Paralysis Induced by Feeding Synthetic Glycerides to Chicks.* (29988)

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The ability of chickens to utilize large amounts of dietary neutral fat is well established(1-3). Recent work conducted in this laboratory and elsewhere(4,5) has shown that essentially normal early growth is obtained when chicks are fed simplified diets in which virtually all of the non-protein metabolizable energy is provided as neutral fat. However, the replacement of neutral fat (soybean oil) by the mixture of fatty acids derived from it by hydrolysis produces marked growth retardation which can be partially overcome by adding small amounts of glycerol or glucose. This raises the question whether the retarded growth rate is due to some limitation of the rate of fatty acid utilization or to the absence from the fatty acid diet of some factor present in the original complete oil. Our present studies have investigated this by comparing the nutritional properties of soybean oil with mixed triglycerides synthesized from soybean oil fatty acids. Surprisingly, the synthesized triglycerides have produced very poor growth and a severe leg paralysis, possibly indicating the need for a previously unrecognized nutrient required under these conditions.

Materials and methods. For these experiments, a sample of reconstituted triglycerides was specially prepared for us† by a procedure involving: (a) preparation of methyl esters by methanolysis of soybean oil in alkaline medium; (b) purification of the methyl esters by distillation under reduced pressure; (c) transesterification of the methyl esters with glycerol in alkaline medium followed by purification of the resynthesized triglycerides by removing soaps and unreacted materials. The fatty acid composition of the intermediate and final products was the same as the original soybean oil according to analysis by gas-liquid chromatography.

The composition of our reference high-carbohydrate diet was: 48.85 glucose; 31 isolated soybean protein (ADM C-1 Assay Protein); 10 soybean oil; 7.41 mineral mixture; 0.72 vitamin mixture; 0.7 DL-methionine; 1 glycine; 0.02 antioxidants (BHT, DPPD), and 0.3 chromic oxide; the latter was included as an inert index substance for determinations of fat absorbability. The mineral mixture supplied, in mg per 100 g diet: 3600 $CaHPO_4 \cdot 2H_2O$; 1000 $CaCO_3$; 680 KCl; 600 Na_2HPO_4; 580 $NaHCO_3$; 400 $KHCO_3$; 350 $MgSO_4$; 100 NaCl; 50 ferric citrate; 25 $MnSO_4 \cdot H_2O$; 20 $ZnCO_3$; 2 $CuSO_4$; 1 KIO_3; 0.8 Na_2MoO_4; 0.022 Na_2SeO_3; 0.18 cobaltous acetate. The vitamin mixture supplied in mg per 100 g diet: 15 niacin; 10 calcium pantothenate; 2 pyridoxine; 1.5 riboflavin; 1 thiamine; 0.3 folacin; 0.3 menadione; 0.1 biotin; 0.01 cyanocobalamin; 200 choline chloride; 500 I.U. vit. A palmitate; 50 I.C.U. vit. D_3; 7.5 mg alphatocopheryl acetate.

The high-lipid experimental diets were prepared by replacing the glucose (48.85 g) and soybean oil (10 g) of the reference diet with an equicaloric amount of the lipid under study (29.95 g triglyceride; 30.70 g fatty acid; or 32.10 g methyl esters) and enough cellulose to give a mealy texture to the diet (19.6 g). The respective high-lipid experimental diets were based on: (a) soybean oil; (b) the reconstituted triglycerides; (c) the distilled methyl esters which served as intermediates in the synthesis of the triglycerides; (d) soybean oil fatty acids prepared by alkaline hydrolysis of the oil used in (a). In each diet protein supplied 32% of the metabolizable energy, and glucose and/or lipid provided the remaining 68%.

Each diet was fed to duplicate lots of 10 one-day-old, broiler type chicks maintained in battery brooder cages provided with wire screen floors and temperature-controlled brooding compartments in a temperature-controlled laboratory. Diets and water were fed *ad libitum*.

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TABLE I. Growth, Food Intake and Paralysis Incidence at 3 Weeks of Age of Chicks Fed Various Lipids.

Diets	Avg wt	Diet consumed	Paralysis incidence
	(g)	(g)	(%)
Reference (high carbohydrate)	408[a]*	452	0
(a) Soybean oil	354[b]	366	0
(b) Reconstituted triglycerides	205[c]	226	100
(c) Soybean oil fatty acids	212[c]	227	0
(d) Methyl esters of soybean oil fatty acids	202[c]	222	0

* Figures with differing superscript are significantly different ($P < 0.01$).

Results and discussion. Growth and food consumption data to 3 weeks of age from the first experiment comparing these lipids are summarized in Table I. In agreement with our previous observations, the diet based on soybean oil promoted nearly as rapid growth as the reference high carbohydrate diet. In contrast, chicks fed the diets based on soybean oil fatty acids or methyl esters exhibited markedly retarded growth, their average weights approximating half of the normal controls. Aside from the difference in growth rate, however, the chicks fed these lipids were normal in appearance. The chicks fed the diet based on reconstituted triglyceride also showed greatly retarded growth, and in addition exhibited 100% incidence of a severe incoordination and leg paralysis. The disability was progressive. By the end of the first week, some incoordination and a stilted posture and walk were evident. Paralysis, with one or both legs extended in an outstretched position, affected some individuals by the tenth day and all of the chicks before the end of 3 weeks. The most severely affected chicks could move only with great difficulty and with the use of their wings. Even these chicks were able to eat and drink when within reach of water and food; the incoordination was apparently confined to the legs. As shown in Table I, growth and food intake were similar for the 3 derived lipids up to 3 weeks of age; replication was excellent within treatments, and mortality to this age was negligible. During the fourth week, some of the paralyzed chicks lost weight and became moribund.

All the above observations were confirmed by a second experiment which also included a diet containing the mixture of free fatty acids derived by alkaline hydrolysis from the reconstituted triglyceride (Table II). As before, all the chicks fed the reconstituted triglyceride diet exhibited incoordination and paralysis. Surprisingly, none of the chicks receiving the fatty acids derived from the same reconstituted triglycerides showed any symptoms of incoordination or paralysis.

In a further experiment, we found that repeated washing of the reconstituted triglyceride preparation with aqueous solutions containing EDTA in order to remove possible metallic contaminants did not prevent the development of paralysis or produce any detectable change in its nutritional properties.

Chicks receiving the reference diet prepared with 10% of reconstituted triglyceride did not show any symptoms of incoordination and were normal in appearance. However, their rate of growth lagged significantly (about 10%) behind that exhibited by chicks receiving the reference diet containing soybean oil. This suggests the possibility that the resynthesized triglycerides lack the factor(s), other than the essential fatty acids, which are responsible for the well established growth stimulating effect of soybean oil and some other fats for chicks(6-10).

TABLE II. Growth, Food Intake and Paralysis Incidence at 2 Weeks of Age of Chicks Fed Triglycerides and the Mixture of Fatty Acids Derived From Them.

Diets	Avg wt	Diet consumed	Paralysis incidence
	(g)	(g)	(%)
Reference (high carbohydrate)	230[a]*	221	0
Soybean oil	217[a]	197	0
Reconstituted triglycerides	124[b]	110	100
Soybean oil fatty acids	144[c]	125	0
Fatty acids from reconstituted triglycerides	149[c]	135	0

* Figures with differing superscript are significantly different ($P < 0.01$).

It is possible that the syndrome we have observed is due to a severe deficiency of an unidentified essential nutrient present in soybean oil. However, such deficiency symptoms have not been obtained by feeding purified fat-deficient diets to chicks, even in prolonged experiments(11). Therefore, if we are dealing with a deficiency, high lipid diets such as we have used may induce the paralysis by increasing the requirement for the missing nutrient. At present, however, we cannot exclude the possibility of a paralysis-inducing toxic factor present in the reconstituted triglycerides.

Summary. Feeding mixed triglycerides synthesized from soybean oil fatty acids as the sole non-protein energy source in a simplified diet produced severe growth retardation, incoordination, and a crippling leg paralysis in young chicks. The possibility that these effects may be due to the lack of an unidentified essential nutrient is discussed.

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Studies on Experimental Infection of Weanling Mice with Reoviruses.* (29989)

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The role of reoviruses in human and animal disease has been studied by many workers, but is not yet well defined(1). There have been several well documented cases of enteric disease in children where no other agent was isolated, the most notable of which involved 3 separate outbreaks of respiratory illness and enteritis in a Washington, D. C. nursery(1). Lerner *et al* reported evidence of reovirus type 2 infection in 7 children, 6 of which had exanthems(2).

Type 2 reovirus has been isolated from patients with steatorrheic diarrhea and from apparently healthy school children(3).

These viruses have been isolated from a variety of animal species including cattle, dogs, monkeys, chimpanzees, wild and laboratory mice and man(4,5,6,7), in some cases with overt clinical illness(1,5,8,9).

The purpose of this study was to determine whether or not respiratory illness could be established in mice *via* the intranasal route of administration, to study the clinical, gross and histological pathology and finally to attempt to demonstrate the virus by fluorescent antibody (FA) procedures, correlating such procedures with conventional virus detection methods.

Experimental animals. Young rabbits and adult roosters were used for reovirus antibody production. Weanling White Swiss mice (3-4 weeks old) were used as the test animals.

Virus. The viruses employed in this study were obtained from the American Type Culture Collection (ATCC). They were reovirus type 1 Lang Strain(13), passed 26 times in primary monkey kidney (PMK) cells; reo-

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