, and the whole placed in a wooden box. The 2.5 cm. space between the walls of the bath and the wooden box was filled with suitable lagging material. The glass windows were left uncovered.

Thermostat. This was a toluene-Hg thermostat constructed from approximately 5 yards of thin-walled Pyrex

Fig. 1. Diagram of toluene-Hg thermostat made of Pyrex-glass tubing and used in bath for determination of D_2O by the falling-drop method. *A*, Hg reservoir for coarse adjustment of Hg level; *B*, capillary tubing of 3 mm. bore; *C*, glass tubing, 10 mm. internal diameter; *D*, capillary tubing of 1.5 mm. bore; *E*, tungsten wire sealed into capillary; *F*, benzene reservoir; *G*, small Hg reservoir for fine adjustment of Hg level.

Fig. 2. Diagram of centrifugal stirrer. The stem is made of 0.5 in. (12.5 mm.) brass tubing, the jets of 0.25 in. (6 mm.) copper tubing and the funnel of thin copper sheet.

Fig. 3.

Fig. 3. Micropipette for the delivery of small drops. *A*, metal ring which compresses rubber sleeve *B*, placed around the glass tubing; *C*, neoprene gland washer which provides air-tight seal between piston and barrel. Other parts are self-explanatory. Scale approx. 2/3.

Fig. 4.

Fig. 4. The drawn-out end of the glass part of the micropipette. The diagram illustrates how the correct shape of the tip is obtained: first the tubing is drawn out into a stout capillary which is drawn out further over a micro-flame. The arrows indicate the line where the tip should be cut; the tip is then ground around with a dentist's drill fitted with a fine carborundum disk. Water drops delivered hang from such tips.

tubing of 10 mm. internal diameter. The glass tubing was bent in ∞ shapes and was placed on three sides of the bath. The thermostat head was of the usual design. Fig. 1 illustrates the thermostat diagrammatically. The thermostat was operated with a Sunvic electronic relay and Proportioning Head. The temperature of the bath can be maintained for months within $\pm 1.5 \times 10^{-3}^\circ$ with this regulator at 27° . The heating element was bare Nicrome wire wound on glass tubing and distributed on three sides of the bath at the bottom; the total resistance of the heating element was 4Ω draining 3 amp. at 12 V. No trouble was encountered on account of lack of insulation of the heating element at this low voltage. Ordinary distilled water was used in the bath.

The stirrer was a centrifugal type driven by a small 24 V. motor (see Fig. 2). This stirrer was found very efficient without causing troublesome vibrations. A drop of permanganate placed under the funnel of the stirrer appears through the top jets within a few seconds and stains the water of the bath uniformly in about 10 sec. No difference in temperature at the top and bottom of the bath could be detected with a Beckmann thermometer.

Maintenance of bath. In the last instance this bath has been in constant operation day and night for 1 year without change of water. No preservative was added to the water, but a layer (about 1 cm. thick) of liquid paraffin was poured over the surface. The anaerobic condition suppressed completely the growth of moulds, etc. Before the use of liquid paraffin the water had to be changed about every month in spite of various preservatives added.

It was found that a layer of benzene (free of S compounds)

between the Hg column and contact needle prevented oxidation of the surface of Hg and ensured a clean contact and smooth running of the thermostat. For the continuous replacement of this benzene the cup of the thermostat head was connected through a small side arm with a reservoir.

The micropipette used for the delivery of the water drops was designed by Dr B. W. Robinson (see Fig. 3). It consisted of a calibrated micrometer screw which moved a piston in a snugly fitting barrel filled with mercury. A Pyrex capillary (1.5 mm. bore) bent in $\cap$ shape and drawn out at the end into a fine tip was fixed air tight to the metal parts with an expandable rubber washer. The metal parts were made of stainless steel.

When the tip of the Pyrex capillary has the correct shape shown in Fig. 4 the water drops delivered under the surface of the *o*-fluorotoluene hang from the tip, otherwise the drops creep up on the outside of the capillary and lead to inaccuracy of delivery. The pipette assembly was clamped in a rack and pinion.

While the construction of this pipette requires precision machining it has great advantages over the one described by Keston, Rittenberg & Schoenheimer (1937-8) as no suction is required when filling. It can be filled with water sufficient for the delivery of at least three drops and can easily be rinsed between successive determinations with 2-3 cu.mm. of the new water sample. The accuracy of delivery is better than 1% in the region of 10 cu.mm. The water drops are delivered under the surface of the *o*-fluorotoluene and are detached by the surface tension of the latter on raising the pipette.

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APPENDIX 2

Preparation of Solid Samples for Assay of ^{14}C

By G. POPJÁK

All radioactivity measurements reported in the previous investigation were carried out on solid samples (cholesterol, cholesterol-digitonide, fatty acids, Pb salts and Na salts of fatty acids). 'Infinite thickness' samples, i.e. 25 mg. of material/sq.cm. were prepared on disks of identical geometry. The measurements were carried out with a He-filled bell-shaped Geiger-Müller counter which had a thin mica window. Under our conditions of counting a solid sample which contained $10^{-3} \mu\text{C}$ of ^{14}C /mg. of substance gave approximately 1800 counts/min. in an 'infinite thickness' sample of 2 sq.cm. area. The background of our apparatus was 8-10 counts/min. The calibration of the counter was carried out with a sample containing known amounts of ^{14}C .

When sufficient material is available, as in the investigation described, assay of ^{14}C in 'infinite thickness' samples has the great advantage that the radioactive counts obtained on the various samples are linearly proportional to the specific activities and hence the ^{14}C contents may be expressed without self-absorption corrections. Further, the assay of ^{14}C directly on organic compounds of high C content

avoids the undesirable dilution when the organic compounds are first combusted and the CO_2 obtained is collected as BaCO_3 . The assay of ^{14}C on organic materials has the added advantage that the samples may be stored in common atmosphere, whereas with $\text{Ba}^{14}\text{CO}_3$ special measures are required to avoid exchange with atmospheric CO_2 .

Several methods have been described for the preparation of solid samples for the assay of ^{14}C (Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949) to suit individual requirements. The technique to be described has been used in this Institute for the assay of ^{14}C in a variety of crystalline and amorphous compounds (apart from those named above) and found very satisfactory.

Mounting material. Disks made of plastic material (Perspex or Polythene) and moulded to the shape shown in Fig. 1a were used. Two types were employed: (i) with solid, and (ii) with perforated, bottom.

Preparation of samples on solid disks ('pellet' technique). The solid disks were used for preparation of 'infinite thick-