Radioactive Eggs. II. Distribution of Radioactivity in the Yolks. (19020)

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The distribution of radioactivity in the yolks of eggs laid by a hed fed sodium acetate-1-C^{14} has been determined. The method of feeding and the gross distribution of radioactivity in the shell, yolk and albumen has already been described(1). The 10 yolks discussed below include 7 eggs laid during the period of feeding, 2 large ova taken from the hen (a Single Comb White Leghorn) after sacrifice and the last fraction consists of the combined small ova.

The dried yolks were separated into protein, phospholipid, fatty acid, cholesterol, and glycerol fractions. In each case, activity was present in every fraction. The rapidity of incorporation of radioactivity is demonstrated by the finding that all fractions of the first yolk, laid within 24 hours after feeding was begun, contained measurable amounts of activity. The fact that this yolk was, presumably, completely formed about the time that feeding was initiated(2) further accentuates the speed with which acetate is incorporated into all fractions. In every case the specific activity of the cholesterol was higher than that of any other fraction. Generally, the order of specific activity of the other fractions was fatty acids, phospholipid, glycerol and protein, although in the second egg the glycerol specific activity was second to that of cholesterol. Only the specific activity of the glycerol was the same order of magnitude throughout, the specific activity of all other fractions increased with each succeeding egg. The peak activity was reached in the sixth egg in the case of protein and glycerol; the seventh in the case of cholesterol and the fatty acid activity was at the same high level in the fifth and sixth eggs. The high specific activity of the cholesterol may be explained in either of two ways. Either the acetate is directly incorporated into this component, which view might be supported by the higher specific activity of this fraction in the first egg and also that the peak activity of the cholesterol occurs in the seventh yolk and not in the fifth or sixth, as with other fractions. The seventh egg was laid after all feed had been exhausted and it could be argued that the acetate was already incorporated into cholesterol, whereas conversion to the other components had not yet occurred. On the other hand, there is evidence(3-5) that fatty acids can be converted to cholesterol and the theory that fatty acids, once formed, are further metabolized to cholesterol could also explain the higher specific activity of this component.

In a previous publication(1) the abnormally low value obtained for the specific activity of yolk 5 was ascribed as probably being due to an error in combustion. The present results seem to confirm this conclusion, namely, that the original low value was the result of an error in technic rather than being due to any abnormality in egg production.

Experimental. Protein. The dried egg yolks were pulverized and extracted with ether-alcohol 1:3 in a Soxhlet apparatus for 100 hours. Extraction for longer periods caused no weight loss in the protein residue. The dried residue was converted to carbon dioxide by the Van Slyke wet combustion procedure (6). The carbon dioxide was collected as

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TABLE I. Specific Activities of Yolk Fractions (μc/mg).

Yolk #	Protein	Phospholipid	Cholesterol	Fatty acids	Glycerol
1	2.85×10^{-5}	1.74×10^{-4}	3.51×10^{-3}	7.77×10^{-4}	3×10^{-3}
2	4.38×10^{-4}	1.17×10^{-3}	3.87×10^{-3}	3×10^{-3}	6×10^{-4}
3	1.13×10^{-3}	3.44×10^{-3}	7.86×10^{-3}	7.86×10^{-3}	1.90×10^{-3}
4	1.67×10^{-3}	6.16×10^{-3}	2.99×10^{-2}	1.47×10^{-2}	3×10^{-3}
5	2.28×10^{-3}	1.27×10^{-2}	4.59×10^{-2}	2.68×10^{-2}	2.38×10^{-3}
6	3.23×10^{-3}	1.11×10^{-2}	6.77×10^{-2}	2.67×10^{-2}	3.80×10^{-3}
7	2.80×10^{-3}	1.02×10^{-2}	1.01×10^{-1}	2.41×10^{-2}	2.25×10^{-3}
8	2.52×10^{-3}	1.07×10^{-2}	6×10^{-2}	1.83×10^{-2}	2.36×10^{-3}
9	2.07×10^{-3}	7.15×10^{-3}	5.56×10^{-2}	1.33×10^{-2}	1.82×10^{-3}
10	1.85×10^{-3}	7.22×10^{-3}	4.54×10^{-2}	6.29×10^{-3}	1.22×10^{-3}

barium carbonate. All determinations for radioactivity were made using barium carbonate plates and a "Nucleometer" windowless counter.

Phospholipid. The solvent from the initial extraction was distilled at reduced pressure in a current of nitrogen. The residue was taken up in dry petroleum ether and the phospholipid precipitated with acetone and magnesium chloride(7). The phospholipid was reprecipitated prior to assay for radioactivity. For radioactivity determinations, an aliquot of a petroleum ether solution of the phospholipid was transferred to a quartz combustion vessel, the solvent evaporated and the dried residue combusted in a copper oxide filled, quartz tube in a stream of air. The evolved carbon dioxide was collected and assayed as described above.

Cholesterol and fatty acids. The solvent was removed from the supernatant following precipitation of the phospholipid by distillation at low pressure in a stream of nitrogen. The residue was refluxed with a 15% solution of potassium hydroxide in alcohol for 48 hours. The cholesterol was then separated in several different ways. In one experiment, the cholesterol was precipitated as the digitonide directly from the basic saponification mixture. In another instance, the saponification mixture was acidified, extracted with ether and the ether solution was passed over an alumina column. The cholesterol and non-saponified material were eluted together with petroleum ether-benzene 9:1, the column was stripped with a solution of methanol-acetic acid 4:1. There was no detectable activity left on the alumina. The cholesterol was separated as the digitonide.

The most convenient method for separating the cholesterol and non-saponified material from the fatty acids was one involving the use of the ion exchange resin Amberlite IRA-400 (8). The saponification mixture was acidified and ether extracted. The ether extract was passed over a column of the resin; the non-acidic material passed directly through. The acids were eluted with a solution of ether-ethanol-conc. hydrochloric acid 70:30:8.

The cholesterol was precipitated from the neutral fraction as the digitonide. The cholesterol was recovered from the digitonides and recrystallized from acetone. Paper chromatography on "Quilon" impregnated paper(9) using methanol as the developing solvent gave R_f values between 0.53 and 0.58 for all 10 fractions. Radioautography was used(10) to determine R_f values and also to show that no radioactive contaminants were present. The cholesterol specific activities were determined by plating the cholesterol directly from an acetone solution and counting the plates in a "Nucleometer." The fatty acids obtained on elution were dried and converted to carbon dioxide by wet combustion.

The saponification procedure used did not give complete saponification in any case but one. In several instances the neutral residue remaining after precipitation of the choles-

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terol was subjected to a second saponification with 20% aqueous potassium hydroxide for 60 hours. Repetition of the ion exchange separation still gave some neutral material, which did not contain a digitonin precipitable component. The specific activities of the acids obtained upon elution corresponded closely with the activities of the acids obtained after the first saponification. In the cases of yolks 1, 2 and 7, where a second saponification was carried out, the specific activities were 7.37×10^{-4} μc/mg, 2.60×10^{-3} μc/mg, and 2.61×10^{-2} μc/mg, respectively.

Glycerol. The aqueous solution remaining after the saponification mixture had been extracted was distilled to dryness at reduced pressure. In several cases, the salt residue was extracted with hot pyridine and, after cooling, benzoyl chloride was added to this solution. Acidification and ether extraction yielded the glycerol tribenzoates of yolks 1, 2, 3, 4 and 6. These derivatives had melting points in the range 73-76° and none depressed the melting point of an authentic sample of this material.‡ For yolks 5, 7, 8, 9 and 10, the salt residue was extracted with hot acetone. Evaporation of the acetone in a current of nitrogen left the desired glycerol, which was also converted to the tribenzoate in a few cases. Wet combustion was used to assay radioactivity. In cases where a sample was assayed both as free glycerol and as tribenzoate the specific activities were in agreement.

Summary. (1) The distribution of radioactivity in the yolks of eggs laid by a hen fed sodium acetate-1-C^{14} has been determined. (2) The radioactivity appears in all fractions of all yolks in the following order of increasing specific activity: protein, glycerol, phospholipids, fatty acids, cholesterol.

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The Mechanisms of the Choline Oxidase and Transmethylase Enzyme Systems.* (19021)

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In an earlier investigation of the choline oxidase system(1) various requirements of the enzyme system for maximum activity in rat liver homogenates were studied. Since the work of Muntz(2) implied that in order for transmethylation from choline to homocysteine to take place choline must first be converted to betaine, an investigation of the role of all 3 possible donors, *i.e.*, choline, betaine aldehyde, and betaine, in forming methionine from homocysteine was undertaken.

Since previous work concerning transmethylation to homocysteine was carried out anaerobically(2,3), it appeared desirable to compare the roles of choline and its oxidation products in transmethylation both aerobically and anaerobically and to correlate oxidation data with transmethylation data. In the earlier study of the choline oxidase system(1), the results indicated that the oxidation of choline to betaine did not occur as smoothly as might be expected since after the first 10

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