Role of Linoleic Acid in Infant Nutrition

Clinical and Chemical Study of 428 Infants Fed on Milk Mixtures Varying in Kind and Amount of Fat

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Copies of this Supplement may be obtained for $1.00 each, from The American Academy of Pediatrics, 1801 Hinman Avenue, Evanston, Illinois

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INTRODUCTION

The study reported here attempts to evaluate the role of fat and to determine the relative importance of the kind and amount of fat included in the diet of infants. Clinical and chemical findings are presented concerning more than 400 infants maintained on diets without fat and with fat having different fatty acid compositions. The study embraced a 4-year period.

Many attempts have been made to develop milk mixtures that emulate human breast milk in composition. One of the recognized differences between human and cow's milk concerns the nature of the fat. Significant in this regard is the fact that the linoleic acid content of human mother's milk is four to five times greater than that in cow's milk. Previous studies from this laboratory[1-3] strongly support the hypothesis that linoleic acid is an essential nutrient for infants. The results of the present study provide further confirmation of this concept.

MATERIALS AND METHODS

Milk Preparations Used

The original plan of study was to feed each of four groups of 100 babies on one of four different milk formulas for the first 6 months to 1 year of life. Three formulas contained the same total amount of fat but variable amounts of linoleic acid obtained from appropriate mixtures of vegetable oils and from butterfat. The fourth preparation was a skimmed-milk formula which contained almost no fat, hence, only traces of linoleic acid. The pediatricians did not know the specifications regarding the nature of the fat in the different formulas. Relatively early in the study, because of evidences of fat deficiency in the infants fed the low fat milk, a fifth formula was substituted which was equal in fat content to the other three mixtures, but was composed of saturated fatty acids, a small amount of oleic acid, and only traces of linoleic acid. The composition of the five different formulas after dilution with equal parts of water is presented in Table I.

The milk mixtures were sterilized in cans containing 13 fluid ounces and labeled "Infant Formula #1, #2, #3, #4, or #5." Each label was a different color and contained the statement, "Prepared especially for the Department of Pediatrics, University of Texas School of Medicine."* The same chemist and plant superintendent

* Dr. Hansen died on October 16, 1962.

* Kindly supplied by The Baker Laboratories, Inc., Cleveland, Ohio.

Supported by grants-in-aid from The Baker Laboratories, Inc., Cleveland, Ohio, and Gerber Products Company, Fremont, Michigan.

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PEDIATRICS, January, Part II, 1963

TABLE I

COMPOSITION OF MILK FORMULAS AT FEEDING DILUTION

Components*	Formula Number				
	1	*2*	*3*	*4*	*5*
Fat (%)	3.0†	3.0†	3.0‡	0.1‡	3.0§
Protein (%)	2.5	2.6	2.3	2.4	2.5
Carbohydrate (%)	7.4	7.3	7.5	13.5	7.0
Ash (%)	0.7	0.7	0.7	0.7	0.7
Water (%)	86.4	86.4	86.5	83.3	86.8
Calories/oz‖	20	20	20	19	20
Linoleic acid (% cal)	2.8	7.3	1.3	0.04	0.07
Total fat (% cal)	41	41	41	1	42
Protein (% cal)	15	15	14	15	15
Carbohydrate (% cal)	44	44	45	84	53

* Vitamin Content: vitamin A, 2500 U.S.P. units; vitamin D, 800 U.S.P. units; ascorbic acid, 50 mg; thiamine, 0.6 mg; niacin, 5 mg; riboflavin, 1.1 mg; and vitamin B_6, 0.18 mg. Iron ammonium citrate was added to supply 7.5 mg elemental iron per quart.

† Blend of corn and coconut oils.

‡ Butterfat.

§ Hydrogenated coconut oil.

‖ Carbohydrate (corn syrup) was added to equalize the caloric value of the mixtures.

supervised the preparation of all the mixtures. Supplies were shipped at monthly intervals in order to prevent the possibility of age thickening.

The infants were given solid foods according to the schedule in Table II. An adequate supply of the different products was prepared at one time,° so each type of solid food had the same composition throughout the study. The composition of the solid foods is shown in Table III.

Subjects of Study

Before any infant was placed on a diet of one of the milk mixtures, the nature of the study was explained to the mother by one of the staff physicians participating in the co-operative venture. The members of the staff had agreed to provide, as private physicians, all pediatric care for the first year of life, including periodic examinations and routine immunizations. The pediatricians also answered calls for emergency conditions. The milk and solid foods were provided gratis for the duration of participation in the study.

The subjects were infants seen in the well baby clinics under the supervision of members of the pediatric staff and included children of members of the staff, of medical students, of nurses, and of technicians. In 1956, 132 were introduced to the study; 174 were added in 1957, 106 in 1958, and 16 in 1959. The distribution between premature and full-term infants was 109 and 319 respectively. The birthweights of five of the premature infants were so close to being marginal (2,500 gm) they were included with the full-term group because they had been started on the special milk mixtures relatively late compared to most of the infants and had gained very well. Eighty-six per cent of the premature infants and nearly 50% of the full-term infants were started on the special milk mixtures while still in the hospital nurseries. There were 355 infants who began the special formula within the first 3 weeks of age, 64 between 3 and 6 weeks, 9 between 6 and 12 weeks. The distribution of 20, 108, and 300, respectively, among Latin American, white, and Negro infants was approximately the same as seen in the outpatient service. There were 202 males and 226 females. The study included observations on 10 sets of twins. In order to prevent confusion for

° Kindly supplied by Gerber Products Company, Fremont, Michigan.

TABLE II

SCHEDULE OF SOLID FOODS

Age of Infant (mo.)	*Type of Food Added*
2–3	Cereals: rice, oatmeal, mixed
4–5	Vegetables: peas, green beans, mixed vegetables, carrots, beets, squash
	Fruits: prunes, pears, bananas, applesauce, pears-pineapple, applesauce-apricots, plums-tapioca, fruit-dessert
6	Meats: veal, beef, lamb
	Breads: teething biscuits

TABLE III

COMPOSITION OF INFANT FOODS PER 100 GRAMS

Type	*Calories*	*Protein*	*CHO*	*Fat*		*Linoleic Acid*		*Crude Fiber*	*Ash*	*Total Solids*	*Average Serving, 3–6 months (gm)*†
				Total	*% Cal.*	*Total*	*% Cal.*				
Cereals											
Oatmeal	400	15.3	64.6	7.8*	18.1	2.9	6.7	1.2	4.3	93.2	10–12
Rice	381	6.6	75.1	5.1*	12.1	1.7	4.1	0.8	5.4	93.0	10–12
Mixed	388	13.8	69.4	5.1*	11.9	2.2	5.2	1.1	4.3	93.7	10–12
Vegetables											
Peas	52	4.0	8.3	0.3	5.3	..	..	0.4	0.8	13.8	67
Green beans	24	1.4	4.3	0.1	3.9	..	..	0.8	0.9	7.5	67
Mixed vegetables	37	2.4	6.3	0.2	5.0	..	..	0.7	1.0	10.6	67
Carrots	24	0.7	5.1	0.1	3.7	..	..	0.5	1.0	7.4	67
Beets	38	1.1	8.1	0.1	2.4	..	..	0.6	1.2	11.1	67
Squash	24	0.7	5.0	0.1	3.9	..	..	0.7	0.9	7.4	67
Fruits											
Prunes	86	0.6	20.2	0.3	3.2	..	..	0.8	0.6	22.5	47–67
Pears	59	0.2	14.2	0.1	1.5	..	..	1.2	0.2	15.9	47–67
Bananas	92	0.5	22.0	0.2	2.0	..	..	0.2	0.3	23.2	47–67
Applesauce	85	0.2	20.8	0.1	1.1	..	..	0.6	0.2	21.9	47–67
Pears/pineapple	73	0.4	17.6	0.1	1.3	..	..	1.3	0.3	19.7	47–67
Applesauce/apricot	97	0.4	23.2	0.3	2.9	..	..	0.6	0.5	25.0	47–67
Plums/tapioca	98	0.4	23.8	0.1	0.9	..	..	0.3	0.2	24.8	47–67
Meats											
Veal	98	16.6	..	3.5	33.0	0.09	0.8	..	1.1	21.2	50
Beef	94	14.4	..	4.0	40.0	0.10	1.0	..	1.0	19.4	50
Lamb	99	16.3	..	3.7	35.0	0.15	1.4	..	0.8	20.8	50

* Determined by Acid Hydrolysis.
† ½ can = 67 gm; ⅓–½ can = 47–67 gm; ½ can = 50 gm.

the mother, both twins were assigned the same milk mixture.

Each subject was given a case number to facilitate record keeping. During the first year of the study, random sample procedures determined assignment of one of the four original milk formulas to the individual infant. Because of the necessity of change in the formulas, only 32 subjects were started on formula 4 and 65 subjects on formula 5, instead of the 100 or more as for the other three formulas. In 26 instances because of intercurrent illness, clinical judgment, death, parental complications, or failure to keep appointments, the infant was dropped from the study before any significant clinical or chemical observations could be made. In other words, of 454 infants given a case number, there were 428 on whom clinical evaluations were made for an average period of 9½ months. Approximately 60% were followed through the first year of life. Throughout the study, careful attention was given to the maintenance of a continued healthy state of all the infants. Each of the pediatricians followed the semi-demand basis in regard to the timing and amount of milk offered. Solid foods were usually withheld for the first 3 months in order to evaluate better the effect of the particular milk mixture. However, if the physician deemed the earlier use of solid food advantageous to the infant's progress, the necessary additions were made. No pediatrician had more than 60 patients under supervision at any one time.

Clinical Observations

The plan of study called for outpatient visits to a special clinic at 3-week intervals for the first 12 weeks, then at monthly intervals. The same personnel were in charge of the clinic throughout. The following clinical features were given careful attention, and the observations were recorded on charts, with special attention to skin (moist and soft, scaly and dry, irritated or infected), stools (soft and regular, frequent, copious and foul, hard but regular, hard and infrequent), intercurrent illness (coryza, upper respiratory infection, otitis media, pneumonia), general features, (vomiting, regurgitation), and satiety (happy, content, placid, fretful, irritable). Measurements of weight, length, and head circumference were made, and, when indicated, temperatures were taken. The body measurements were plotted on Wetzel grids.

The mother was requested to record the dietary intake of the infant for a 4-day period between visits to the special clinic. Mimeographed forms "Mother's Record of Feeding," were provided. These showed subjects' name, formula number and dilution, and the date. Most infants were seen on Friday, and it was recommended that the dietary intake be recorded for the 4 days immediately preceding the visit. Spaces on the charts were delineated for noting the feeding time, the number of ounces taken at each feeding and the ounces of water given in addition. The usual household measures (teaspoonful, tablespoonful, cup or can) were used for recording the intake of cereals (dry), vegetables, fruits, and meat. In order to calculate the average daily weight gain, the gain between each visit was divided by the number of days in the interval.

When the mother and/or the physician felt that an infant was not thriving satisfactorily, one of the alternate milk mixtures was substituted. In a number of instances, when symptomatology of fat deficiency was suspected, a specific fatty acid (linoleic, palmitic, oleic, or arachidonic) was given either in the form of an ester or as the triglyceride. This was mixed with a small amount of food or placed directly in the mouth with a dropper at the time of feeding. More often, however, when manifest signs of linoleic acid deficiency appeared, they were corrected by switching the infant to one of the mixtures containing liberal amounts of linoleic acid.

Laboratory Procedures

Determinations of the serum lipids were made at 3-month intervals beginning with the third month, or before changing formulas. Mothers were instructed to withhold feedings from midnight on, prior to these tests, allowing water ad libitum, however. The method of Wiese and Hansen[4] was used for determination of the total fatty acids in the blood serum, employing the alkaline isomerization technique for the distribution of the dienoic, trienoic, and tetraenoic acids. Total proteins of the serum were measured by nesslerization. Hemoglobin and total and differential leukocyte counts were made at the same time. Other laboratory studies (such as urinalysis, serum electrolytes, roentgenographic and bacteriologic procedures) were made when indicated.

RESULTS

Clinical Features

Information is deposited with the American Documentation Institute Auxiliary Publications Project as Table C (see footnote on title page) concerning each individual infant, including birth date, sex, race, birthweight, age started on milk mixture, duration on the study, data regarding changes to other milk mixtures, and serum lipid values. Summary comments concerning significant observations made by staff physicians also are given.

General Considerations: Data on the number of infants started on each mixture and the number changed to other mixtures during each 3-month period are summarized in Table IV. The percentage of infants requiring a change in formula were: #1, 2.6; #2, 0.0; #3, 3.8; #4, 50; and #5, 41.5%. Detailed data of formula changes for full-term infants are given in Table V-A, and for premature infants in Table V-B. The

TABLE IV

Milk Formula Changes

Subjects	Formula Number				
	1	*2*	*3*	*4*	*5*
Total started	116	112	103	32	65
>2,500 gm	93	86	74	20	46
<2,500 gm	23	26	29	12	19
Changed to other mixtures					
During 0–3 months					
Total	3	0	2	12	13
>2,500 gm	3	0	2	5	9
<2,500 gm	0	0	0	7	4
During 4–6 months					
Total	0	0	1	4	6
>2,500 gm	0	0	1	3	4
<2,500 gm	0	0	0	1	2
During 7–9 months					
Total	0	0	0	0	6
>2,500 gm	0	0	0	0	4
<2,500 gm	0	0	0	0	2
During 10–12 months					
Total	0	0	1	0	2
>2,500 gm	0	0	0	0	2
<2,500 gm	0	0	1	0	0
Total changes for year	3	0	4	16	27

percentage of full-term infants remaining on the study in each of the five groups at the end of each 3-month period (Table V-A) was greater for the mixtures containing linoleic acid than for those lacking linoleic acid. For premature babies, the parallel data (Table V-B) reveal a similar, although less clear-cut, pattern. In both groups, the percentage of infants who remained on formulas 4 and 5 increased somewhat after the first quartile. The relative stability with aging may be attributed, at least in part, to the addition of solid foods or, in several instances, to dietary supplementation with linoleic acid.

Satiety: The mothers' periodic descriptions of satiety (happy, content, placid, fretful, irritable) showed no significant group differences in the reactions of the babies to any of the five formulas.

Growth and Devlopment Records: Most of the subjects had satisfactory growth patterns as indicated by chartings on the Wetzel grid. Inspection of data for head circumference and body length revealed no particular trends.

At the completion of the investigation, the participating pediatricians jointly studied the growth curve for each subject and selected those showing marked deviation from the normative channels. These deviations presumably represent exceptionally poor or exceptionally favorable growth trends. The findings are summarized in Table VI. Although the numbers involved are small, the higher proportions of greater-than-anticipated growth rates in groups 1, 2, and 3, and of lower-than-anticipated growth rates is groups 4 and 5 are significant.

Since the values for the average gain in weight per day, at 6, 9, and 12 weeks were almost identical for groups 1 and 2, they were combined for statistical comparison with groups 3 and 5. The data for group 4 were too few for consideration. The statistical summary of weight gains is given in Table VII. There was no difference in mean weight gain between groups 1 and 2 combined and group 3. As indicated in the *P* values the differences were significant for groups 1 plus 2 versus 5, and for group 3 versus 5.

Caloric Consumption: Because of the many variabilities in the feeding of premature infants, data regarding food consumption are presented only for the full-term infants.

The staff physicians individually evaluated the social and personal factors which were believed to influence the reliability of the records made by the mothers. The physicians then collectively performed further screening. After results of serum lipid de-

TABLE V-A

DATA REGARDING NUMBER OF FULL-TERM INFANTS GIVEN DIFFERENT MILK MIXTURES, WITH CHANGES MADE

Changes	Formula Numbers				
	1	2	3	4	5
1st 3 months					
Started (no.)	93	86	74	20	46
Shifted to (no.)	+6	+9	+1	+1	+2
Shifted from (no.)	−3	0	−2	−5	−9
Dropped (no.)	−1	−1	−1	−6	−3
Remaining (no.)	95	94	72	10	36
Completing quartile (%)	96	99	97	48	75
Remaining at 3 months (%)	96	99	97	48	75
4–6 Months					
At end of 3 months (no.)	95	94	72	10	36
Shifted to (no.)	+3	+1	+2	0	+2
Shifted from (no.)	0	0	−1	−3	−4
Dropped (no.)	−10	−8	−4	−2	−3
Remaining (no.)	88	87	69	5	31
Completing quartile (%)	90	92	93	50	82
Remaining at 6 months (%)	86	91	91	24	62
7–9 months					
At end of 6 months (no.)	88	87	69	5	31
Shifted to (no.)	0	+3	+1	0	0
Shifted from (no.)	0	0	0	0	−4
Dropped (no.)	−5	−10	−14	0	−3
Remaining (no.)	83	80	56	5	24
Completing quartile (%)	94	89	80	100	77
Remaining at 9 months (%)	81	81	73	24	48
10–12 months					
At end of 9 months (no.)	83	80	56	5	24
Shifted to (no.)	0	+2	0	0	0
Shifted from (no.)	0	0	0	0	−2
Dropped (no.)	−13	−17	−4	−1	−1
Remaining (no.)	70	65	52	4	21
Completing quartile (%)	84	79	93	80	88
Remaining at 12 months (%)	69	64	68	19	42
Total full-term infants receiving formula	102	101	78	21	50

terminations had been ascertained, each case was again evaluated by the staff physicians and the biochemist. In several instances the results of serum lipid values were such as to arouse suspicion that strict adherence to the special formula had not been maintained. Caloric intake data for these cases were eliminated. This step

TABLE V-B

DATA REGARDING NUMBER OF PREMATURE INFANTS GIVEN DIFFERENT MIXTURES, WITH CHANGES MADE

Changes	*Formula Number*				
	1	*2*	*3*	*4*	*5*
1st 3 months					
Started (no.)	23	26	29	12	19
Shifted to (no.)	+2	+5	+3	0	+1
Shifted from (no.)	0	0	0	−7	−4
Droppped (no.)	−9	−12	−12	−2	−7
	—	—	—	—	—
Remaining (no.)	16	19	20	3	9
Completing quartile (%)	64	58	61	25	45
Remaining at 3 months (%)	64	58	61	25	45
4-6 months					
At end of 3 months (no.)	16	19	20	3	9
Shifted to (no.)	+1	+1	0	0	+1
Shifted from (no.)	0	0	0	−1	−2
dropped (no.)	−2	0	−2	0	−1
	—	—	—	—	—
Remaining (no.)	15	20	18	2	7
Completing quartile (%)	88	100	90	67	70
Remaining at 6 months (%)	58	61	55	17	33
7-9 months					
At end of 6 months (no.)	15	20	18	2	7
Shifted to (no.)	0	+1	+1	0	0
Shifted from (no.)	0	0	0	0	−2
Dropped (no.)	−1	−3	−2	0	0
	—	—	—	—	—
Remaining (no.)	14	18	17	2	5
Completing quartile (%)	93	85	89	100	71
Remaining at 9 months (%)	54	55	50	17	24
10-12 months					
At end of 9 months (no.)	14	18	17	2	5
Shifted to (no.)	0	+1	0	0	0
Shifted from (no.)	0	0	−1	0	0
Dropped (no.)	−3	−2	−3	0	−1
	—	—	—	—	—
Remaining (no.)	11	17	13	2	4
Completing quartile	79	94	76	100	80
Remaining at 12 months	42	50	38	17	19
Total premature infants receiving formula	26	34	33	12	21

proved to be necessary in surprisingly few cases. Such eliminations were made, however, if a period of recording intake coincided with an acute illness or some questionable episode.

In considering the caloric intake, per se, the high standard deviations in the individual groups made differences in the mean values not statistically significant. However, when the caloric intake was related to

TABLE VI

INTERPRETATION OF GROWTH AND DEVELOPMENT RECORDS

Records	*Formula Number*				
	1	*2*	*3*	*4*	*5*
Full-term infants	93	86	74	20	46
Exceptionally favorable	7	9	8	1	0
Exceptionally poor	4	2	4	6	20
Premature infants	23	26	29	12	19
Exceptionally favorable	1	1	0	0	0
Exceptionally poor	2	1	2	2	10
Totals	116	112	103	32	65
Exceptionally favorable	8	10	8	1	0
Exceptionally poor	6	3	6	8	30

weight gain, significant differences were noted (Table VIII).

INTERCURRENT ILLNESS: Infections of the respiratory tract were recorded quite frequently, but the incidence did not appear to be influenced by the character of the diet. The frequency of vomiting, regurgitation, unusual feeding problems, or uncommon behavioral characteristics revealed no trends that appeared to be related to the diet.

Twenty-six infants were hospitalized during the study: five with pneumonia; three, bronchitis; four, skin infections; seven, diarrhea; two, hernia; one, slow weight gain; one, pyloric stenosis; one, meningitis; one, burn; and one, otitis media. Hospitalization occurred more frequently with infants receiving milk mixtures 4 and 5 (four of the five with pneumonia, three of the four with skin infections, and six of the seven with diarrhea) than with the other formulas. The incidence of staphylococcal skin infections, with or without severe complications was not influenced by the type of diet. The response to therapy likewise was not different whether or not linoleic acid was in the diet. Among the 454 infants started on the study there were 7 deaths, including 4 prematures. Three died within the first week of life, hence, could not be included in the final tabulation. The fourth (Case 443) died at 41 days of age of bronchopneumonia. He had been placed on formula 5 at 2 days of age and developed diarrhea at 14 days and again 2 weeks later. All three deaths in the full-term infants were associated with staphylococcal infections which coincided with an epidemic due to hemolytic Micrococcus aureus, multiple antibiotic resistant organisms of phage types 80-81, 42B and 44A, which occurred in the hospital newborn nursery.[5-7]

CONDITION OF STOOLS: The most notable feature in regard to bowel movements was the prevalence of loose stools among infants on the formulas lacking linoleic acid, particularly during the first quartile, and

TABLE VII

MEAN VALUES WITH STANDARD DEVIATIONS FOR DAILY WEIGHT GAIN AT 6, 9, AND 12 WEEKS FOR INFANTS GIVEN FORMULAS 1 AND 2 COMPARED WITH FORMULA 3 AND WITH FORMULA 5

Formula Number	*6 Weeks*		*9 Weeks*		*12 Weeks*	
	Infants (no.)	*Mean weight gain/day (gm)*	*Infants (no.)*	*Mean weight gain/day (gm)*	*Infants (no.)*	*Mean weight gain/day -gm)*
#1+#2	113	35.4±11.8	119	32.7±10.6	125	28.0±8.2
#3	48	33.6± 9.9	52	31.5± 9.9	51	29.2±7.9
#5	23	25.9± 8.4	23	22.1± 6.6	20	22.9±8.2
Probability	#1+2 vs #5 P<0.001		#1+2 vs #5 P <0.001		#1+2 vs #5 P <0.01	
Probability	#3 vs #5 P <0.01		#3 vs #5 P <0.001		#3 vs #5 P <0.01	

TABLE VIII

Mean Values with Standard Deviation for Calories Consumed per Kilogram per Day for Each Gram Gain in Weight, at 6, 9, and 12 Weeks for Infants on Formulas 1+2 Compared with Formula 3 and with Formula 5

Formula Number	*6 Weeks*		*9 Weeks*		*12 Weeks*	
	Infants (no.)	*Cal/kg/gm/day*	*Infants (no.)*	*Cal/kg/gm/day*	*Infants (no.)*	*Cal/kg/gm/day*
#1+#2	113	3.82±1.80	119	3.93±1.64	125	4.47±1.80
#3	48	4.50±2.49	52	4.71±2.05	51	4.50±1.77
#5	23	5.32±2.41	23	6.33±2.28	20	6.63±2.74
Probability	#1+2 vs #5 P <0.001		#1+2 vs #3 P <0.01; #1+2 vs #5 P <0.001; #3 vs #5 P <0.01		#1+2 vs #5 P <0.001; #3 vs #5 P <0.001	

especially among those on formula 4 (Table IX). The stools were watery and sometimes sirupy in character. The extremely high carbohydrate intake undoubtedly was a factor in producing loose stools in infants on formula 4; however, the incidence was greater in infants receiving formula 5 than 1, 2, or 3. Hard stools were rarely noted except for term infants in groups 2 and 3 during the early months of life. In the first quartile, attacks of diarrhea were slightly more common for infants on formulas 3 and 4 than for those on 1, 2, or 5.

Condition of Skin: The occurrence of dry and scaly skin with thickening was by far the most striking and significant abnormality observed among infants receiving formulas low in linoleic acid. This feature was most easily discernible in the Negro infants because of the contrast of the white scales against the dark background (Figs. 1 & 2). During the first 3 months, dryness of the skin with desquamation, redness, and oozing in the intertriginous folds was observed in all full-term infants receiving formulas 4 (no fat) and in 40% receiving formula 5 (saturated fat) (Table X-A). During the second quartile only five full-term infants were still on formula 4, and all five manifested the typical skin signs. Of the 31 infants on formula 5, 55% showed the dry and scaly skin. During the third quartile, in a large proportion of the infants receiving milk mixtures 4 and 5, clearing of the skin was gradual but continuous. During the first and second quartiles, dryness

TABLE IX

Condition of Stools

Condition of Stool	*Formula Number*				
	1	*2*	*3*	*4*	*5*
	Number of Infants				
1st 3 months					
Full-term infants	93	86	74	20	46
Loose	5	8	4	14	23
Hard	5	16	14	1	2
Premature infants	23	26	29	12	19
Loose	2	1	3	7	6
Hard	1	0	0	0	0
4–6 months (all infants)	111	112	92	13	45
Loose	1	3	0	6	10
Hard	2	3	3	0	1
7–9 months (all infants)	103	106	87	7	38
Loose	1	1	0	0	1
Hard	0	4	3	0	2
10–12 months (all infants)	97	97	73	7	29
Loose	0	2	0	0	0
Hard	1	1	1	0	0
Total Infants	116	112	103	32	65

TABLE X-A

FULL-TERM INFANTS WITH SYMPTOMS REFERABLE TO THE SKIN AT VARIOUS AGES

Symptoms	Formula Number									
	1		2		3		4		5	
	No.	Visits	No.	Visits	No.	Visits	No.	Visits	No.	Visits
3–6 weeks	93	93	86	86	73	73	17	17	40	40
Total with skin symptoms	4		6		6		8		11	
Dry and scaly	1		2		3		7		10	
Perianal irritation	0		2		0		4		2	
Bacterial skin infection	3		2		3		0		2	
6–9 weeks	95	95	91	91	72	72	13	13	35	35
Total with skin symptoms	6		5		9		8		11	
Dry and scaly	5		2		6		7		9	
Perianal irritation	0		0		0		1		1	
Bacterial infection	1		3		3		0		1	
9–12 weeks	95	95	94	94	72	72	10	10	36	36
Total with skin symptoms	2		7		2		10		15	
Dry and scaly	1		3		2		10		14	
Perianal irritation	0		0		0		1		0	
Bacterial infection	1		4		0		1		1	
4–6 months	88	264	87	261	69	207	5	15	31	93
Total with skin symptoms	11		9		9		5		17	
Dry and scaly	4		3		4		5		17	
Perianal irritation	0		0		0		1		0	
Bacterial infection	8		6		5		0		0	
7–9 months	83	249	80	240	56	168	5	15	24	72
Total with skin symptoms	8		6		2		1		10	
Dry and scaly	2		2		0		1		9	
Perianal irritation	0		0		0		0		0	
Bacterial infection	6		4		2		0		2	
10–12 months	70	210	65	195	52	156	4	12	21	63
Total with skin symptoms	6		4		3		1		3	
Dry and scaly	1		2		2		1		2	
Perianal irritation	0		0		0		0		0	
Bacterial infection	5		2		1		0		1	
Total symptoms	102	1,082	101	1,041	78	812	21	99	50	375
Total with skin symptoms	24		26		24		14		34	

of the skin as a transient episode was noted in 14 of the infants receiving formulas 1, 2, and 3. In the premature infants, dry scaly skin was recorded infrequently on formulas 1, 2, and 3 and in nearly all receiving mixtures 4 and 5 (Table X-B). At 4 to 5 months in the premature infants receiving mixtures 4 and 5, the incidence of skin abnormalities was high but became less severe with increasing age.

Perianal irritation also tended to occur somewhat more frequently among infants on diets low in linoleic acid, but the incidence was not so great as with the dry, scaly skin syndrome. Perianal irritation disappeared after the first 3 months.

TABLE X-B

Premature Infants with Symptoms Referable to the Skin at Various Ages

Symptoms	*Formula Number* 1		2		3		4		5	
	No.	*Visits*	*No.*	*Visits*	*No.*	*Visits*	*No.*	*Visits*	*No.*	*Visits*
3–6 weeks	18	18	21	21	21	21	6	6	12	12
Total with skin symptoms	2		0		2		6		6	
Dry and scaly	0		0		0		5		3	
Perianal irritation	2		0		0		1		2	
Bacterial infection	0		0		2		2		2	
6–9 weeks	17	17	19	19	20	20	5	5	10	10
Total with skin symptoms	1		1		1		3		6	
Dry and scaly	1		0		0		3		6	
Perianal irritation	0		1		0		0		0	
Bacterial infection	0		0		1		0		0	
9–12 weeks	16	16	19	19	20	20	3	3	9	9
Total with skin symptoms	1		0		1		3		7	
Dry and scaly	1		0		1		3		7	
Perianal irritation	0		0		0		0		0	
Bacterial infection	0		0		0		0		1	
4–6 months	15	45	20	60	18	54	2	6	7	21
Total with skin symptoms	1		0		2		2		7	
Dry and scaly	1		0		0		2		7	
Perianal irritation	0		0		0		0		0	
Bacterial infection	0		0		1		0		0	
7–9 months	14	42	18	54	17	51	2	6	5	15
Total with skin symptoms	2		2		1		0		2	
Dry and scaly	1		1		0		0		2	
Perianal irritation	0		0		0		0		0	
Bacterial infection	1		1		1		0		0	
10–12 months	11	33	17	51	13	39	2	6	4	12
Total with skin symptoms	1		1		2		0		1	
Dry and scaly	0		0		0		0		1	
Perianal irritation	0		0		0		0		0	
Bacterial infection	1		1		2		0		0	
Total symptoms	26	192	34	244	33	226	12	44	21	94
Total with skin symptoms	7		6		9		8		10	

Laboratory Data

Serum Lipids: In Table XI are summarized quartile data for the total fatty acids as well as diene, triene, and tetraene fatty acids in the blood serum of infants on the various milk mixtures. The mean, standard deviation, and standard error of the mean are presented for each group of subjects. When the diet was very low in linoleic acid content, the total fatty acids in the serum tended to be high. This is especially true for infants receiving formula 4 (no fat). In Figure 3 the inverse relationship between the amount of linoleic acid in the diet as well as the dienoic acid in the blood serum and the level of total

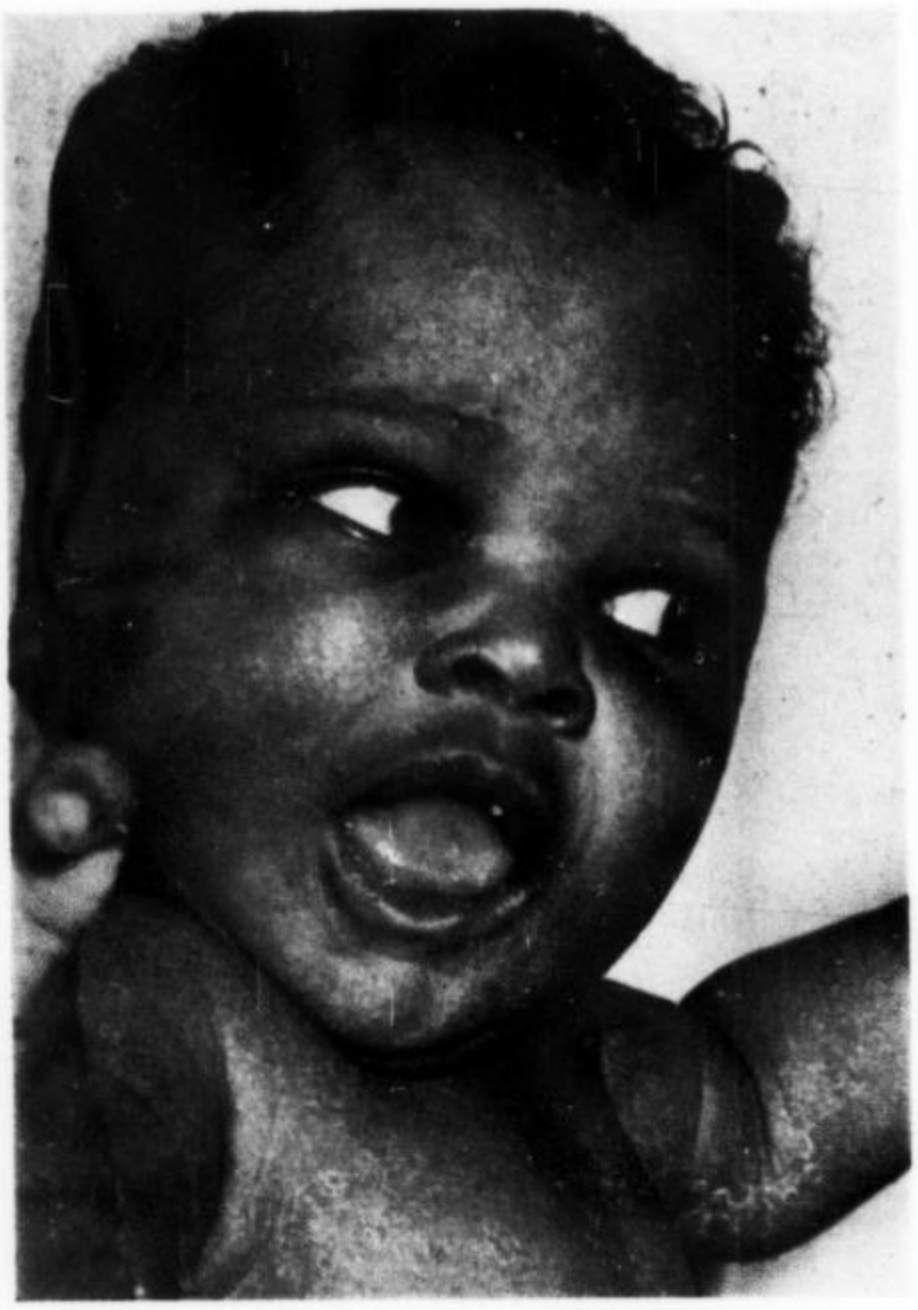

FIG. 1. At one week of age this female infant (Case 269) was started on a milk mixture (Formula 5) containing 42% of the calories as fat, but no linoleic acid. At 3 weeks a scaly dry skin was definitely discernible, becoming more pronounced with time (age in photograph, 10 weeks). At 2 months, pustules containing staphylococcus aureus developed; the child was referred to the hospital. Response to antibiotic therapy was satisfactory. At 2½ months, linoleic acid (trilinolein) was given in an amount equivalent to 2% of the caloric intake. Within 2 weeks there was great improvement in the skin, which soon became entirely clear. The linoleic acid supplement was discontinued after 32 days, and within 6 weeks the skin again became red and scaly, which condition gradually cleared. Solid foods were started at 3 months, and, as indicated from the serum lipid findings, there was an increase in dienoic acid level of the serum.

FIG. 2. Typical skin changes developed in a premature infant (Case 447) who received milk mixture 5. In an attempt to evaluate the role of oleic acid, triolein was given in an amount equal to 2% of the caloric intake, but no improvement occurred during a 2-week period, at which time the photograph was taken. No change in the serum lipid values followed the addition of oleic acid to the diet. Linoleic acid, as trilinolein, in an amount equal to 2% of the caloric intake was then added to the diet, and within 2 weeks the skin returned to normal.

TABLE XI

MEAN, STANDARD DEVIATION, AND STANDARD ERROR FOR BLOOD SERUM TOTAL FATTY ACIDS (TFA) (IN MG/100 ML), WITH DIENE, TRIENE, AND TETRAENE ACIDS AS A PERCENTAGE OF THE TOTAL FATTY ACIDS, FOR ALL INFANTS

	Formula Number				
	1	*2*	*3*	*4*	*5*
3 months					
TFA (no. of determinations)	66	58	60	11	30
Mean	237	231	269	306	263
S. D.	45.5	37.8	48.0	79.2	66.6
SE_m	5.6	5.0	6.4	25.1	12.4
Di (no. of determinations)	66	58	60	11	30
Mean	28.97	35.40	12.87	2.80	5.55
S. D.	3.41	5.16	2.59	1.29	1.75
SE_m	0.42	0.68	0.34	0.41	0.32
Tri (no. of determinations)	66	58	60	11	30
Mean	1.77	1.39	2.50	5.54	3.74
S. D.	0.61	0.75	0.66	1.62	1.05
SE_m	0.08	0.10	0.09	0.51	0.19
Tetra (no. of determinations)	66	58	60	11	30
Mean	9.07	10.81	8.01	2.56	2.69
S. D.	2.17	2.14	1.37	1.00	1.45
SE_m	0.27	0.28	0.18	0.32	0.27
6 months					
TFA (no. of determinations)	60	62	52	4	27
Mean	245	235	282	301	265
S. D.	44.2	38.8	48.6	32.9	54.0
SE_m	5.8	5.0	6.8	19.0	10.6
Di (no. of determinations)	60	62	53	4	27
Mean	30.53	37.64	15.67	7.88	10.89
S. D.	3.79	3.48	2.05	1.95	3.89
SE_m	0.49	0.45	0.28	1.13	0.76
Tri (no. of determinations)	61	62	53	4	27
Mean	1.67	1.35	2.23	4.40	3.54
S. D.	0.79	0.80	0.73	0.68	0.61
SE_m	0.10	0.10	0.10	0.39	0.12
Tetra (no. of determinations)	61	62	53	4	27
Mean	9.81	10.50	8.63	3.30	3.92
S. D.	2.08	1.91	1.45	1.35	1.76
SE_m	0.27	0.25	0.20	0.78	0.35
9 months					
TFA (no. of determinations)	53	51	51	5	22
Mean	248	236	288	345	255
S. D.	39.1	35.7	32.9	94.8	43.9
SE_m	5.4	5.1	4.7	47.4	9.6
Di (no. of determinations)	53	51	52	5	23
Mean	30.77	36.87	17.65	9.18	17.13
S. D.	3.85	3.53	3.51	2.65	4.53
SE_m	0.53	0.50	0.49	1.33	0.97
Tri (no. of determinations)	53	51	52	5	23
Mean	1.82	1.38	2.28	3.10	3.39
S. D.	0.85	0.83	0.88	0.75	0.80
SE_m	0.12	0.12	0.12	1.37	0.17
Tetra (no. of determinations)	53	51	52	5	23
Mean	10.48	11.60	9.14	4.60	5.93
S. D.	2.05	2.05	1.28	1.24	2.15
SE_m	0.29	0.29	0.18	0.62	0.46

TABLE XI—(*Continued*)

	Formula Number				
	1	*2*	*3*	*4*	*5*
12 months					
TFA (no. of determinations)	60	57	52	5	17
Mean	254	251	275	309	264
S. D.	37.4	42.5	37.7	60.7	59.7
SE_m	4.9	5.7	5.3	30.4	14.9
Di (no. of determinations)	60	57	53	5	16
Mean	30.58	36.46	20.89	12.48	19.94
S. D.	3.40	3.16	3.55	3.28	4.15
SE_m	0.44	0.42	0.49	1.64	1.07
Tri (no. of determinations)	60	57	53	5	17
Mean	1.83	1.80	1.99	3.14	2.48
S. D.	0.88	0.89	0.85	0.91	0.82
SE_m	0.12	0.12	0.12	0.46	0.21
Tetra (no. of determinations)	60	57	53	5	17
Mean	10.73	11.17	10.13	6.46	8.35
S. D.	1.78	1.80	1.20	1.71	2.32
SE_m	0.23	0.24	0.17	0.85	0.58

fatty acids in the blood serum is shown graphically for the infants at 3 months of age.

Of special interest are the diene fatty acid levels, which reflect so remarkably the linoleic acid content of the diet. The blood serum values for diene fatty acids were much higher for infants receiving formulas 1 and 2 than for the others. Although the serum values were much lower in groups 4 and 5, the lowest values were found in infants receiving formula 4. Intermediate values were found for the infants receiving milk mixture 3 (linoleic acid about 1.3% of the calories). The dienoic acid level of the blood serum changed very little at 6, 9, and 12 months for infants receiving formulas 1 and 2, but it increased markedly with increasing age for infants on the other formulas. Figure 4 portrays graphically the changes occurring with time for the infants on formula 5. The increase in the dienoic acid level of the blood serum coincided with the clinical improvement of the skin condition.

The level of trienoic acid in the blood serum varied inversely with the dienoic acid. Summary data are presented in Figure 5. The level of tetraenoic acid in the blood serum tended to follow the pattern of the dienoic acid, although the change was not so great proportionately. Not shown in the compilation of serum lipid data (Table XI) were the results obtained from 24 premature infants in whom the serum lipids were determined before the age of 7 weeks. These infants were evenly distributed among the five dietary groups, and the 7-week values for total fatty acids, and dienoic, trienoic, and tetraenoic acids agreed very closely with the results obtained at 3 months of age.

Statistical comparisons of the serum lipid

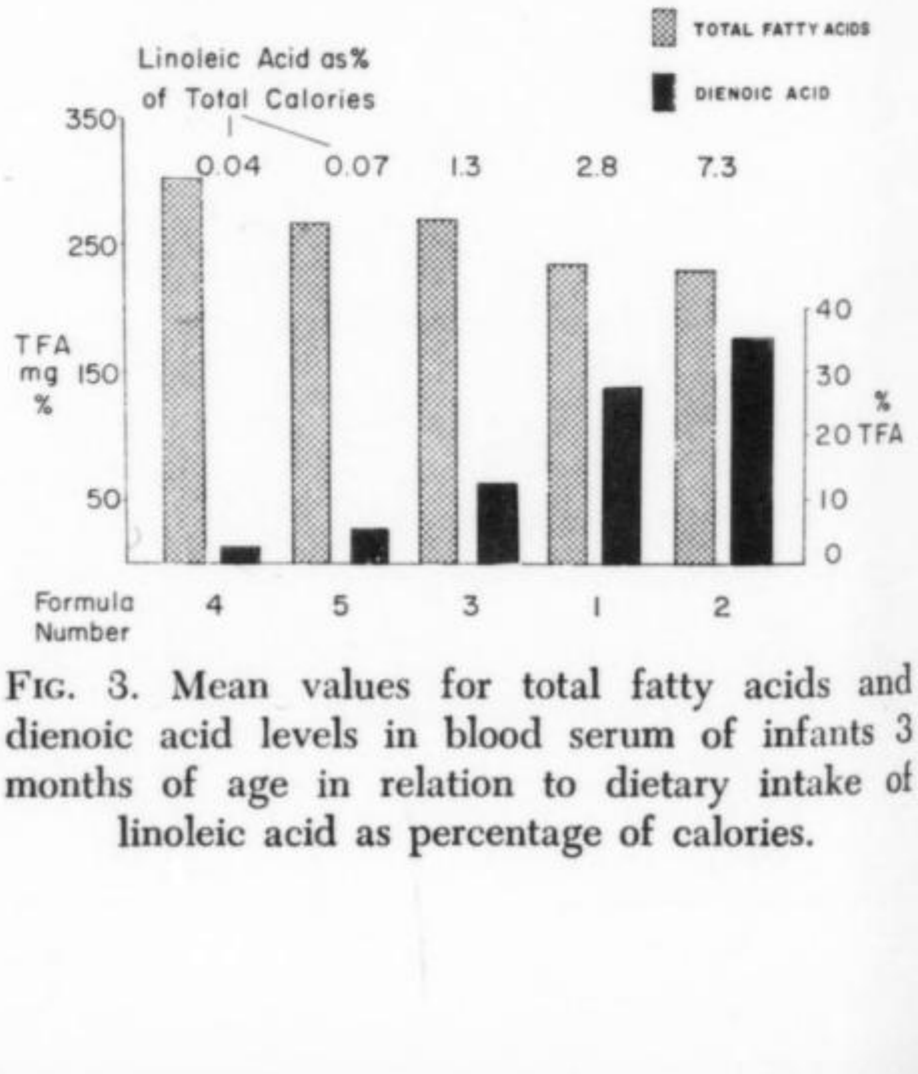

FIG. 3. Mean values for total fatty acids and dienoic acid levels in blood serum of infants 3 months of age in relation to dietary intake of linoleic acid as percentage of calories.

values were made between various subgroups such as full term and premature, white, Negro and Latin American, and males and females, in all possible combinations. There was no evidence that length of gestation, race, or sex had any influence on serum lipid values.

Serum Proteins: The values for the total proteins of the serum as well as albumin and globulin were summarized. (This information has been deposited with the American Documentation Institute. See footnote on title page.) Consideration of mean values, standard deviations, and probable errors of the means revealed no significant differences among the infants on the different formulas at the various age levels.

Routine Blood Counts: The mean values, standard deviations, and standard errors of the means for hemoglobin, total leukocyte count, and polymorphonuclear and mononuclear cells showed no relationship to the kind or amount of fat in the diet. (These data are deposited with the American Documentation Institute. See footnote on title page.)

Histologic Features: No attempt was made to examine skin biopsy specimens routinely. However, on several occasions, it was possible to obtain slivers of skin for histologic study. Sections of skin were obtained from eight infants, but in only one case was a biopsy specimen obtained from an infant on a diet containing liberal quantities of linoleic acid. In order to make a more careful evaluation, sections of skin were obtained from an additional eight infants who formed the basis for the control histology. The usual methods of preparation and staining procedures (hematoxylin and eosin) were used.[*] The findings for control infants and those receiving milk mixtures low in linoleic acid content were remarkably similar to the histologic features demonstrated in previous studies with dogs,[8,9] as well as with rats on control and low fat diets.[10–12]

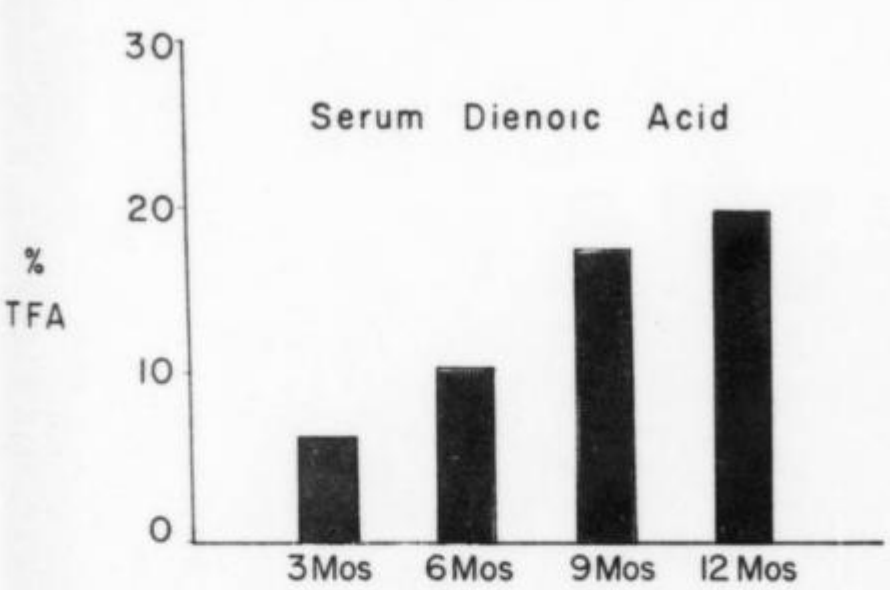

Fig. 4. Influence of solid food with increasing age on the mean dienoic acid levels of the blood serum of infants receiving a milk mixture having 42% of the calories as fat but extremely low in linoleic acid content (Formula 5).

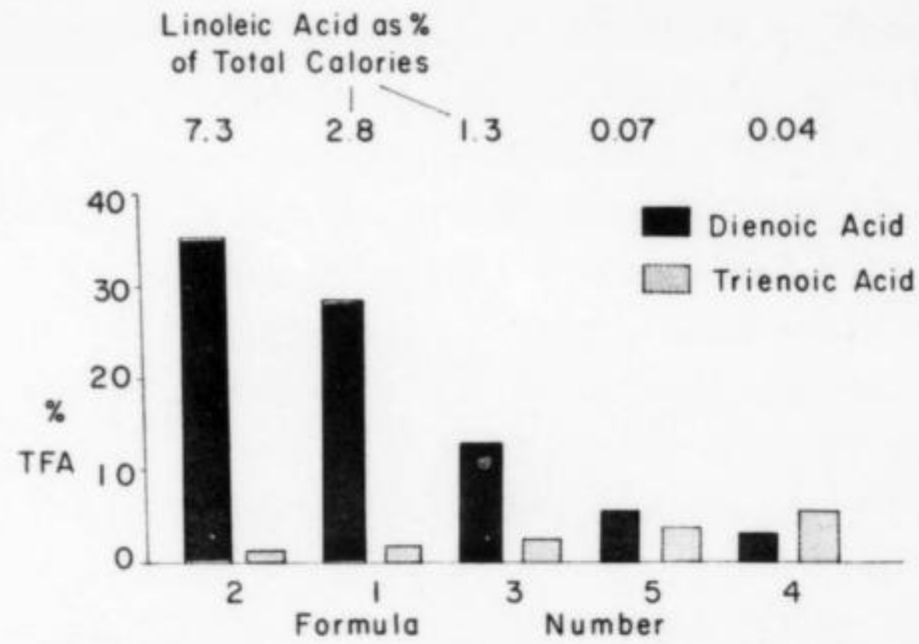

Fig. 5. Mean values for dienoic and trienoic acids as per cent of total fatty acids of the serum for infants at age 3 months fed on milk mixtures containing negligible amounts ($< 0.1\%$) to 7.3% of the calories as linoleic acid.

Typical histologic sections of skin are illustrated in Figures 6 and 7. Figure 6 is for an infant (Case 177) who had received formula 5 (low in linoleic acid) for 3 months. Figure 7 is one of the 8 infants who served as control.

COMMENT

Published results of comparable infant feeding studies are not available; hence, it is not possible to interpret our findings in the light of previous investigative work. Moreover, in this type of study it is admittedly difficult to control such features as environment, social and economic conditions, psychological and emotional reac-

[*] Prepared and interpreted by Dr. John G. Sinclair, Professor of Histology, University of Texas School of Medicine, Galveston, Texas.

tions, or to make consistently accurate objective observations for prolonged periods on a large number of infants.

In persuing the literature regarding the subject of infant feeding it is evident that numerous attempts have been made to compare the adequacy of feeding many types and kinds of milk with that of breast milk. In a comprehensive review, Aitken and Hytten[13] cited data comparing the relative effectiveness of breast and artificial feeding. In an extensive prospective study, Mellander *et al.*[14] in Sweden evaluated certain clinical and chemical features in breast and in artificially fed infants. It is surprising to note, however, that, insofar as we could discover, no long-term controlled studies have been carried out wherein an evaluation was made concerning the nature of the fat in the milk, especially in relation to its linoleic acid content. Although it is recognized that there is a marked difference in the amount of linoleic acid in human and cow's milk, the distinct differences in the protein, mineral, and vitamin composition of the two milks may profoundly influence the results of comparative studies. In the study reported here the attempt was made, therefore, to vary the linoleic acid intake of the infants while holding other nutrients constant among the several groups. It should be pointed out, however, that it is difficult to evaluate completely the specific nutritional role of linoleic acid, because when the linoleic acid content of a fat is changed it must be replaced by fatty acids having different chain lengths and degrees of unsaturation. In our studies, inas-

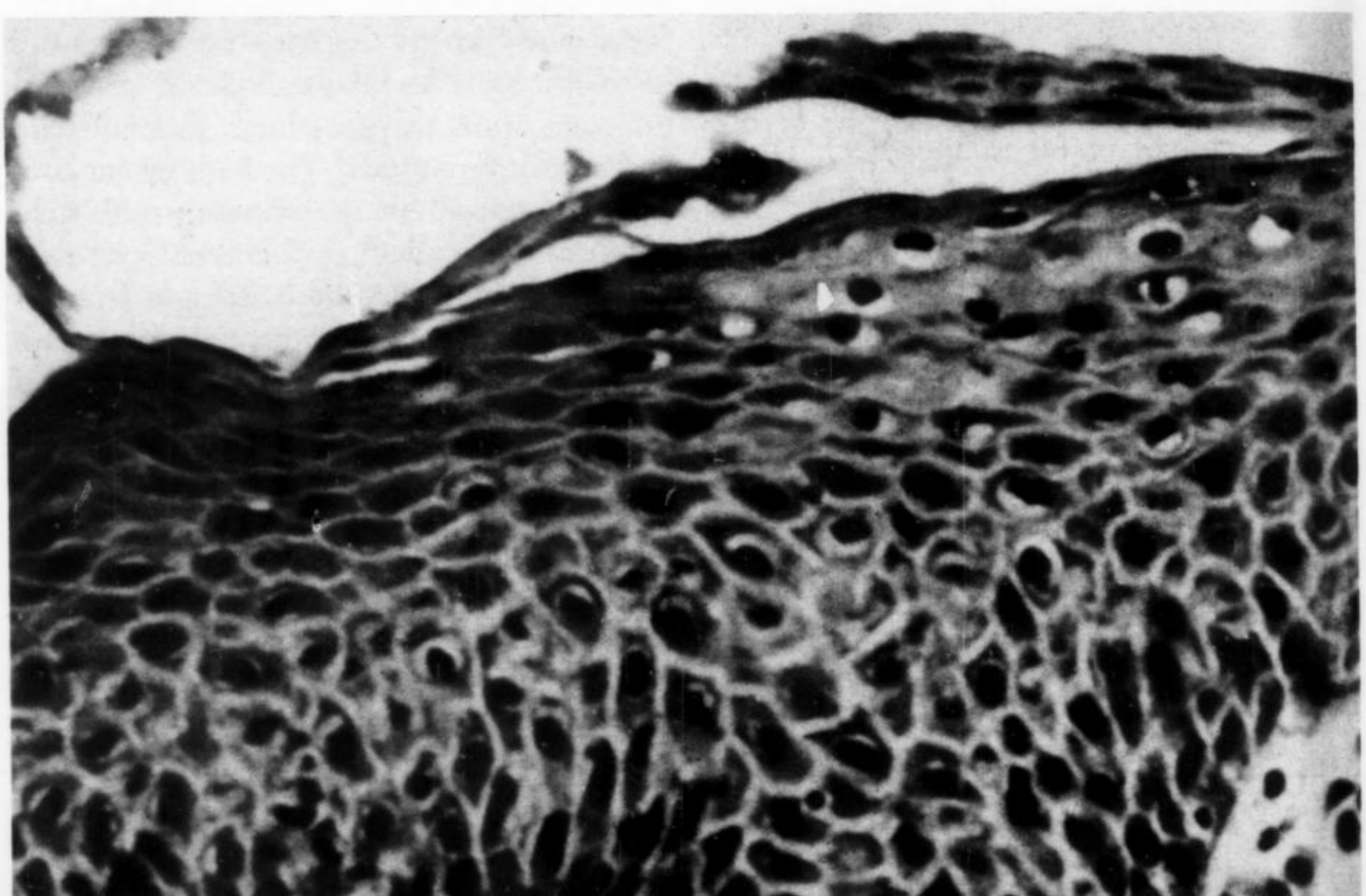

FIG. 6 Photomicrograph of histologic sections of skin from infant receiving $< 0.1\%$ of the calories as linoleic acid (Formula 5). ($\times 600$).

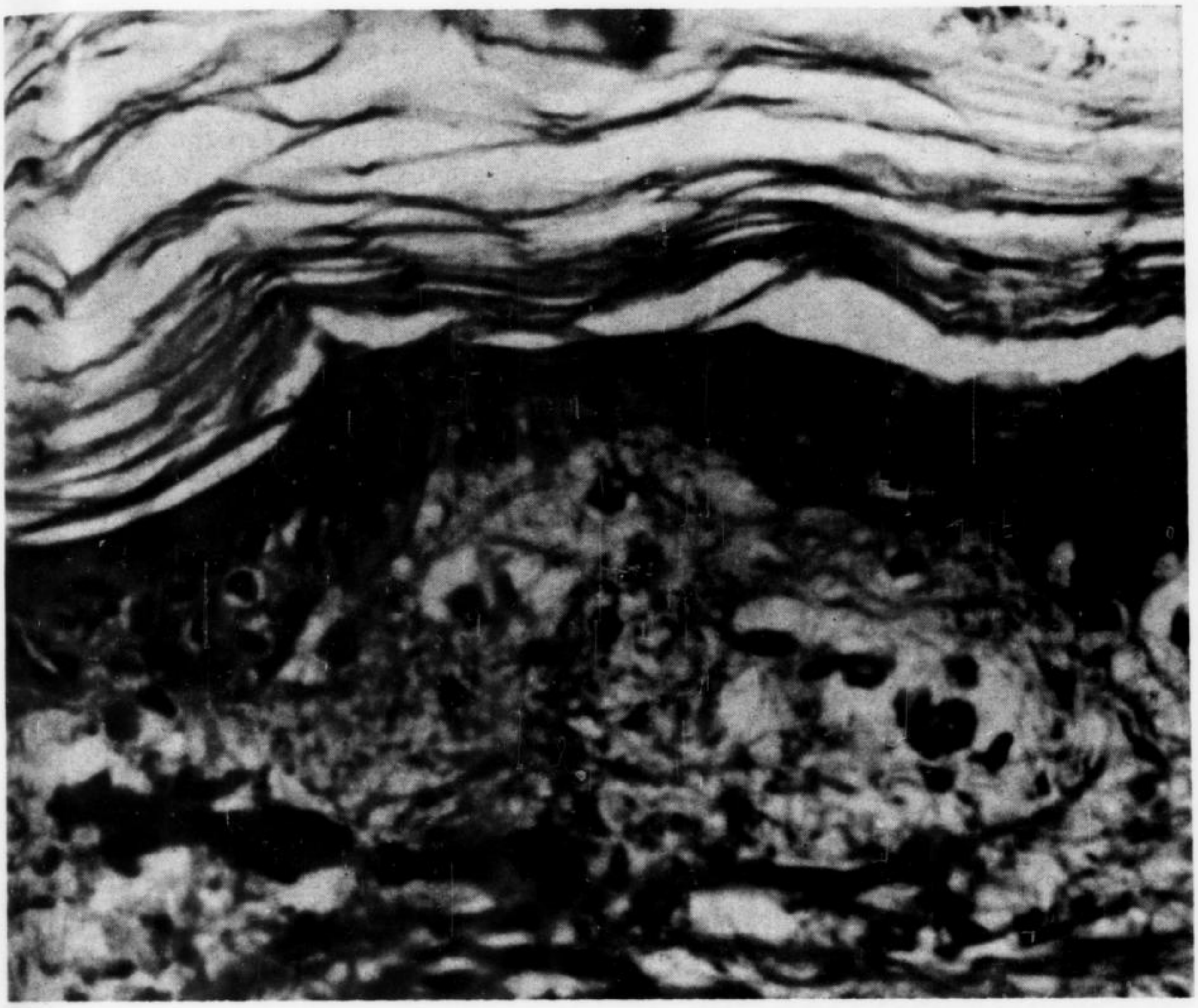

FIG. 7. Photomicrograph of skin from a newborn infant. (×600).

much as different fats were used to effect variations in the linoleic acid content, it was necessary to change to some extent the proportion of other fatty acids in the fat; nevertheless, the other nutrients (protein, minerals, and vitamins) remained the same in all the formulas. In one mixture carbohydrate was substituted isocalorically for fat. By and large, the clinical and chemical observations appear significant particularly when interpreted in the light of findings from studies with experimental animals. Data from the literature have been summarized by several authorities.[15-17] A recent review is that of Aaes-Jørgensen.[18]

The development of a dry and scaly skin with thickening as a persistent symptom in the very young, rapidly growing infant points to this phenomenon as a typical manifestation of linoleic acid deficiency. The relative incidence of the occurrence of skin changes suggestive of fat deficiency for the infants receiving the different milk mixtures is presented graphically in Figure 8. In compiling these data only those subjects have been considered who were started on a particular milk mixture before the age of 6 weeks and remained on the same mixture for at least 3 months or until development of skin changes occurred. It is felt by the observers that if an infant is started on a diet lacking linoleic acid at a very young age, it would be suprising if he did not develop definitive skin changes within the matter of 2 to 3 months. On the basis of recent findings by Wiese *et al.*[19] with young puppies, rate of growth is important in the development of skin abnormalities in linoleic acid deficiency states.

Infants who received the milk mixture

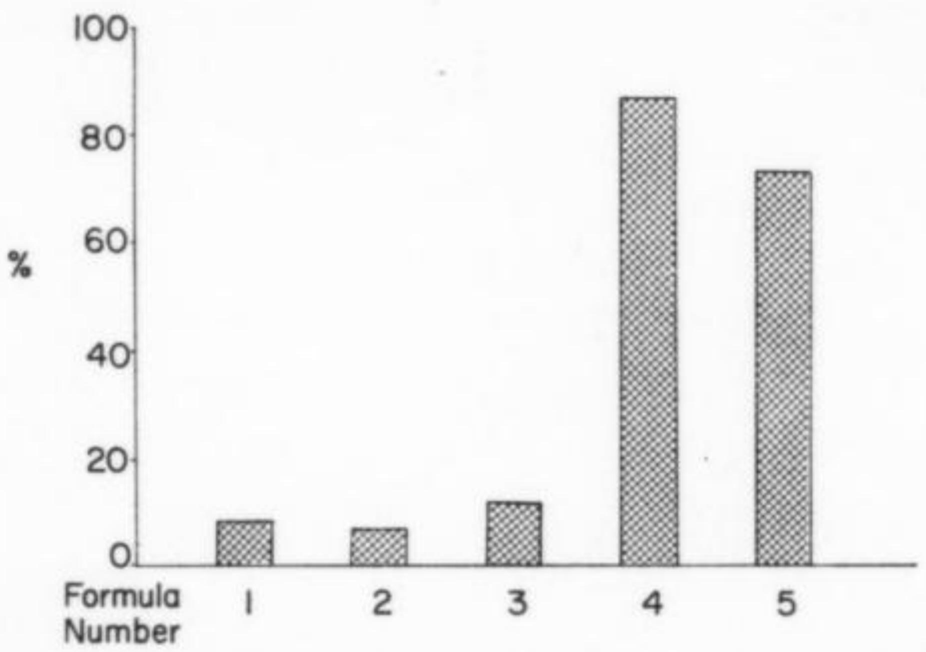

FIG. 8. Schematic representation of incidence of the occurrence of skin changes suggestive of fat deficiency in infants who were given the different milk mixtures before the age of 6 weeks and who were given the same mixture for a 3-month period or until dry scaly skin with thickening was definite.

containing saturated fat to equal 42% of the calories developed skin changes more slowly than the infants who received the skimmed milk mixture. This observation suggests either that linoleic acid even in small amounts such as in formula 5 delays the development of skin manifestations or that dietary fat per se has a protective effect on the skin. Also, the finding that the addition of solid foods to the diets was associated with a gradual amelioration of the skin abnormalities indicates that the linoleic acid provided by solid foods is effective in curing skin manifestations. The changes in serum lipid values with increasing age support this conclusion (Table XI). Particularly significant is the fact that the administration of linoleic acid per se effected not only clinical improvement in the skin condition, but also an increase of dienoic acid in the blood serum.

The incidence of bacterial infections of the skin was not significantly different among the various groups of infants (Tables X-A and X-B). Serious complications, however, occurred in several infants receiving the milk mixtures low in linoleic acid content, but by no means were they confined to this group, and the episodes did not occur concurrently. Experience in our own laboratories with experimental animals indicates increased susceptibility to bacterial infections resulting from fat deficiency.

The dermatologic evidences of fat deficiency which have been demonstrated in a great variety of experimental animals were emphasized in the original contributions of Burr and Burr[20, 21] in their studies with rats. Adult animals on fat-free diets are known not to develop classical signs of fat deficiency readily. On the other hand, Barki *et al.*[22] showed that if severely depleted adult rats (reduced to half their starting weight) are given fat-free diets ad libitum, they manifest classical fat deficiency symptoms along with the gain in weight.

In 1919 von Gröer[23] and in 1937 von Chwalibogowski[24] maintained two infants each on low fat diets for prolonged periods. Skin changes were not observed in these four subjects. However, from their data it is not clear to what extent fat had been removed from the diet. All four infants were given solid foods early, especially cereals, and it may well be that there was sufficient linoleic acid in the diet to prevent skin manifestations. In 1935, Holt and coworkers[25] maintained three infants, 4 to 6½ months of age, on diets low in fat for periods of 2 to 7 days. One infant developed eczema. The eruption disappeared when fat was added to the diet again, only to recur when the low fat diet was resumed. The longest period of time an infant has been known to remain on a diet very low in fat was the patient reported in 1944 by Hansen and Wiese.[26] The special diet was given for its possible effectiveness in the management of his chylous ascites. As a young infant he developed impetigo, which was very resistant to treatment, compared with the results obtained with the other infants in the same ward. Likewise, with the occurrence of prickly heat during the hot weather season, a distinct dermatitis developed which persisted for months after the onset of cool weather and long after the skin of other children similarly affected had cleared. When the child was about 6 months of age, eczematous patches developed on the cheeks, scalp, and various parts of the body; however, the lesions responded readily to local therapy. Recently Warwick and co-workers[27] reported the development of abnormal skin symptoms in a child with chylous ascites, and lymphedema. This 4½-year-old girl had been maintained on a low fat diet, and the ectodermal changes were suggestive of a deficiency of unsaturated fatty acids.

As indicated from the work of Burr and Burr and confirmed by subsequent workers, impairment of growth appears to be a characteristic feature of fat deficiency in experimental animals. It cannot be concluded from the findings of von Gröer and von Chwalibogowski relative to the poor growth of their infants that this resulted from a deficiency of linoleic acid. In fact, it is difficult to ascertain from the information available to what extent other possible nutritional factors were lacking in the diets of their infants. Inasmuch as in our studies the diets were complete other than for fat and linoleic acid, there seems to be little doubt that the rate of gain in weight was related to the linoleic acid intake.

The finding of a greater caloric intake in relation to gain in body weight in infants lacking linoleic acid in the diet as compared to infants receiving dietary linoleic acid confirms not only earlier observations[28] on human subjects, but also early studies with rats by Wesson and Burr[29] and Burr and Beber.[30] This phase of the linoleic acid problem has been pursued more recently by Panos and co-workers.[31] They demonstrated increased basal oxygen consumption in rats reared on diets deficient in linoleic acid. Actually these authors observed that this was the earliest of all aberrations to appear in the fat deficient state.[32] By paired feeding of rats, Naismith[33] found food consumption of linoleic acid deficient animals to be 40 to 60% greater than that of the controls receiving 7.5% of their salories as linoleic acid. Combes *et al.*,[34] in studies with premature infants, found a lesser caloric consumption for each gram gain in body weight when the milk contained liberal quantities of linoleic acid than when this fatty acid was not provided. In recent studies by Panos[35] differences in insensible weight loss have not been demonstrable in infants with and without linoleic acid in the diets. On the other hand, determination of respiratory quotients on an adult subject maintained on a diet extremely low in linoleic acid for a period of 6 months revealed that the human being reacted metabolically in the same manner as the rat.[36]

It is quite obvious from the present study as well as previous observations both with experimental animals and human subjects that the serum lipid pattern readily reflects nutritional status regarding linoleic acid. Unfortunately, no attempt was made by von Gröer or von Chwalibogowski to determine the iodine number of the blood fat in the infants studied by them. Early work in our laboratories had demonstrated conclusively that the iodine number of the fat in the blood serum reflects the degree of unsaturation of the fat in the diet. The true magnitude of the change in specific fatty acids was not appreciated, however. It was some years later that Wiese *et al.*[37] showed that the diene and tetraene fatty acids in the blood serum varied directly with linoleic acid in the diet while the triene fatty acids varied inversely. This observation was found to hold true not only for young puppies but also for the human infant.[38] Study of the serum lipids appears to give quite conclusive evidence that rather liberal quantities of linoleic acid may be contributed by the addition of solid foods to the diet. This is particularly significant for the infants receiving milk mixtures low in linoleic acid. For example, in Figure 4 one may note the marked increase in the dienoic acid level of the blood serum with the passage of time, resulting no doubt from the addition of cereals and meats to the diets. Equally significant is the fact that the alterations in serum lipid findings are in keeping with the clinical improvement noted in these patients.

Specificity of Linoleic Acid: It is quite apparent from the results of this study that young human infants do not thrive when placed on diets lacking linoleic acid. The inadequacy of the milk mixture containing 42% of the calories as fat, yet extremely low in linoleic acid content, speaks for the specificity of dietary linoleic acid, rather than for fat per se. Especially significant, of course, is the observation that the administration of linoleic acid, given as an ester or as trilinolein or as present in the formula or in solid foods, restored to normal the skin of infants who had received diets deficient in linoleic acid.

Requirement for Linoleic Acid: Before the recognition of specific requirements for many nutrients, it was generally believed that fat served only as a concentrated source of energy and had no other function. A later concept was that fat acted as a carrier for certain vitamins, but since fat per se is readily synthesized from carbohydrate and protein, it was thought to serve no other purpose. However, in 1918, Aron[39] reported that in rats, growth was unsatisfactory when the animals were deprived of fat (butter). It was this observation that prompted the studies by von Gröer employing human infants. In 1927 Evans and Burr,[40,41] also found that rats failed to thrive when given a diet low in fat in spite of the addition of vitamin E to the diet. These authors[42] suggested that a gap be left in the developing vitamin nomenclature, namely "F," for an unknown nutrient. Although McAmis *et al.*[43] in 1929 reported unsatisfactory progress of rats on diets low in fat, it remained for Burr and Burr[20,21] to prove the dietary essentiality of linoleic acid.

In estimating the requirement for linoleic acid in experimental animals, the criteria used have been growth and changes in the skin, hair and tail. Although rate of weight gain has been a valuable tool for the laboratory animal, it would be difficult, indeed, to depend upon this criterion alone for the human infant. As previously stated the serum levels of certain unsaturated fatty acids reflect to a remarkable degree the dietary intake of linoleic acid, both for the young infant and the puppy and are of particular significance in view of the excellent correlations with clinical and histologic findings.[37,38] Various studies carried on by the authors suggest that the requirement for linoleic acid by the human infant depends on the rate of growth as well as the composition of the diet. On the basis of observations regarding ectodermal changes and serum lipids as reported here, young rapidly growing infants developed evidences of deficiency when receiving less than 0.1% of their calories as linoleic acid and did not show these signs when receiving 1.0% or more of the calories as this fatty acid. The optimum dietary level is yet to be determined. That mother's milk supplies about 4 to 5% of the calories as linoleic acid bears special consideration in relation to establishment of nutritional requirements.

SUMMARY AND CONCLUSIONS

Four hundred and twenty-eight infants over a 4-year period were studied for approximately 4,000 patient-months of observation while receiving five different milk mixtures varying in linoleic acid content from less than 0.1 to 7.3% of the calories. It was found that linoleic acid is a required nutrient for the young human infant. The following pertinent observations were made. (1) Evidences of linoleic acid deficiency developed in young infants who received either a diet practically devoid of fat or one providing 42% of the calories as fat but extremely low in linoleic acid (less than 0.1% of the calories). (2) Manifestations of the deficiency state disappeared promptly when linoleic acid was given as the ester or triglyceride or in a milk mixture providing 1% or more of the calories as linoleic acid. (3) The most characteristic feature of the deficiency state was dryness of the skin with desquamation, thickening and later intertrigo. (4) The incidence of bacterial skin infections was the same in the different groups. It was noted that young infants receiving diets very low in linoleic acid seemed to react severely when outbreaks of staphylococcal infection developed in the hospital environment. (5) Records for rate of growth showed unsatisfactory progress

for many of the infants on low linoleic acid intakes, whereas the course of events was satisfactory in almost all of the infants who received 1.3 to 7.3% of the calories as linoleic acid. (6) Blood serum levels for dienoic acid of 5.6 ± 1.8% of the total fatty acids were indicative of the deficiency state, whereas values of 12.9 ± 2.6% of the total fatty acids represented the minimal normal. (7) Histologic alterations in the skin of infants on diets low in linoleic acid showed the same characteristics as seen in experimental animals. (8) Infants given milk mixtures extremely low in linoleic acid had a gradual amelioration of fat deficiency manifestations as well as increasing levels of dienoic acid in the blood serum following the introduction of cereals to their diet.

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Acknowledgment

The authors wish to express their appreciation for the helpfulness of Mrs. Charles Plyler in carrying on the activities of the special infant feeding clinic, of Miss Billie Rekoff and Dr. Michael G. Rekoff, Jr., for technical assistance, of Mrs. T. C. Mather and Dr. Lillian Lockhart for compilation of records, and of Miss Barbara Hirschler and Mr. Malcolm Roemer for preparation of the data. We are grateful to Dr. John Sinclair for his help with the histological studies. We feel especially indebted to Dr. Walter L. Thompson of The Baker Laboratories, Inc., and to Dr. Robert A. Stewart of Gerber Products Company for their complete cooperation in the maintenance of supplies and assistance in obtaining data regarding the food analyses. We are grateful to Dr. George M. Briggs of the Department of Nutrition, University of California, Berkeley, for suggestions regarding the manuscript. Special thanks are due to members of the pediatric resident staff at the University of Texas for their assistance in obtaining blood samples and to the many mothers for their excellent co-operation in making this study possible.

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