This might reflect a slow reversal of anatomical changes in the kidneys. By light microscopy the kidneys of D.I. rats appear normal, but ultra-microscopic changes in the nephrons of normal rats undergoing acute water diuresis were described recently(6). Adaptation of an enzyme system(s) needed for the action of vasopressin might also require considerable time.

We have observed striking hypertrophy of neurons in the supraoptic and paraventricular nuclei of untreated hereditary D.I. rats and partial reversal of these changes after 28 days of vasopressin administration(7). These changes might reflect adjustment to a new steady state in the feed-back system which regulates activity in the hypothalamo-neurohypophysial system. For example, either oxytocin, which these D.I. rats probably produce and release in excess(4), or an analogue of vasopressin which these rats might synthesize in lieu of this hormone, might inhibit the antidiuretic effect of vasopressin, perhaps by competition for binding sites(8). Initially, under the influence of constant osmotic stimulation, either or both of these compounds might be released in excessive amounts. Then, as normal fluid balance is approached under treatment with exogenous vasopressin, progressively less hypothetical inhibiting compound(s) might be released until finally the urine was concentrated normally.

These possible mechanisms, which might influence the degree of hypertonicity of the renal medullary interstitium and/or the permeability of various renal membranes to water, are speculative and by no means mutually exclusive.

Summary. When given large doses of vasopressin tannate in oil, rats with hereditary hypothalamic diabetes insipidus at first do not concentrate urine normally. This defect is progressively corrected as daily injections of hormone are continued for 4 weeks.

We thank Mrs. R. A. Garrity for valuable help.

1. Alexander, C. S., Filbin, D. M., Fruchtman, S. A., J. Lab. Clin. Med., 1959, v54, 566.
2. Alexander, C. S., Proc. Soc. Exp. Biol. and Med., 1958, v99, 142.
3. Valtin, H., Schroeder, H. A., Am. J. Physiol., 1964, v206, 425.
4. Sawyer, W. H., Valtin, H., Sokol, H. W., Endocrinology, 1964, v74, 153.
5. De Wardener, H. E., Herxheimer, A., J. Physiol., 1957, v139, 42.
6. Sabour, M. S., MacDonald, M. K., Lambie, A. T., Robson, J. S., Quart. J. Exp. Physiol., 1964, v49, 162.
7. Sokol, H. W., Valtin, H., Am. Zoologist, 1964, v4, 290.
8. Morel, F., Jard, S., Am. J. Physiol., 1963, v204, 227.

Received October 26, 1964. P.S.E.B.M., 1965, v118.

Alterations of Lipogenesis in the Intact Chick by Dietary Malonic Acid.* (29872)

WILLIAM E. DONALDSON (Introduced by Henry W. Garren)
Department of Poultry Science, North Carolina State College, University of North Carolina, Raleigh

Previous experiments have shown that dietary biotin deficiency can depress the rate and alter the pattern of lipogenesis in the intact chick as measured by total carcass fatty acids, fatty acid composition and acetate incorporation into long-chain fatty acids (LCFA). The inhibition of lipogenesis appeared to be at the acetyl-CoA to malonyl-CoA step(1). Malonic acid has been shown to stimulate LCFA synthesis *in vitro*(2,3).

This paper reports the effects of dietary malonic acid on LCFA synthesis in normal and biotin-deficient intact chicks.

Methods. Day-old female White Rock

* Contribution from Poultry Science Dept., North Carolina Agri. Exp. Station, Raleigh. Published with approval of Director of Research as Paper No. 1863 of the Journal Series. This investigation was supported in part by PHS Research Gant AM-07833 from Nat. Inst. of Arthritis and Metab. Dis.

TABLE I. Effects of Dietary Biotin Deficiency and Malonic Acid Supplementation on Growth and Dermatitis of Chicks.

Diet	24-day body wt* Exp 1	Exp 2	Avg	24-day dermatitis score, Exp 2†
		(g)		
Control (casein)	209	208	209	.00
+ .8% malonic acid	—	193	—	.00
+ 1.6% " "	—	174	—	.00
Biotin def	146	150	148	2.09
+ .1% " "	129	—	—	—
+ .2% " "	142	—	—	—
+ .4% " "	149	—	—	—
+ .8% " "	138	167	153	2.14
+ 1.6% " "	—	147	—	2.12

* Two groups per treatment. Twelve and 8 chicks per group in Exp 1 and 2, respectively.

† Dermatitis was independently scored by 2 persons using a 0 to 3 scale where 0 = no dermatitis and 3 = severe dermatitis.

chicks were used in both experiments. The chicks were maintained in electrically heated batteries with raised-wire floors. The casein-sucrose diets used were reported earlier(1). Malonic acid† was added to all diets at the expense of sucrose. The levels of malonic acid supplementation are listed in the tables under results. The casein-sucrose diet, with and without 2 mg of biotin/kg was fed *ad libitum* in Experiments 1 and 2. At 24 days, the chicks were weighed and subjectively scored for severity of dermatitis. In Experiment 2, five chicks fed each diet were selected at random and killed by dislocating the neck. Total fatty acids were determined for each chick by a method outlined previously(1). Aliquots of the isolated fatty acids were converted to methyl esters and subjected to gas chromatographic analysis(4).

Results and discussion. The growth and dermatitis data from Experiments 1 and 2 are shown in Table I. Biotin deficiency depressed growth and resulted in the typical marked dermatitis of the legs and feet. Malonic acid supplementation had no effect on growth rate or dermatitis in deficient chicks but reduced growth rate in control chicks.

The carcass fatty acid data are shown in Table II. In confirmation of a previous report(1), biotin deficiency resulted in an increase of the ratio of 16-carbon to 18-carbon

† Mann Research Laboratories, Inc., New York.

TABLE II. Effects of Dietary Biotin Deficiency and Malonic Acid Supplementation on Fatty Acid Distribution of Chicks (Exp 2).*

Diet	Carcass fatty acid, dry basis	Fatty acids 14:0†	16:0	16:1	18:0	18:1	Ratio Saturated: Unsaturated	Ratio 16 carbon: 18 carbon
	(%)	(%)	(%)	(%)	(%)	(%)		
Control	28.9	1.0 ± .14	27.8 ± 1.18	6.8 ± .52	13.9 ± 1.07	50.5 ± .94	.74	.55
+ .8% malonic acid	29.1	1.0 ± .05	28.4 ± .98	8.8 ± .52	9.9 ± .66	51.8 ± .93	.65	.62
+ 1.6% " "	28.7	1.1 ± .09	29.7 ± .54	9.0 ± .52	10.7 ± .48	49.4 ± .44	.71	.66
Biotin deficient	22.8‡	1.1 ± .12	29.6 ± 1.10	11.4 ± .78	8.8 ± .29	49.1 ± 1.58	.65	.73
+ .8% malonic acid	23.2‡	1.0 ± .06	28.9 ± .92	10.1 ± .84	9.8 ± .63	50.2 ± 1.15	.66	.67
+ 1.6% " "	27.3	1.1 ± .08	30.4 ± .84	12.0 ± 1.10	8.2 ± .45	48.3 ± 1.49	.66	.77

* Each value represents the average of 5 chicks ± standard error.

† First number denotes number of carbon atoms; second number denotes number of double bonds. Acids higher than 18:1 were detected in negligible quantities.

‡ Significantly different from control (P = .05).

fatty acids. In the previous report, statistically significant increases in 16:0‡ and 16:1 and decreases in 18:0 and 18:1 acids were observed. Because of the increased variability of the present results, only the changes in 16:1 and 18:0 acids are statistically significant ($P=0.01$). The LCFA content of the carcass was significantly reduced ($P=0.05$) by biotin deficiency. Supplementation of the biotin-deficient diet with 1.6% malonic acid increased the LCFA content of the carcass to a level not statistically different from control. Malonic acid did not alter the LCFA composition of deficient chicks. Conversely, malonic acid had no statistically significant effect on carcass LCFA content of control chicks but altered LCFA composition so that it approached that of deficient chicks.

The change in LCFA composition that occurred in these experiments was accompanied by a concomitant decrease in growth rate. That differences in growth rate *per se* are responsible for the LCFA composition changes is doubtful since total carcass fatty acid content was unrelated to growth rate. In the control groups, 0.8% malonic acid reduced growth and altered LCFA composition. The further reduction in growth of control chicks fed 1.6% malonic acid was not accompanied by a further change in LCFA composition. This view is supported by the work of Wiese *et al*(5) who showed that the levels of saturated and mono-ene fatty acids of blood serum from dogs is unaffected by caloric intake of low-fat diets. Marion and Edwards(6) fed low-fat diets containing 15 or 25% protein to chicks. The lower protein level reduced growth to a degree similar to the present experiments. Liver fatty acid composition was unaffected by protein level at 14 days. At 28 days, composition varied between protein levels, but the pattern of change did not resemble the pattern observed in the present experiment.

Fat feeding has been shown to inhibit acetyl-CoA incorporation into LCFA by a cell-free system isolated from rat liver(7). Malonyl-CoA was readily incorporated in this inhibited system. Using a similar system from rat mammary glands, Dils and Popjak (3) observed that malonate stimulated LCFA synthesis but did not incorporate due to a lack of conversion of malonate to malonyl-CoA. Spencer and Lowenstein(2) found that their rat mammary gland system incorporated malonate into LCFA but that the rate of incorporation was low as compared to citrate or acetate incorporation. Their work suggested that the conversion of malonate to malonyl-CoA was a rate limiting step. More recently, Hulsmann(8) reported the existence of a carboxyltransferase reaction in rat-heart sarcosomes. This system can synthesize malonyl-CoA from acetyl-CoA and oxalosuccinate, however, the system requires biotin. In the work cited above(1), the incorporation of malonate-C^{14} into carcass and liver fatty acids and into respiratory CO_2 of chicks was poor as compared to acetate-C^{14} incorporation.

The above results suggest the possibility that although malonate does incorporate into LCFA, malonate is not a major precursor of LCFA due to its relatively poor conversion to malonyl-CoA.

If malonate is not readily converted to malonyl-CoA, the results presented here suggest that malonate stimulation of LCFA synthesis is indirect. Kallen and Lowenstein(9) showed that malonate stimulates the synthesis of malonyl-CoA from acetyl-CoA. Presumably, this reaction was inhibited by the biotin deficiency state of the chicks used in the present experiments, therefore stimulation of this reaction by malonate should not occur. Evidence for the existence of non-biotin dependent pathways of LCFA synthesis has been reviewed elsewhere(10). The change in LCFA composition that occurs with biotin deficiency may reflect the products of such a pathway. If the stimulatory effect of dietary malonic acid were mediated through a non-biotin pathway, it would account for the increase in total LCFA content of deficient chicks and the failure to alter LCFA composition when malonate is fed. Under these conditions in normal chicks, malonate would result in altered composition without necessarily affecting total synthesis of LCFA. The above concept is supported by the work

‡ First number denotes chain length; second number denotes number of double bonds.

of Iliffe and Myant(11). These workers used a cell-free preparation from rat liver in which LCFA were synthesized from acetate by way of the biotin-dependent malonyl-CoA pathway. This preparation contained phosphorylating mitochondria and did not require the cofactor additions necessary for synthesis in soluble, cell-free systems. Under these conditions, which more closely resemble the intact cell, malonate had no effect on LCFA synthesis.

Summary. Biotin deficiency in intact chicks resulted in lower total carcass fatty acid deposition and altered fatty acid composition as compared to normal chicks. Dietary malonic acid stimulated total fatty acid deposition in the carcass of biotin-deficient chicks but did not alter the fatty acid composition of the carcass. In normal chicks, malonic acid had no effect on total fatty acid deposition but altered fatty acid composition such that it approached the composition of deficient chicks. Growth and biotin-deficiency dermatitis were unaffected by malonic acid in deficient chicks, but malonic acid depressed the growth of normal chicks.

The author gratefully acknowledges the technical assistance of Mrs. Faye Suggs and Mr. Paul Ziglar and the comments and suggestions of Drs. Gennard Matrone and C. H. Hill.

1. Donaldson, W. E., Proc. Soc. Exp. Biol. and Med., 1964, v116, 622.
2. Spencer, A. F., Lowenstein, J. M., J. Biol. Chem., 1962, v237, 3640.
3. Dils, R., Popjak, G., Biochem. J., 1962, v83, 41.
4. Donaldson, W. E., Poultry Sci., 1964, v43, 784.
5. Wiese, H. F., Hansen, A. E., Coon, E., J. Nutrition, 1962, v76, 73.
6. Marion, J. E., Edwards, H. M., ibid., 1962, v77, 23.
7. Bortz, W., Abraham, S., Chaikoff, I. L., J. Biol. Chem., 1963, v238, 1266.
8. Hulsmann, W. C., Biochim. et Biophys. Acta, 1963, v77, 502.
9. Kallen, R. G., Lowenstein, J. M., Arch. Biochem. Biophys., 1962, v96, 188.
10. Wakil, S. J., Bressler, R., Metabolism, 1962, v11, 742.
11. Iliffe, J., Myant, N. B., Biochem. J., 1964, v91, 369.

Received October 26, 1964. P.S.E.B.M., 1965, v118.

Hemagglutination by Reoviruses Propagated in Various Cell Lines.* (29873)

JOHNG S. RHIM,† JAMES I. KATO AND WILLIAM PELON
(Introduced by G. J. Buddingh)
Virology Section, Louisiana State University, New Orleans, and International Center for Medical Research and Training, San José, Costa Rica

Although primary cultures of renal cells from the Rhesus monkey have been shown to be the most sensitive host system for the study of reovirus types(1), both expense and availability limit the use of tissue from this animal in the virus laboratories of many countries. An alternate solution for this problem would be the use of cells in continuous passage for the study of reoviruses. This report describes the results obtained with respect to hemagglutinin production and development of infectious virus in 3 such cell systems.

* This investigation was supported by USPHS Grant GM 11330-20, Office of International Research.

† Present address: Laboratory of Tropical Virology, Nat. Inst. of Allergy & Infect. Dis., N.I.H., Bethesda, Md.

Materials and methods. Virus. The 3 prototype reovirus strains, type 1 "Lang," type 2 "D-5" and type 3 "Dearing," employed in these studies were supplied by Dr. A. B. Sabin, Children's Hospital Research Foundation, Cincinnati. In addition, the "Abney" strain (type 3) was obtained from Dr. L. Rosen, Pacific Research Station, NIAID; Honolulu, strain "48/4" (type 2) from Dr. C. Moscovici, University of Colorado Medical Center, Denver; strain "988" (type 2) from Dr. A. M. Lerner, Wayne State Uni-