Summary. A procedure is described which utilizes a combination of filter paper and ion exchange chromatography to separate the urinary metabolic products of adrenaline. After intravenous infusion of dl-adrenaline-2-C^{14} in 6 human subjects, 73 ± 5% of infused radioactivity was recovered in 24 hour urine. Radioactivity which appeared in urine was distributed among various metabolic products as follows: adrenaline, 4%, 3-methyl-O-adrenaline, 5%, 3-methyl-O-adrenaline conjugate, 42 ± 7%; 3-methoxy-4-hydroxymandelic acid, 27 ± 3%; 3, 4-dihydroxymandelic acid, 12 ± 4%; 2 unidentified fractions, 4%.

ADDENDUM: Recent work on the separation procedure has resolved the 0.3M eluate and the 3.0M eluates each into two fractions. Preliminary work indicates that the metadrenaline conjugate accounts for about 80% of the radioactivity in the 0.3M eluate and one unidentified compound accounts for the remainder. In the 3.0M eluate, 3,4-dihydroxymandelic acid accounts for 30 to 50% of the radioactivity. This work is still in progress and will be reported at a future date.

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Plasma Cholesterol in Growing Chicken as Influenced by Dietary Protein and Fat.*† (24130)

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In the human, Ahrens *et al.*(1), Beveridge *et al.*(2), and others have indicated a relationship between degree of saturation of ingested fat and resulting serum cholesterol level. Studies with "normal" experimental animals (those not receiving dietary cholesterol or other cholesterolemic agents) have shown essentially no differential response to dietary fat(3). Hegsted and coworkers(4) presented evidence of a significant negative correlation between the product of the essential fatty acids and total saturated fatty acids (EFA x TSFA) of dietary fats and serum cholesterol level. Serum cholesterol level can be experimentally elevated not only through dietary cholesterol but also by feeding low protein diets(5,6,7). Our objective was to evaluate the effect of level and type of dietary fat on plasma cholesterol in growing chickens receiving either 1) low protein diet plus 0.3% cholesterol or 2) high protein diet with 2% cholesterol. Since differences in serum cholesterol as a result of varying the dietary fat (as observed by Hegsted *et al.*) might be related to differences in absorption of dietary cholesterol it was of particular interest to find out whether the low protein induced hypercholesterolemia would respond differen-

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TABLE I. Effect of Type and Level of Fat on Plasma Cholesterol, Weight Gain, and Feed Consumption of Growing Chicks Fed 2 Levels of Protein and Cholesterol.

Measurements	8% protein + .3% cholesterol					25% protein + 2% cholesterol				
	Fat					Fat				
	Level, %	Olive (55)*	Corn (491)	Coconut + cottonseed§ (1290)	Coconut + safflower§ (2120)	Level, %	Olive (55)	Corn (491)	Coconut + cottonseed§ (1290)	Coconut + safflower§ (2120)
Plasma cholesterol, mg %	3	738 ± 76†	744 ± 85	739 ± 64	504 ± 43	3	220 ± 19	177 ± 16	273 ± 92	153 ± 6
	6	779 ± 42	600 ± 74	782 ± 66	846 ± 60	6	347 ± 30	158 ± 10	224 ± 22	196 ± 12
	9	829 ± 56	816 ± 42	679 ± 65	866 ± 82	9	212 ± 31	386 ± 44	254 ± 38	251 ± 18
Avg wt gain, g/16 days	3	40 ± 11 †	51 ± 6	24 ± 8	51 ± 12	3	217 ± 11	222 ± 14	213 ± 10	179 ± 6
	6	12 ± 6	63 ± 8	40 ± 9	19 ± 6	6	202 ± 8	183 ± 6	201 ± 10	175 ± 12
	9	24 ± 7	35 ± 8	39 ± 12	42 ± 7	9	187 ± 14	186 ± 10	213 ± 16	225 ± 10
Avg feed consumption, g/16 days	3	278‡	273	244	284	3	423	426	440	395
	6	207	312	280	247	6	389	378	389	388
	9	240	256	267	264	9	397	365	406	426

* Product of essential fatty acids × total saturated fatty acids. † Mean ± stand. error. ‡ Avg consumption determined from total feed consumption of each lot. § Equal amounts of both fats were used.

tially to various fats.

Methods. White Leghorn cockerels were reared for 2 weeks on a practical starting ration, at which time they were divided into groups of 8 chicks/treatment. Two-week-old chicks were used to avoid any effects from unabsorbed yolk material. Percentage composition of basal diet was as follows: isolated soybean protein (Drackett Assay Protein C-1) 10, DL-methionine 0.10, mineral mix (8) 5.34, fiber 3, choline Cl 0.20, B vit. mix (8) 0.15, Vit. A, D and E mix(8) 0.10, glucose 72.61, cholic acid 0.20, cholesterol 0.3. The basal diet served as the low protein diet while the high protein diet was formulated by addition of 20% isolated soybean protein, 0.2% DL-methionine and 1.7% cholesterol to the basal diet at the expense of glucose. By including 3 levels of 4 different fats or fat-combinations, at the expense of glucose, into each of these 2 diets, 24 different treatments were obtained. Fats and fat combinations were chosen to cover a wide range of the product EFA x TSFA as given by Hegsted *et al.*(4). The design of the experiment is found in Table I. The birds were maintained on experimental diets for 16 days in electrically heated wire cages. They were weighed individually at beginning and end of experimental period. Group feed consumption was recorded. Blood samples were taken at end of experimental period by heart puncture using a heparinized syringe. After centrifugation to separate plasma and cells, 0.05 ml aliquots of plasma were taken for cholesterol analysis by modification of the method of Zlatkis(9) using stable iron reagent of Rosenthal *et al.*(10).

Results. At each of the 2 protein levels there were essentially no effects from either the type or level of dietary fat (Table I). It is thus apparent that Hegsted's finding in the rat is not applicable to the chicken. The hypercholesterolemia induced by combination of low protein level and small amount of dietary cholesterol was not affected by any of the fats. The observed plasma cholesterol levels on high protein diet were essentially normal, indicating ability of the chicken to adjust to large amounts of dietary cholesterol in the presence of otherwise adequate diet. By con-

trast Katz and Stamler(11) induced marked cholesterolemia in the growing chick by addition of the same level of cholesterol (2%) and 5% cottonseed oil to a diet containing 18% protein. This level of protein, in the authors' experience, is inadequate for proper metabolism of the absorbed cholesterol.

Weight gains and feed consumption in our study (Table I) indicate uniformity at each protein level. This is of particular interest since the caloric density of the diets varies considerably between the 3 and 9% levels of dietary fats. The uniformity of feed consumption might well be a factor in uniformity of the plasma cholesterol values.

Summary. The effects on plasma cholesterol level of 4 fats each supplied at 3 levels in a high and a low protein diet were studied in the cholesterol-fed chicken. On the low protein diet a marked hypercholesterolemia was observed which was not affected by type of fat studied or level of supplementation. On the high protein diet, plasma cholesterol levels were essentially normal regardless of source and level of dietary fat. Since the fats used represented a wide range in the product EFA x TSFA, a lack of relationship between this product and plasma cholesterol level in the growing chicken is indicated.

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Use of Ionophoretic Mobility Equilibria in Fractionation of Mixtures Containing Indoles.* (24131)

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Chromatographic separations of indoles in urine samples are often complicated by interfering substances which mark the spot, distort the Rf value and spot size, or cause streaking (1,2). It is also quite difficult to separate certain alkaloids from pigments and other "interfering substances" in urine by the conventional methods of paper electrophoresis; however, good separations are practicable by the mobility equilibrium method (unpublished data, 1952). The phenomenon of mobility equilibrium (M.E.) was first described in open-hanging-strip electrophoresis by Durrum (3). Separations by this method are time-consuming and require high current flow with volatile electrolyte systems. Some of the theoretical considerations presented in this paper have also been discussed by Macheboeuf, *et al.*(9). The purpose of this report is to describe a less time-consuming method in which low current flow may be utilized; the use of horizontal or vertical types of open paper electrophoresis units is practicable; and volatile or non-volatile electrolytes may be employed. It is thus possible to separate neutral molecules and charged ions (amphoteric and non-

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