TABLE II. Relationship between Litter Series, 14th Day Weight of Young and Number of "Functional" Mammary Glands.

Position of litter series	I	II	III	IV
No. of females	89	59	39	34
Avg No. of functional glands	9.6	10.2	10.3	10.2
S.E.	0.12	0.17	0.17	0.20
Avg wt of 6 young on 14th day (g)	122.5	137.5	143.1	138.3
S.E.	1.59	2.30	2.69	3.14
Avg birth wt of 6 young (g)	29.35	30.28	31.38	30.71
S.E.	0.223	0.173	0.696	0.243

ter was the lowest as compared to the weights of those of the subsequent series. The average 14th day weight of 6 young born in the third order of birth was the highest. It is interesting to note that the average number of "active" mammary glands in the females of the first group was the least. It may seem, therefore, that there is a correlation between the number of "active" mammary glands and the position of litter series.

Discussion. The wide variation in the weights of young rats from different litters has been mainly due to the maternal milk supply. A closer study revealed that there was a linear relationship between the number of "milking" mammary glands of the mother rats and weights of their offspring. Actually it is possible to explain on the basis of the number of "active" or "milking" mammary glands the observed differences in the weaning weights of young born in the different order of birth. It should, however, be admitted that a corresponding study of milk yield of the lactating mothers would have been of considerable importance, but certain technical difficulties did not permit of extensive studies being made on this aspect.

Summary. An attempt has been made to determine the cause(s) for variation in weight of suckling rats. It was found that the 14th day weight of rats did depend upon the number of "milking" glands of the mother rats. The average 14th day weight of young born in the first litter was lowest when compared with the weights at corresponding periods of those delivered in the subsequent litters. The average number of "functional" mammary glands of mothers nursing their first born was also the lowest as compared with those of mothers rearing their second, third and fourth litters.

The authors wish to express their gratitude to Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor, for keen interest and valuable suggestions. It is a pleasure to acknowledge the technical assistance of Mr. S. Rajamanickam.

1. Schultze, M. O., *J. Nutr.*, 1954, v54, 453.
2. Hubbell, R. B., *ibid.*, 1954, v53, 429.

Received August 27, 1956. P.S.E.B.M., 1956, v93.

Essential Fatty Acids in the Chick. I. Development of Fat Deficiency.* (22718)

J. G. BIERI, G. M. BRIGGS, M. R. SPIVEY FOX, C. J. POLLARD, AND L. O. ORTIZ
Laboratory of Nutrition and Endocrinology, N.I.H., P.H.S., Bethesda, Md.

Symptoms of essential fatty acid deficiency in various animal species in which it has been produced are rather similar. Thus, in the rat(1), dog(2), guinea pig(3), and mouse (4), onset of deficiency is relatively slow and becomes apparent in changes in skin and fur as well as in decreased growth rate. The periods required for depletion of essential fatty

* Preliminary report appeared in *Poultry Sci.*, 1955, v34, 1231.

TABLE I. Composition of Purified Diet.

Ingredient	g/kg
Purified casein*	200
Gelatin	80
Glucose (cerelose)	615
Corn oil†	40
DL-methionine	3
Salts A(6)	60
Vitamins‡	2
Aureomycin§	

* General Biochemicals, Inc.

† Mazola.

‡ Vitamins added as follows (in cerelose premix), mg/kg diet: Vit. B_1 8, vit. B_2 8, calcium pantothenate 20, choline chloride 2000, niacin 100, vit. B_6 8, d-biotin 0.3, folic acid 3, vit. B_{12} 0.02, vit. D_3 0.02, α-tocopherol acetate 100, and 2-methyl-1,4-naphthoquinone 1. Vit. D, E, and K added in ethanol. 30 mg/kg of vit. A acetate added in ethanol in first exp. 1 mg vit. A acetate given orally, weekly, in 2nd and 3rd exp. See text.

§ 200 mg/kg diet added after 8 wk of 2nd and 3rd exp.

acids, as detected by these symptoms, are 4 weeks in the mouse and guinea pig, 8-12 weeks in the rat, and 12-40 weeks in the dog. Morbidity and death in these animals occur considerably later. It is apparent that tissues of these species relinquish their polyunsaturated fatty acids at a slow rate. In contrast, it has been reported that chicks on a synthetic-type, fat-free diet grew slowly from the first week, feathered poorly, and died within 3 to 4 weeks(5). These observations suggest that the chick has a considerably greater essential fatty acid requirement than other species. Contrary to this viewpoint, our studies indicate that chicks grow normally on a fat-free diet for at least 6 to 8 weeks, and do not develop symptoms of deficiency until later.

Methods. Day-old female New Hampshire chicks in groups of 6 were placed in electrically heated brooders with screen mesh floors and fed the synthetic-type diet given in Table I, with or without inclusion of 4% corn oil. Both feed and water were given *ad lib.* The air-conditioned room was kept at 75 ± 1° F and 55% relative humidity. In initial experiments it was found that 3 mg of vit. A acetate/kg, mixed into the diet in ethanol, was so rapidly destroyed (as found by analysis) that vit. A deficiency frequently developed in the chicks in 3-4 weeks. This could be prevented by increasing the level to 30 mg/kg. However, occasional appearance of vit. A deficiency symptoms even under these conditions prompted a study of parenteral and oral administration of this vitamin. A single, weekly oral dose of 1 mg vit. A acetate in 0.4 ml of 20% aqueous Tween 40† gave a sufficiently high liver storage of the vit. to prevent deficiency. Chemical analyses of livers from chicks in these experiments showed that both vits. A and E were absorbed in adequate amounts. Chicks were weighed and examined weekly. When 8 weeks old, the groups were reduced to four birds each because of space limitations.

Results. In preliminary experiments lasting 4 weeks, birds on fat-free diet grew as well as those receiving 4% corn oil in the diet and appeared normal in all respects as indicated in a preliminary report(7). Experiments of longer duration were then carried out and growth data from 3 separate trials are averaged in Table II. Although control chicks were slightly heavier throughout, the difference at 8 weeks was not significant ($P = >0.1$). By 12 weeks chicks fed the fat-free diet were definitely smaller ($P = <0.01$). It was apparent from the growth curves that effects of fat-deficiency appeared between the 6th and 8th weeks. The first visible symptom attributed to fat deficit was a lightening of normally dark brown plummage, occurring from about the 8th week. This was not marked and did not progress or occur uniformly in all birds. Changes in skin were not readily detectable. In several birds an increased scaliness occurred after 16-20 weeks.

Of the 8 fat-deficient chickens kept beyond 8 weeks, 4 were maintained as long as 24 weeks. Three others died at 13, 22 and 24 weeks, and one was killed at 17 weeks. Throughout this period the appearance of the birds was normal except for the slight changes noted above. Death of chicks in the first trial was attributed to air sac infection. In succeeding experiments 200 mg aureomycin/kg of diet was fed after the birds were 8 weeks of age and this cause of death was eliminated.

† Polyoxyethylene sorbitanmonopalmitate.

TABLE II. Growth of Chicks Fed a Fat-Free Diet (Avg of 3 Trials).*

	Fat-free diet			Control diet		
Age (wk)	Wt in g	No. surviving	Feed effic.†	Wt in g	No. surviving	Feed effic.
4	295 ± 11‡	18	.549	303 ± 12	18	.537
6	560 ± 13	17		579 ± 16	18	
8	830 ± 26	17		894 ± 30	16	
12	1288 ± 46	8*		1512 ± 49	8*	

* Six chicks/group in each trial. Only 2 trials were continued for 12 wk and longer; the No. of chicks was reduced to 4 in each group after 8 wk.
† Total wt gain/total food consumed.
‡ Mean ± S.E.

In two birds which died in later experiments, no obvious cause of death could be detected. At autopsy, fat-deficient chickens (24 weeks) had considerable amounts of abdominal fat, though less than controls. The oviducts and ovaries in two birds were immature, but in the other two the ovaries contained developed yolks. All other organs appeared normal.

Three male chicks maintained on fat-free diet for 8 weeks had combs and wattles which were smaller and paler than those of three males receiving 4% corn oil in the diet. At autopsy, the testes of fat-deficient chicks were only half as large as those of control chicks (178 vs. 441 mg/kg body weight). Body weights of deficient birds were only slightly less (6%) than those of normal birds.

Water consumption measurements made during the ninth week revealed that fat-deficient birds drank only about 15% more than the controls. In two trials made during the 11th and 12th weeks deficient chicks consumed 137 ml/kg/day compared with 103 ml/kg/day for normal chicks (4 per group).

Discussion. These experiments show that when chicks are fed a synthetic-type diet devoid of fat but adequate with respect to all other nutrients, growth continues normally for at least six weeks. Furthermore, the symptoms of fat deficiency that eventually develop are not striking. These results are in marked contrast to the picture presented by Reiser(5), and which has been quoted in recent reviews(8,9), that chicks grow poorly and die within one month when deprived of fat. It would appear that the diet used by Reiser was probably deficient in nutrients other than fat. The difficulty in insuring a sufficient intake of vit. A in a fat-free diet has already been discussed above. In addition to a possible vit. A deficiency, the diet of Reiser may also have been low in thiamine since the soybean protein, used in his diets, contains sulfite which has been shown to destroy thiamine(10). The salts V, which he used, accelerate thiamine destruction(11). Repetition of Reiser's experiments in our laboratory, using egg albumen as the protein but with vit. A given orally as described above, did not result in any difference between control and fat-free birds at 4 weeks

Analysis of purified casein(12) used in these experiments revealed a fat content of 0.23% and examination of the polyunsaturated fatty acids(13) in this lipid showed only 4.4% of dienoic and no trienoic or tetraenoic acid. This would amount to an intake of less than one mg dienoic acid daily (0.046% lipid in the diet). In comparison with the linoleic acid requirement of other species, this amount would be negligible. This same casein in a similar purified diet readily produces essential fatty acid deficiency in the guinea pig(3).

The decrease in growth rate, which occurred after 6-8 weeks on the fat-free diet, was also found by Russell *et al.*(14), although their difference began at 10 weeks and was not as marked. The higher caloric content, or an increased palatability of the control diet with fat, may have contributed to these differences. In the present studies feed efficiencies of the control and fat-free birds were found to be similar at 4 weeks (Table II).

Summary. Day-old chicks raised on a purified diet containing approximately

0.046% fat grew normally for 6-8 weeks, at which time weight gains began to decrease slightly. No marked symptoms of deficiency developed, but depigmentation of feathers and scaliness of skin were observed. Fat-deficient male chicks had smaller testes and some females had immature oviducts, when compared with normal chicks. Water consumption by the deficient birds did not increase markedly until the 11th week on the fat-free diet.

1. Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, v82, 345.

2. Hansen, A. E., and Wiese, H. F., *Texas Rept. Biol. Med.*, 1951, v9, 491.

3. Reid, M. E., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 708.

4. White, E. A., Foy, J. R., and Cerecedo, L. R., *ibid.*, 1943, v54, 301.

5. Reiser, R., *J. Nutrition*, 1950, v42, 319.

6. Briggs, G. M., Spivey, M. R., Keresztesy, J. C., and Silverman, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 113.

7. Briggs, G. M., and Fox, M. R. S., *Poultry Sci*, 1955, v34, 1231.

8. Holman, R. T., *The Vitamins*, vol. II, W. H. Sebrell and R. S. Harris, ed., Academic Press, New York, 1954, 296.

9. Deuel, H. J., Jr., and Reiser, R., *Vitamins and Hormones*, vol. XIII, R. S. Harris, G. F. Marrian and K. V. Thimann, ed., Academic Press, New York, 1955, 43.

10. O'Dell, B. L., Boston, M. D., Savage, J. E., and Hogan, A. G., *Poultry Sci.*, 1952, v31, 559.

11. Waibel, P. E., Bird, H. R., and Baumann, C. A., *J. Nutrition*, 1954, v52, 273.

12. Assn. Off. Ag. Chem., *Methods of Analysis*, 1955, 210.

13. Wiese, H. F., and Hansen, A. E., *J. Biol. Chem.*, 1953, v202, 417.

14. Russell, W. C., Taylor, M. W., and Palskin, L. J., *J. Nutrition*, 1940, v19, 555.

Received August 27, 1956. P.S.E.B.M., 1956, v93.

Variations in After-Discharge from the Hippocampus. (22719)

R. E. CORRELL AND W. R. INGRAM

Departments of Psychiatry and Anatomy, University of Iowa

A number of investigators have noted the relative sensitivity of the hippocampal formation to the production of after-discharge(2,5, 8,9). It has also been noted that the after-discharge shows little or no spread to the rostral neocortex(3), although there is some disagreement on the extent of spread. Akert and Andy(1) describe the local after-discharge in the guinea pig as characterized by 18-25 c/sec. regular waves with occasional activity at slower or faster frequencies. Liberson and Akert(6) describe a tonic-clonic sequence. The tonic phase consists of 10-30 c/sec. activity associated with brief spikes of 100-250 c sec. frequency. The relation of the DC potentials, described earlier by Liberson and Cadilhac(7), to the clonic phase was indicated. Crutzfeldt and Meyer-Mickelett(3), using cats, describe 3 characteristic phases preceded by a period of 50-100 c/sec. activity. The first phase consists of 8-12 c/sec. waves and is followed by 18-28 c/sec. activity. The final phase consists of short pauses and single spikes.

The purposes of the present study were to determine the components of hippocampal after-discharge in the cat, and more specifically the different patterns which these components may form. A study was also made of variations in after-discharge threshold within the hippocampal formation, and an attempt was made to relate variations in pattern of after-discharge to a number of antecedent conditions.

Material and methods. Bilateral bipolar electrode placements were made in a series of 20 cats with the aid of a Horsley-Clark stereotaxic instrument. All operations were performed under aseptic conditions using sodium pentobarbital (Nembutal) anesthesia. In all cases placements were as nearly symmetric as possible, and at the level of the posterior portion of the lateral geniculate body. Some variation was introduced in the vertical plane