markedly enlarged and exclusion of this organ abolished the absorption enhancing effect of sorbitol seem to provide clues for elucidation of the mechanism. Hypertonic solutions of sorbitol act like a cathartic and increase the speed of descent of coadministered B_{12} in the digestive tract. Physiological absorption of B_{12} which is executed by the small intestine is reduced by accelerated passage(9). It is possible that an enlarged cecum with liquid contents alters the physiology of the intestine in such a way as to effect unphysiological absorption of B_{12} involving no intrinsic factor. Changes of intestinal flora and hence altered availability of B_{12} molecules to the mucosa may be an important factor.

The possibility still remains that a chemical conjugation of sorbitol to B_{12} as suggested by Heinrich(10) may be involved in an unknown fashion. As to the absorption efficiency of the mucosa for B_{12}, there is no evidence that sorbitol increases permeability of the mucosa. The data of the loop experiment suggested reduced permeability by a large dose of sorbitol. Whether the mechanisms working in the rat will directly apply in man awaits further investigation.

Summary. The effect of D-sorbitol on intestinal absorption of $Co^{60}B_{12}$ was studied in rats. The data demonstrated that sorbitol, when given by mouth in large quantities together with supraphysiological doses of B_{12}, enhanced absorption of B_{12} in intact animals. However, absorption of small, physiological doses of B_{12} was reduced by the same dose of sorbitol. The dependence of the sorbitol effect on the B_{12} dose may be explained on the basis of the 2 different mechanisms for B_{12} absorption. No intrinsic factor activity was demonstrated in sorbitol as studied with isolated intestinal loops. When the cecum was excluded from the absorptive route by ileostomy, the conditions that increased absorption in intact animals resulted in reduction of absorption. Possible involvement of the cecum and the altered physiological state of the intestinal contents in enhancement of B_{12} absorption by sorbitol have been discussed.

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Received October 10, 1961 P.S.E.B.M., 1961, v108

Depletion and Synthesis of Fatty Acids in Chickens Fed a Diet Low in Unsaturated Fatty Acids. (27079)

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Linoleic (18:2)* and arachidonic (20:4) acids are considered to be essential fatty acids (EFA). Use of this term infers that either of these compounds will meet the dietary requirement for a fatty acid(s) which cannot be synthesized by the body in sufficient quantities for normal metabolism and growth. Although arachidonate may be the only fatty acid necessary for the function of the cell(1) it is not a specific dietary essential since linoleate is readily converted to arachidonate(2, 3). In contrast linoleate either cannot be syn-

* First number denotes chain length; the second, number of double bonds.

thesized sufficiently to be detectable by the methods used(4,5,6) or is synthesized in only minute amounts(7,8,9,10). The long periods of time involved in detecting symptoms of an EFA deficiency in the dog (12-40 weeks), rat (8-12 weeks), mouse (4 weeks) and guinea pig (4 weeks) (11,12,13,14) are usually interpreted as an indication of a slow rate of catabolism or excretion of EFA. In contrast to these species it has been observed(1) that in chickens, growth differences between EFA depleted and repleted groups can be detected in less than 2 weeks. Is this early response caused by a rapid decrease in tissue level of EFA due to catabolism of EFA or by rapid dilution of the EFA originally present with other fatty acids synthesized by the chick? In the present studies it was found that there is a rapid decrease in the proportion of 18:2 and 20:4 in tissues of chickens fed fat-free diets and this was largely a consequence of dilution of these acids with monoenoic and saturated fatty acids which were readily synthesized in the EFA-deficient chicken.

Procedure. The studies were conducted with Nichols 108 pullets reared in electrically-heated cages, maintained in an air-conditioned room provided with continuous illumination. In the first experiment day-old chicks were fed a low-fat diet containing 20% vitamin-free casein and 8% gelatin(1) for 7 days. They were then divided into 2 groups, one continued on the basal diet, the other the same diet except that 2% safflower oil plus 3.8% cellulose replaced 5.8% glucose. At various time intervals 3 to 5 chicks from each group were sacrificed and the heart and liver removed, frozen and stored at −15°C until analyzed for fatty acids by the gas chromatographic methods previously described(16). The results are expressed as percent by weight of total fatty acids excluding any with a retention time higher than that of 20:4.

In the second experiment a purified diet(1) containing 21% vitamin-free casein as the protein source and 1% methyl myristate as the only fat source was fed *ad libitum* for 28 days. In the third experiment the methyl myristate was deleted. The diets contain less than 0.005% linoleate and no detectable amount of arachidonate. The newly hatched chicks or 28 day-old chicks were sacrificed by putting them in a choloroform atmosphere and the entire carcass and feathers were ground in a large meat grinder until a relatively homogeneous mixture was obtained. The ground carcasses were frozen immediately and stored at −15°C until analyzed. Four grams of tissue were saponified by addition of 30 ml 25% KOH and heating at 50°C for 24 hours under nitrogen. The resulting solution was extracted 3 times with 10 ml portions of ether, brought to acid pH with concentrated HCl and extracted 3 times with 10 ml portions of pentane. The pentane extract was dried with anhydrous sodium sulfate, evaporated to dryness and weighed. This weight was considered to be total fatty acids. The percentage composition of the fatty acids was determined by gas chromatography by methods described previously(16). The grams of each fatty acid were calculated by multiplying total fatty acid content by percent of the particular fatty acid determined by gas chromatography(16). Four or five chickens from each treatment were analyzed.

Results and Discussion. The results are given in Table I. The change with time in

TABLE I. Change in Carcass Content of Fatty Acids with Age of Chickens Fed a Diet Free of Unsaturated Fatty Acids.

Fatty Acid*	Day Old %	Day Old mg	28-Day Old %	28-Day Old mg	Change mg
		Exp 1			
12:0	.0	0	.30	96	96
14:0	.6	18	13.60	3780	3762
14:1	.0	0	3.00	817	817
16:0	30.9	894	32.70	9030	8136
16:1	4.6	140	19.90	5470	5330
18:0	7.1	214	3.10	865	651
18:1	38.6	1165	26.80	7425	6270
18:2	16.4	517	.50	143	−364
20:4	1.4	39	.05	13	−26
TOTAL		2967		27639	24670
		Exp 2			
12:0	.1	2	.20	60	58
14:0	.4	9	1.90	571	562
14:1	.0	0	1.10	331	331
16:0	28.4	634	35.50	10675	10041
16:1	5.6	125	20.90	6284	6159
18:0	11.9	266	3.50	1052	786
18:1	37.9	847	35.50	10675	10614
18:2	14.6	326	.80	240	−86
20:4	1.0	22	.06	18	−4
TOTAL		2234		30070	27836

* First number denotes chain length; the second, number of double bonds. Analyzed by gas chromatography. Each number is average of at least 4 replicate analyses.

the proportions of fatty acids in livers of chicks fed EFA deficient diets is given in Fig. 1. Values for EFA (18:2 plus 20:4), the major monoenoic acids (16:1 and 18:1), and a fatty acid with a retention time compatible with a 20:3 structure are summarized. The decrease in the proportion of EFA is very rapid during the first 11 days and thereafter decreases quite slowly. As EFA decreased the monoenoic acids increased. This confirms the reciprocal relationship observed between 18:2 and the monoenoic acids(15). The 20:3 acid increased slowly but continually during the experimental treatment. When linoleic acid (as safflower oil) was added at 7 days EFA percent increased rapidly (Fig. 2). Within 4 days after addition of the linoleic acid (as safflower oil) to the diet the percentage of 18:2 and 20:4 was 16.0 ± 0.48% and 8.6 ± 0.82%, respectively, in the livers of chicks fed the basal diet. The differences between dietary treatments was statistically significant. The data again demonstrate the reciprocal relationship between EFA and the monoenoic acids. Similar results were obtained with heart tissue (Fig. 3 and 4).

During the 28 day growth period average fatty acid content of the chicken increased 24.7 g in Experiment 1 and 27.8 g in Exp. 2 (Table I). Most of this increase was the result of synthesis of saturated and monoenoic acids. Palmitate (16:0) was the predominant

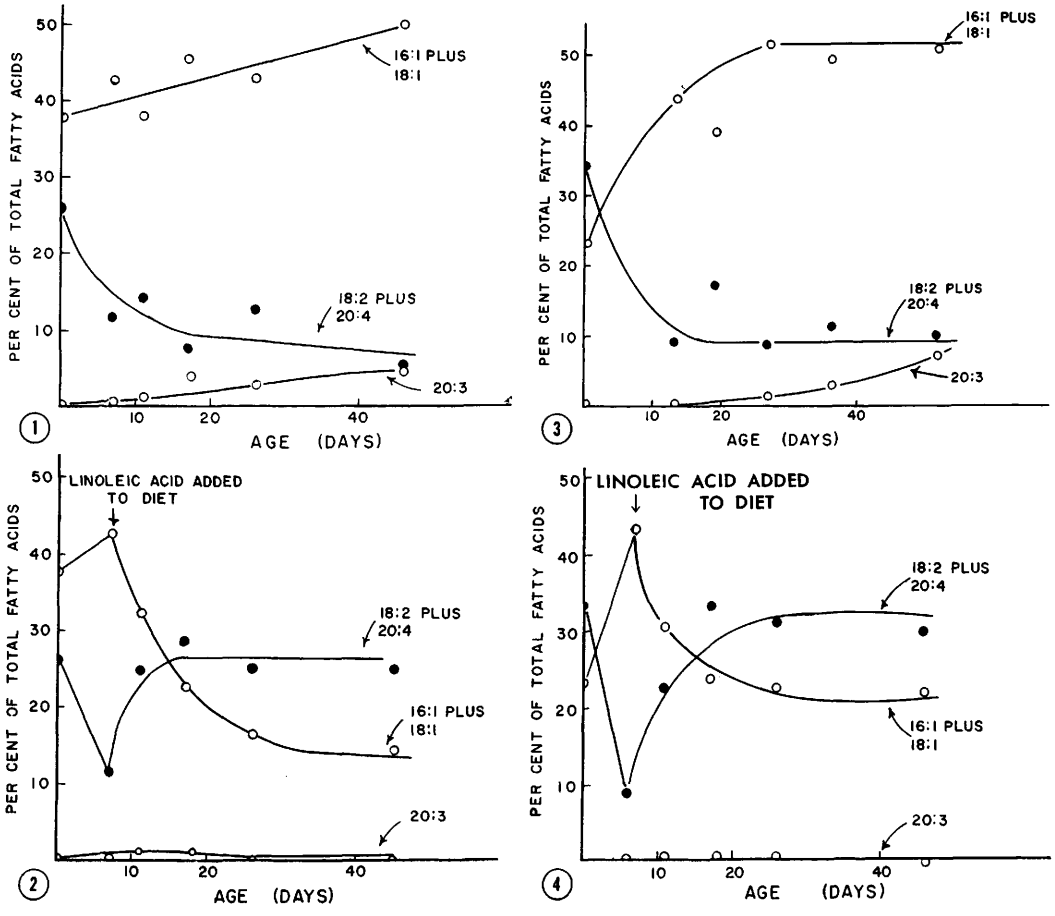

FIG. 1. Change in fatty acid composition of liver from chicks fed a low-fat diet.
FIG. 2. Effect of dietary linoleic acid on the fatty acid composition of liver.
FIG. 3. Change in fatty acid composition of heart from chicks fed a low-fat diet.
FIG. 4. Effect of dietary linoleic acid on the fatty acid composition of heart.

saturated fatty acid synthesized and palmitoleate (16:1) and oleate (18:1), the major monoenoic acids. This confirms the observation of Meade et al.(17), who found by use of a combination of silicic acid chromatography and alkaline isomerization procedures that the fats of rats fed EFA-deficient diets are rich in palmitoleate. The large amount (3780 mg) of myristate (14:0) recovered in the 28 day old chickens in Exp. 1 evidently was a result of deposition of dietary 14:0 since in the second experiment with no 14:0 in the diet only 571 mg was recovered in the 28-day old carcass.

In contrast to the saturated and monoenoic acids, the amount of 18:2 and 20:4 in the carcass of the 28 day-old chicken decreased to less than the initial amount present in the day old chickens. This suggests that there is some catabolism or excretion of 18:2 and 20:4. It is known that 18:2 is oxidized at a high rate by EFA-deficient mice(18), rats(19), and chickens(20). No data on 20:4 oxidation are available. Webb et al.(10) presented evidence for synthesis of 18:2 from acetate in fowl blood. However, based on the present studies such synthesis does not contribute significantly to the EFA economy of the young chicken.

As a result of the depletion of 18:2 and 20:4 concurrent with synthesis of saturated and monoenoic acids, the proportion of EFA in the fat of the body decreased drastically. In both experiments with 28-day old chicks the proportion of 18:2 or 20:4 was less than 1/16 of that of the newly-hatched chick. The rapid change in concentration of EFA may help explain why the chicken is sensitive to EFA deficiencies.

The chicken responds to supplements of EFA long before all 18:2 or 20:4 is depleted from the tissues(1). This suggests that merely a change in the proportion of 18:2 or 20:4 in some lipid structures may be sufficient to affect a critical function. Such functions may include regulation of enzymatic reactions(21, 22) or transport of critical metabolites in the blood. Addition of linoleic acid to a fat-free diet increases the deposition of short chain saturated fatty acid in adipose(1,23) and other tissues(24). This suggests another possible function for EFA, regulation of cell membrane permeability.

Summary. The proportion of linoleic and arachidonic acid in liver and heart tissue of chickens fed fat-free diets decreased to near minimal values within the first 2 weeks after hatching. Addition of linoleic acid to a fat-free diet at 7 days of age resulted in a significant increase of both linoleic and arachidonic acid in the liver of chicks fed the supplement within 4 days. Chickens fed a diet free of unsaturated fatty acids from 1 to 28 days of age synthesized over 24 g of fatty acids. This consisted mainly of palmitate, palmitoleate and oleate. During the same period the linoleate and arachidonate content of the entire body decreased, resulting in a reduction in the percent of these acids to less than 1/16 of that originally present in the newly-hatched chick.

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Received October 5, 1961 P.S.E.B.M., 1961, v108

Encephalomyocarditis Virus Hemagglutination-Inhibition Test Using Antigens Prepared in HeLa Cell Cultures. (27080)

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Hemagglutinins can be demonstrated in suspensions prepared from the brain tissue of mice infected with the encephalomyocarditis (EMC) virus(1). Although cell cultures of many types support the growth of the virus (2), they have been utilized to only a limited extent for production of hemagglutinating (HA) antigen(3). During our investigations on occurrence of EMC hemagglutination-inhibition (HI) antibody in human sera, a need arose for large quantities of antigen. When reproducible results were not obtained with mouse brain suspensions, cell cultures were tried as a source of hemagglutinin. This report describes a method for preparation of EMC HA antigen in HeLa cells. Conditions necessary for optimal hemagglutination have been investigated and the findings applied to development of an HI test.

Materials and Methods. Virus. The EMC strain was recovered in this laboratory from the lung tissue of a naturally infected pig(4). It was isolated in HeLa cells and identified by neutralization tests employing a hyperimmune antiserum prepared against the American Type Culture prototype strain of EMC virus.‡ At the time of these studies the virus had received 5 consecutive HeLa passages.

HeLa Cells§ were grown as monolayers in 36 oz bottles in Eagle's medium containing 10% horse and 10% calf serum. Before virus inoculation cell sheets were washed twice with Hanks' solution; Eagle's medium containing 4% chicken serum was then added. Heated serum (56°C for 30 minutes) and antibiotics were routinely used in all media.

Diluents. The following solutions were prepared in distilled demineralized water: 0.15 M NaCl, 0.15 M KCl, 0.05 M H_3BO_3 in 0.12 M NaCl, 0.2 M Na_2HPO_4 − 0.2 M NaH_2PO_4 in 0.15 M NaCl (PO_4 buffer − NaCl) or 0.15 M KCl (PO_4 buffer − KCl) over the range pH 7.0 to 8.0, and 0.05 M H_3BO_3 in 0.12 M NaCl (BO_3 buffer − NaCl) or 0.12 M KCl (BO_3 buffer − KCl), adjusted with 1.0 M NaOH to pH 8.0 and 8.5. Occasionally, these solutions contained non-specific hemagglutinins which were readily removed by adsorption with small amounts of kaolin.

Red Blood Cells. (RBC) were collected from sheep, guinea pigs and humans in an acid-citrate-dextrose solution(5), and washed 3 times with isotonic saline. They were stored as a pack at 4°C for not longer than one week and resuspended in diluent as needed.

HA Tests. Four-tenths ml of antigen was serially diluted in 2-fold steps in 12 × 100 mm tubes. Four-tenths ml of a 0.4% suspen-

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‡ Kindly supplied by Dr. W. L. Pond, Nat. Inst. Allergy and Infect. Dis., Bethesda, Md.

§ Original seed obtained from Microbiological Associates, Inc., Bethesda, Md.