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ABSTRACT

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1. The distribution of radioactivity in the egg yolks layed by a hen fed sodium acetate-1-C^{14} has been determined.
2. The radioactivity appears in all the fractions investigated in all the yolks. The general order of decreasing activity being: cholesterol, fatty acids, phospholipids, glycerol, protein.
3. A procedure for the separation of the dried yolk into these fractions is described.

(*) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

For publication in the Journal of Biological Chemistry.

RADIOACTIVE EGGS. II. DISTRIBUTION OF RADIOACTIVITY IN THE YOLKS

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The distribution of radioactivity in the yolks of eggs laid by a hen being 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 been already described (1). The ten yolks discussed below include seven eggs layed during the period of feeding, two 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, cholesterol, fatty acid and glycerol fractions. In each of the ten cases activity was present in every fraction. The rapidity of incorporation of radioactivity is demonstrated by the finding that all fractions of the first yolk, layed within twenty-four 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.

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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, protein, although in the first yolk the glycerol specific activity was second to that of the cholesterol. In all fractions save glycerol, whose specific activity was of the same order of magnitude throughout, the specific activity 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 both the fifth and sixth eggs.

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}	3.00×10^{-3}
2	4.38×10^{-4}	1.17×10^{-3}	3.87×10^{-3}	3.00×10^{-3}	6.00×10^{-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}	3.00×10^{-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^{-2}
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}	6.00×10^{-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}

The data seem to indicate direct incorporation of acetate into cholesterol. The higher specific activity of this fraction in the first egg points to this, as well as the finding that the cholesterol activity reaches its peak in the seventh yolk rather than in the fifth or sixth as with the other fractions. The seventh egg was layed after all feed had been exhausted and the hen was sacrificed shortly thereafter. Presumably, the acetate was already incorporated into cholesterol by that time, whereas it had not yet been converted to the other components. That the fatty acids, once formed, are further metabolized to cholesterol may also explain the higher specific acitivity of this component. Conversion of various fatty acids to cholesterol has been demonstrated (3,4,5). It would not be unreasonable to assume that the five components isolated were biosynthesized in the order of their specific activities.

In a previous publication (1) the abmormally 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.

Although total activities can hardly be determined accurately, since the quantitative recovery of many of the components could not be achieved, calculation of the probable total activities from the average percentage composition of egg yolk (6) gives a smooth curve with the peak at yolk 7, as might be expected for a gradual increase in radioactivity with time and increased incorporation of the acetate. The probable total activities are plotted in graph I.

For calculation of the probable total activities, the percentages of each component were multiplied by the weight of dried yolk and the specific activity of each component. The percentages used were: protein, 33%; phospholipid, 22%; cholesterol, 3.3%; fatty acids, 30.2% and glycerol, 9.5%. The calculations have been summarized in table II.

Table II*

	Protein	Phospholipid	Cholesterol	Fatty Acids	Glycerol	Total
1	0.08	0.33	0.99	2.01	0.24	3.65
2	1.24	2.22	1.10	7.80	0.49	12.85
3	3.29	6.68	2.29	20.90	1.59	34.75
4	4.67	11.50	8.37	37.60	2.42	64.56
5	6.24	23.20	12.60	67.20	1.88	111.12
6	8.92	20.40	18.70	67.50	3.02	118.54
7	8.62	20.90	31.20	67.90	1.99	130.61
8	4.86	13.80	11.60	32.30	1.31	63.87
9	2.22	5.11	5.96	13.10	0.56	26.95
10	0.87	2.27	2.14	2.72	0.17	8.17

(*) All activities as microcuries

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 (7). The carbon dioxide was collected as 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, a few drops of a saturated alcoholic solution of magnesium chloride added and the phospholipid precipitated by the addition of acetone. The phospholipid was reprecipitated before assay for radioactivity. To determine the activity, 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 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 percent alcoholic solution of potassium hydroxide for 48 hours. The cholesterol was separated in several different ways. In one experiment, it 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 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 passing through directly. The acids were eluted with a solution of ether-ethanol-concentrated hydrochloric acid 70:30:8.

The cholesterol was precipitated from the neutral fraction as the digitonide. The digitonides obtained from all ten yolks were split with pyridine and the cholesterol recrystallized from acetone. Paper chromatography on "Quilon" impregnated paper (9) using methanol as the solvent gave the R_f values listed in Table II. Radioautography (10) was used to determine R_f values and also to show that no radioactive contaminants were present. The cholesterol spots were confirmed by the red color obtained with a 20 percent aqueous silicotungstic acid spray. The cholesterol specific activities were determined by plating the cholesterol directly from an acetone solution and counting in a "Nucleometer."

The fatty acids obtained on elution were dried and converted to carbon dioxide by wet combustion.

Table III

R_f Values for Cholesterol Fractions

Yolk	R_f	Yolk	R_f
1	0.57	6	0.54
2	0.55	7	0.56
3	0.54	8	0.58
4	0.54	9	0.54
5	0.55	10	0.53

The saponification procedure used did not give complete saponification in any case but one. In several instances the neutral residue remaining after digitonide precipitation of cholesterol was subjected to a second saponification with 20 percent aqueous potassium hydroxide for 60 hours. Repetition of the ion exchange fractionation still gave some neutral material, which contained no 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. and 2.61×10^{-2} μc/mg. respectively.

Glycerol: - The aqueous solution remaining after acidification and extraction of the saponification mixture 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. Some of these residues were also converted to the tribenzoates. 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.

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SUMMARY

1. The distribution of radioactivity in the egg yolks layed by a hen fed sodium acetate-1-C^{14} has been determined.
2. The radioactivity appears in all the fractions investigated in all the yolks. The general order of decreasing activity being: cholesterol, fatty acids, phospholipids, glycerol, protein.
3. A procedure for the separation of the dried yolk into these fractions is described.

(**) We are indebted to Prof. James Cason for supplying a sample of this material.

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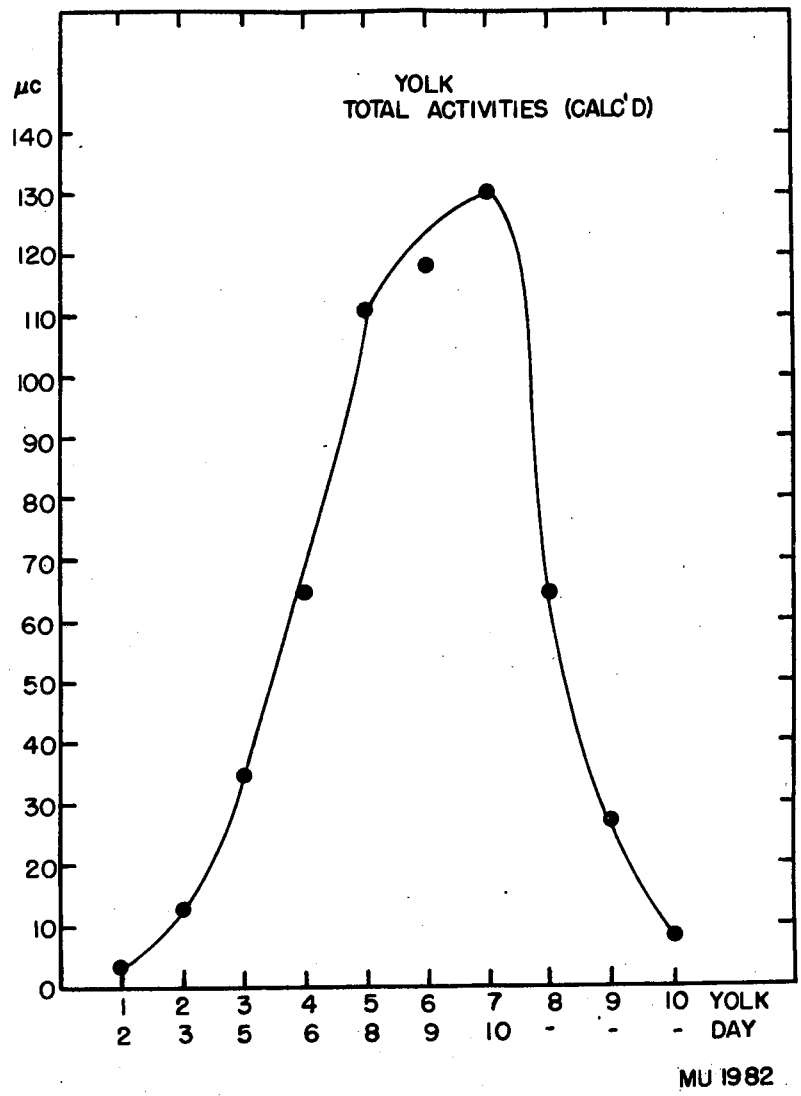
YOLK
TOTAL ACTIVITIES (CALC'D)
μc
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 YOLK
2 3 5 6 8 9 10 - - - DAY
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FIG. 1