the data presented that if only antisera to single strains are used at the dilutions recommended for typing purposes, certain isolates only partially related to the prototypes might be missed, and considered as new types. By subgrouping such partially related types, it may be possible to limit the expansion of picornavirus types, which now number close to 100.

The A20 Coxsackie viruses are global in distribution(2). The first member of the group was isolated from patients with hepatitis in New York State. The A20A prototype was recovered from a patient with suspected poliomyelitis in New Orleans, A20B from a normal child in South Africa, and A20C from a cholera patient in Bangkok(1).

Summary. Thai C-18 virus, previously believed to be a new enterovirus type, has been shown to belong to the Coxsackie virus A20 subgroup. Four antigenic variants are now known for this subgroup. The indicator strain of choice within the subgroup is A20A, as it is most readily neutralized by antisera against all 4 variants.

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In vivo Inhibition of Lipogenesis by Biotin Deficiency.* (29337)

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Biotin deficientcy has been shown to inhibit lipogenesis *in vitro*(1,2,3). It has been suggested that the block in lipogenesis that results from feeding fat occurs between acetyl CoA and malonyl CoA(4), a reaction which requires biotin. Attempts to produce biotin effects on lipogenesis *in vivo* have not been successful perhaps because the diets employed contained 5-12% added fat which itself could have depressed lipogenesis(5,6). This paper reports the effects of biotin deficiency in intact chicks when fat was not included in the control or deficient diets.

Methods. Day-old White Rock × Cornish female chicks were reared in electrically-heated batteries with raised-wire floors and were fed the diet shown in Table I, with or without 2 mg of biotin/kg. Chicks fed the deficient diet exhibited retarded growth and severe dermatitis of the feet and legs at 21 days of age. At 25 days, 12 chicks were selected according to body weight from each group. Six chicks fed each diet were injected intraperitoneally with 2 μc of sodium acetate-

TABLE I. Composition of Experimental Diets.

Ingredient	% of diet
Sucrose	62.8
"Vitamin-free" casein*	25.0
DL-methionine	.3
Glycine	1.0
L-arginine	.5
Cellulose†	4.0
Vitamin mix‡	.4
Mineral mix§	6.0

* Nutritional Biochemicals Corp., Cleveland, Ohio.

† Solka-floc, Brown Co., Boston, Mass.

‡ Same mix less biotin as used by Donaldson, W. E., *J. Nutrition,* 1964, v82, 115. Control diet contained 2 mg of biotin/kg.

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TABLE II. Biotin Deficiency and Incorporation of C^{14} Labeled Acetate and Malonic Acid into Liver and Carcass Lipids.*

Label	Control	Biotin deficient	Statistical significance
Na-acetate-1-C^{14}	% dose in	% dose in	P†
$C^{14}O_2$	45.3 ± 3.8	60.3 ± 1.9	.01
Liver saponifiable	3.7 ± 1.4	1.2 ± .3	n.s.
" non-saponifiable	1.6 ± .8	.4 ± .1	n.s.
Carcass saponifiable	19.1 ± 5.2	4.9 ± 1.2	.05
" non-saponifiable	5.9 ± 1.3	2.9 ± .4	n.s.
Malonic-2-C^{14} acid			
$C^{14}O_2$	16.2 ± .8	18.9 ± 1.8	n.s.
Liver saponifiable	.5 ± .2	.9 ± .2	n.s.
" non-saponifiable	1.3 ± .2	1.4 ± .3	n.s.
Carcass saponifiable	1.9 ± 1.3	1.8 ± .6	n.s.
" non-saponifiable	3.6 ± 1.0	2.3 ± .3	n.s.

* Each value is the avg of 6 chicks ± S.E.

† Probability based on t test.

1-C^{14} (sp. act. 2 mc/mM). The remaining chicks were injected with 4 μc of malonic-2-C^{14} acid (sp. act. 1.24 mc/mM). Respiratory $C^{14}O_2$ production from each chick was measured during a 2-hour collection by a method described previously(7). The chicks were then killed, the livers removed immediately and frozen. The carcasses were frozen after feather removal.

The frozen livers were weighed and homogenized with an equal weight of distilled water for 5 minutes. Duplicate 2 gram samples were withdrawn and moisture determined by freeze-drying and weighing the residue. The dried residue was continuously extracted with 25 ml of chloroform-methanol (2:1, v:v) for 3 hours. The extracts were made up to 50 ml with chloroform-methanol, and 2 ml were pipetted into glass vials. Solvent was removed at 60°C under nitrogen and 10 ml of scintillator solution† was added to the vials before counting for radioactivity. The remaining extract was dried at 60°C under nitrogen, 10 ml of 50% ethanol containing 1.5 g KOH were added, and the samples were placed on a steam bath for one hour. Water was added, and the samples were extracted with petroleum ether, acidified with HCl, and the fatty acids extracted with petroleum ether. Solvent was removed as above and the fatty acids were weighed. A sample was placed in a glass vial for radioactivity analysis as above. The remaining fatty acids were converted to methyl esters and subjected to gas chromatography analysis(8). Carcass samples were treated the same as liver samples.

Radioactivity was measured using a liquid scintillator spectrometer.‡ All samples were counted and recounted after addition of standard C^{14}-toluene. Background was 20-25 counts/min, and counting time was sufficient to reduce counting error to $< 1.0\%$.

Results. The data in Table II show that biotin deficiency resulted in increased incorporation of acetate-C^{14} into respiratory CO_2 and decreased incorporation into carcass fatty acids. The decreased incorporation into liver fatty acids and non-saponifiable lipids of liver and carcass with biotin deficiency is not statistically significant.

Biotin deficiency had no effect on incorporation of malonic acid-C^{14} into respiratory CO_2 or liver and carcass lipids.

The percentage fatty acid content of carcass was depressed by biotin deficiency, but liver fatty acid content was not affected (Table III). Fatty acids are expressed on a dry basis since moisture content did not vary with treatment.

The carcass fatty acid composition data (Table IV) indicate that biotin deficient chicks deposited relatively more 16-carbon and less 18-carbon fatty acids than did control chicks. The ratio of saturated to un-

† Toluene containing 0.01% POPOP (1,4-bis-[2,4-methyl-5-phenyloxazolyl]-benzene) and 0.4% PPO (2,5 diphenyloxazole).

‡ Tri-Carb, Packard Instrument Co., La Grange, Ill.

TABLE III. Biotin Deficiency and Fatty Acid Content of Liver and Carcass.*

	Control	Biotin deficient	Statistical significance
	%	%	P†
Liver fatty acid, dry basis	11.1 ±1.7	9.0 ±1.2	n.s.
Carcass fatty acid, dry basis	20.4 ±1.3	15.1 ±1.3	.01

* Each value is the avg of 12 chicks ± S.E.
† Probability based on t test.

TABLE IV. Biotin Deficiency and Carcass Fatty Acid Composition.*

Acid†	Control	Biotin deficient	Statistical significance
	%	%	P‡
14:0	.5 ±.1	.6 ± .1	n.s.
16:0	27.0 ±.6	29.7 ± .8	.02
16:1	8.4 ±.3	15.4 ±1.2	.001
18:0	9.8 ±.8	5.5 ± .7	.001
18:1	54.3 ±.7	48.8 ±1.3	.001
Total saturated	37.3 ±.3	35.8 ±1.0	n.s.
Ratio			
Sat:Unsat	.59	.56	
16-carbon:18-carbon	.55	.83	

* Each value is the avg of 12 chicks ± S.E.
† First number denotes number of carbon atoms; second number denotes number of double bonds. Acids higher than 18:1 were detected in negligible quantities.
‡ Probability based on t test.

saturated fatty acids was not affected. Similar fatty acid distributions were observed for pooled liver samples.

Discussion. The data from these experiments strongly suggest that biotin deficiency depresses the level of lipogenesis in the intact animal. This result is in agreement with the published observations on the relationship of biotin to lipogenesis *in vitro*(1,2,3), but not with observations *in vivo*(5,6). The diets used in the *in vivo* experiments contained between 5 and 12% fat. Since fat-feeding depresses lipogenesis(4), the use of control diets with fat could depress lipogenesis to the minimal levels expected with a biotin deficient diet.

A comparison of the data on incorporation of acetate-C^{14} and malonate-C^{14} suggests that biotin exerts its primary influence on lipogenesis through the carboxylation of acetate to form malonate since malonate incorporation into fatty acid is not affected by biotin deficiency. It has recently been shown that yeast react to biotin deficiency by synthesizing less 18-carbon and more 16-carbon fatty acids(9). The mechanism responsible for the decrease in 18-carbon fatty acids that occurs with biotin deficiency in yeast and chicks is unknown.

Summary. Biotin deficiency in the intact chick resulted in increased incorporation of sodium acetate-1-C^{14} into respiratory CO_2 and decreased incorporation into carcass long chain fatty acids. Biotin deficiency had no effect on the incorporation of malonic-2-C^{14} acid. The total fatty acid content of the carcass was decreased and the proportion of 16-carbon to 18-carbon fatty acids was increased by biotin deficiency.

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