

TABLE I.
Comparison of Various Routes of Inoculation and the Distribution of Virus.

Route	Passage	Age of embryo	Infectivity of			
			Allantoic membrane	Allantoic fluid	Embryo	Embryo head
Allantoic membrane	1st	10 days	10-6	<10-2	10-5	
	2nd	"	10-5	10-3		10-6
	4th	"	10-5			10-4
	6th	"				10-6
Yolk sac	1st	5 days	10-5*		10-6	
	2nd	"	10-6*		10-6	
	5th	"			10-5	
	7th	"			10-6	
Allantoic sac	1st	10 days	10-4	10-3		10-6

Infectivity determined by intracerebral titration in mice.

< = less than.

*Yolk sac membrane.

rapidly throughout the embryonated egg following inoculation by any of the usual routes. The embryo contains large amounts of virus while the allantoic fluid contains comparatively

small amounts of the active agent. Death of the infected embryos occurs with such regularity that titration of virus can be made as well in eggs as in mice.

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Studies With Dogs Maintained on Diets Low in Fat.*

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The observations and data presented here constitute a part of a general study of the factors which are concerned with the nutrition of the skin. The findings that the degree of unsaturation of the serum lipids tends to be low in patients with eczema^{1,2,3} and that the dietary use of fats rich in unsaturated fatty acids exerts a beneficial effect in certain patients with eczematous skin eruptions^{1,3,4}

together with the observations of Burr and Burr^{5,6} regarding the importance of linoleic and arachidonic acids in nutrition, indicate that particular attention must be given to the unsaturated fatty acids in such a study. The dog seems to lend itself as a suitable experimental animal for observing the effects of a fat deficient diet on the general health and condition of the skin of the animal as well as for studying the distribution of the fatty acids in the various lipid fractions of the blood under these conditions.

Material and Methods. At weaning, 8 puppies born to a collie mother were given a diet low in fat composed of skim milk 73.2%, cottage cheese 7.0%, polished rice 5.7% and sucrose 14.1%. To the daily diet of each

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¹ Hansen, Arild E., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **31**, 160; *Am. J. Dis. Child.*, 1937, **53**, 933.

² Faber, H. K., and Roberts, D. B., *J. Pediat.*, 1935, **6**, 490.

³ Finnerud, Clark W., Kesler, R. L., and Wiese, Hilda, *Arch. Dermat. and Syph.*, 1941, **44**, 849.

⁴ Cornbleet, T., and Pace, E. R., *Arch. Dermat. and Syph.*, 1935, **31**, 224.

⁵ Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; *ibid.*, **82**, 366.

⁶ Burr, G. O., *Federation Proceedings*, 1942, **1**, 224.

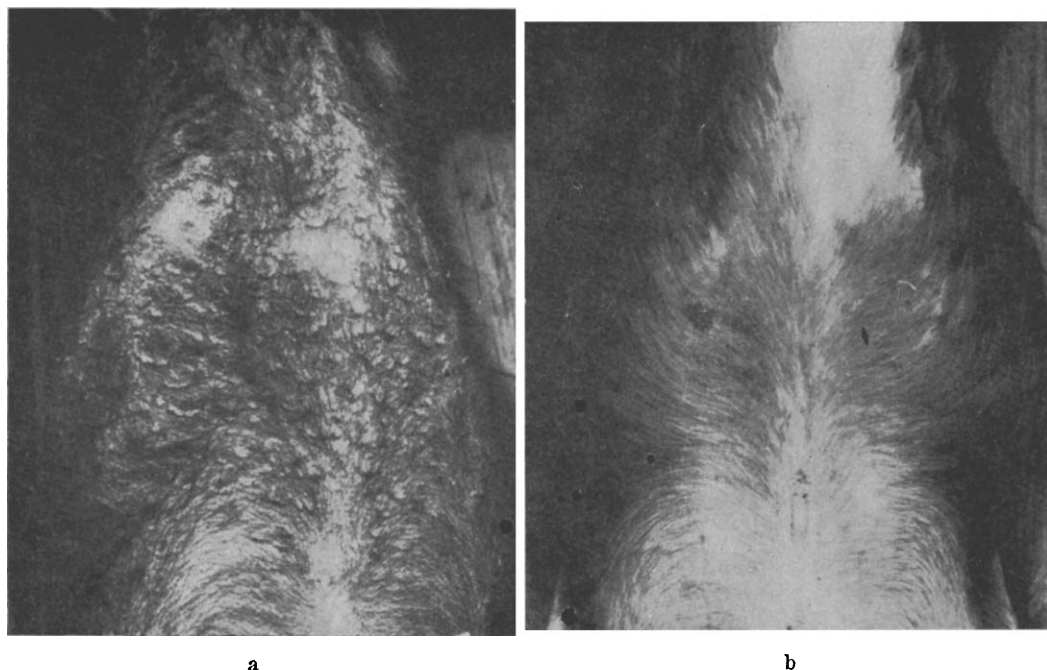


FIG. 1.

Photographs of the ventral surface of (a) puppy on low fat diet; (b) litter mate control puppy receiving 28% of the calories as lard. At this time the animals were 4½ months of age.

animal were added 0.4 g artificial bone ash mixture and 0.2 g Cowgill's salt mixture per kilogram body weight, in addition to the following: Vitamin A (concentrate in natural ester form furnished by General Mills, Inc.), 1600 units; vitamin B complex (furnished by S.M.A. Corporation) to supply individual factors in quantities recommended by Schaefer, McKibbin and Elvehjem;⁷ vitamin D (Drisdol furnished by Winthrop Chemical Company), 250 units; and the weekly administration of vitamin E (Tofaxin, supplied by Winthrop Chemical Company), 25 mg, equivalent to 15 mg of alpha tocopherol. Analysis of the components of the diet showed it contained a total of 0.13% fat and furnished 1% of the total calories. The diet is essentially the same as the one used by an experimental human subject⁸ and previously found to produce the typical fat deficiency symptoms in

rats. Four of the litter mate puppies were used as control animals and in the diet of these, lard was substituted for 28% of the sucrose calories. Burr and his associates^{5,9} showed that lard possesses high curative properties for the fat deficiency symptoms in rats.

For the serum lipid studies, blood specimens were obtained 20-24 hours following a meal. The serum was extracted with the usual 3:1 alcohol-ether mixture of Bloor and the following determinations were made in duplicate: total fatty acids by the microgravimetric technic of Wilson and Hansen;¹⁰ the acetone soluble and the acetone insoluble fatty acids by a technic developed by Hansen;¹¹ the total cholesterol and the cholesterol esters by the method of Bloor¹² in which the Evelyn photo-

⁹ Burr, G. O., Burr, M. M., and Miller, E. S., *J. Biol. Chem.*, 1932, **97**, 1.

¹⁰ Wilson, W. R., and Hansen, A. E., *J. Biol. Chem.*, 1936, **112**, 457.

¹¹ Hansen, Arild E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 376; *ibid.*, 1939, **41**, 205.

¹² Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227; *ibid.*, 1916, **27**, 107.

⁷ Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **47**, 365.

⁸ Brown, Wm. R., Hansen, A. E., Burr, G. O., and McQuarrie, I., *J. Nutrition*, 1938, **16**, 511.

TABLE I.

Comparison of Various Serum Lipid Values of Puppies Maintained on a Diet Low in Fat with Those Obtained from Litter Mate Control Animals on the Same Diet Except for the Isocaloric Substitution of Lard for Sucrose Sufficient to Supply 28% of the Total Calories.

Animal No.	Date 1942	Total fatty acids		Acetone insoluble fatty acids (phospholipid)		Acetone soluble fatty acids (cholesterol esters plus neutral fat)		Cholesterol	
		mg%	I.N.	mg%	I.N.	mg%	I.N.	Total mg%	Ester mg%
Animals on Low Fat Diet.									
19	1-19	311	89.8	155	93.8	142	73.9	158	100
20	1-19	235	81.5	84	84.7	111	76.9	108	62
22	2- 3 *	374	84.8	148	87.3	184	79.1	160	65
22	3-31	574	92.7	303	96.0	214	90.4	273	193
20	4- 9	505	94.7	252	98.6	212	89.3	246	175
22	6- 6	480	92.3	233	96.0	—†	—	261	173
20	6-20	528	97.9	267	98.2	—†	—	382	212
19	6-22	496	95.4	239	102.4	—†	—	262	156
22	8-24	509	93.4	227	94.5	202	88.9	252	170
Avg		455	92.2	220	95.5	176	83.9	243	155
Control Dogs Receiving Lard in the Diet.									
16	1-19	409	115.5	199	113.5	184	117.0	210	147
18	1-19	461	119.6	244	115.6	221	116.5	278	188
17	2- 3 *	463	98.8	213	98.3	208	97.3	246	174
15	2- 3 *	416	100.8	185	104.7	204	97.4	227	173
15	3-31	658	117.1	322	115.7	275	117.6	389	308
15	4- 9	612	123.3	325	116.8	249	126.9	412	283
18	4- 9	578	115.8	309	112.0	249	116.6	354	258
18	6- 6	530	116.9	237	112.4	—†	—	346	256
15	6-20	530	117.9	207	120.0	—†	—	362	266
16	6-22	510	116.6	219	114.9	—†	—	309	180
16	8-24	501	113.5	205	110.5	219	118.0	280	166
Avg		532	117.3	252	114.6	233	118.7	327	228

*Specimens obtained at time of acute infection. (These values are not included in the averages.)

†Fractions used for further study to attempt separation into glyceride and cholesterol ester fatty acids.

electric colorimeter was used; and the iodine number of the fatty acids in each group by the use of the pyridine sulfate dibromide microreagent. The observations and results here reported were made during a 14-month interval.

Discussion of Results. With regard to the general health of the animals, the most distinct finding was that related to the skin. When the animals were 3 months of age, it was noted that the skin of the animals on the low fat diet became dry and the hair dry and coarse. Within a period of 2 weeks the character of the skin of these animals was altered even more; a definite flaky desquamation with large scales and fine scurfy specks appeared over the entire body but was most marked on the ventral surface. The skin and hair of the control animals did not show these changes.

The differences in the appearance of the skin between the animals on the low fat diet and those receiving lard in the diet are shown in Fig. 1.

This abnormality in the dermal structure is similar to that observed in rats kept on a diet practically devoid of fat. The degree of skin change in the dogs however was found to vary somewhat with the season of the year and may be related to the humidity as pointed out by Burr.⁶ The rate of growth of the animals in each group was essentially the same during this period. No differences were noted in activity or in the avidity with which food was consumed. A few periods of anorexia occurred but they were not confined to the dogs on the low fat diet. Repeated urinalysis failed to show either gross or microscopic hematuria. In one puppy which succumbed to an intus-

susception after being on the low fat diet for 4 months, no kidney lesions were found although alterations in the kidneys have been demonstrable at autopsy in 100% of the rats reared on the low fat diet according to Burr.⁶ We have not as yet had the opportunity to observe the reproductive behavior of the animals on the low fat diet.

The results of the analysis of the blood lipids made at intervals on all the puppies are given in Table I.

The average value of the total fatty acids of the serum was found to be somewhat lower in the animals on the low fat diet than that of the control group. (Table I.) This difference, however, may not be significant inasmuch as in both groups of animals two-thirds of the individual values fall in the same range, namely, 400 to 600 mg per 100 cc of serum. The amount of these fatty acids in the phospholipid (acetone insoluble) fraction in both groups was greater than that present in the acetone soluble fraction. Previous studies¹¹ with human subjects revealed that the fatty acids in the acetone insoluble fraction of the serum tend to be in the neighborhood of 30% of the total fatty acids, whereas in these dogs, the phospholipid fatty acids comprise about one-half of the total fatty acids. It will be noted from Table I also that both the total cholesterol and cholesterol ester values are lower in the dogs reared on the low fat regimen. The values of the fatty acids in the various fractions of the serum lipids in the animals reared on a practically fat-free diet indicate that these animals are able to synthesize fatty acids. From the iodine values of the fatty acids in the various fractions as shown in Table I, it is evident that the fatty acids which are synthesized by these animals are materially different from the fatty acids present in the serum of the control dogs. The average iodine number of the total fatty acids in the low fat group was only 92.2 compared with 117.3 for that of the control dogs. There is also a difference in the degree of unsaturation of the phospholipid fatty acids in the two groups. More striking, however, is the difference in the iodine numbers of the fatty acids present in the acetone soluble fraction.

The average iodine number of 83.9 for this fraction in the animals on the low fat diet stands in distinct contrast to 118.7 for the animals given lard in the diet. Since the fatty acids in the acetone soluble fraction contain both glyceride and cholesterol ester fatty acids, the resultant low iodine number of this fraction of the serum lipids of the animals on the low fat diet may be due to the synthesis of either an increased percentage of saturated fatty acids appearing as neutral fat or a decreased percentage and lower degree of unsaturation of the fatty acids combined as cholesterol ester. Preliminary experiments involving fractionation of the acetone soluble portion indicate the latter is the case. The amount of neutral fat in the serum of the two groups of animals has been found to be very nearly the same. The control animals received a plentiful supply of unsaturated fatty acids in their dietary fat and the fatty acids of the serum of these animals were found to be more unsaturated. The fractions of the serum lipids which normally contain highly unsaturated fatty acids, namely, the cholesterol esters and phospholipids, have relatively low iodine numbers in the animals on the low fat regimen which finding suggests that the dog is unable to synthesize fats of a high degree of unsaturation.

Conclusion. Distinct alterations in the appearance of the skin have been observed in puppies reared on a diet low in fat. This skin change became evident at about 3 months of age with the gradual development of a generalized flaky desquamation together with a dryness and coarseness of the hair. The skin and hair of the litter-mate puppies receiving 28% of their calories as lard in the diet remained clear and soft. Coincidental with this phenomenon marked differences in the degree of unsaturation of the fatty acids of the blood serum in the 2 groups of animals was demonstrated. The most marked difference in the iodine number of the fatty acids was found in the acetone soluble fraction, the average value being 83.9 for the animals receiving practically no fat in contrast to 118.7 for the animals receiving lard as a form of fat in the diet.