differences in sensitivity of the assay methods used.

Guinea pig skin-sensitizing γ_1 antibodies were readily detected in milk from actively immunized mothers. However, it remains to be determined whether antibodies reached the milk by specific transport across acinar structures or by simple transudation.

Present experiments confirm earlier studies (reviewed in 13) on fetal transmission of anaphylactic sensitivity in the guinea pig and underscore an additional major difference between guinea pig and human skin-sensitizing antibodies. Although antibodies with tissue-sensitizing properties in guinea pig and man (14) migrate among the fast moving immune globulins on electrophoresis, they differ markedly in other physical properties. Guinea pig skin-sensitizing antibodies are produced in comparatively large amounts, they belong to the 7S group of globulins(7), and in our experiments are resistant to heating at 56°C for 4 hours and to dissociation with mercaptoethanol. The sedimentation characteristics of human skin-sensitizing antibodies are currently being reinvestigated; in one instance, a human skin-sensitizing anti-glucagon antibody was recently shown to have an intermediate sedimentation rate in the range of 8-11 S (15). Furthermore, human reagins are destroyed by heating at 56°C for 4 hours(16) and are readily dissociated with sulfhydryl compounds(17). Finally, in marked contrast to guinea pig skin-sensitizing $7S\gamma_1$ antibodies, reagins have been reported not to be transmitted from mother to child(13,18).

1. Freda, V. J., *Am. J. Obst. and Gynecol.*, 1962, v84, 1756.
2. Hartley, P., *Proc. Roy. Soc. (London) Series B*, 1951, v138, 499.
3. Batty, I., Brambell, F. W. R., Hemmings, W. A., Oakley, C. L., *ibid.*, 1954, v142, 452.
4. Barnes, J. M., *J. Path. Bact.*, 1959, v77, 371.
5. Brambell, F. W. R., Hemmings, W. A., Oakley, C. L., Porter, R. R., *Proc. Roy. Soc. (London) Series B*, 1960, v151, 478.
6. Brambell, F. W. R., Hemmings, W. A., Oakley, C. L., *ibid.*, 1959, v150, 312.
7. Benacerraf, B., Ovary, Z., Bloch, K. J., Franklin, E. C., *J. Exp. Med.*, 1963, v117, 937.
8. Ovary, Z., Benacerraf, B., Bloch, K. J., *ibid.*, 1963, v117, 951.
9. Bloch, K. J., Kourilsky, F. M., Ovary, Z., Benacerraf, B., *ibid.*, 1963, v117, 965.
10. Leissring, J. C., Anderson, J. W., *Am. J. Anat.*, 1961, v109, 149.
11. Leissring, J. C., *ibid.*, 1961, v109, 175.
12. Anderson, J. W., Leissring, J. C., *ibid.*, 1961, v109, 157.
13. Sherman, W. B., Hampton, S. F., Cooke, R. A., *J. Exp. Med.*, 1940, v72, 611.
14. Fireman, P., Vannier, W. E., Goodman, H. C., *ibid.*, 1963, v117, 603.
15. Rockey, J. H., Kunkel, H. G., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 101.
16. Loveless, M. H., *J. Immunol.*, 1940, v38, 25.
17. Leddy, J. P., Freeman, G. L., Luz, A., Todd, R. H., PROC. SOC. EXP. BIOL. AND MED., 1962, v11, 7.
18. Bell, S. D., Eriksson, Z., *J. Immunol.*, 1931, v20, 447.

Received June 26, 1963. P.S.E.B.M., 1963, v114.

A Respiratory Disease Syndrome in Chickens Fed Essential Fatty Acid Deficient Diets. (28592)

D. T. HOPKINS, R. L. WITTER AND M. C. NESHEIM
(Introduced by M. L. Scott)
Departments of Poultry Husbandry and Avian Diseases, Cornell University, Ithaca, N. Y.

Linoleic acid and to a lesser extent, arachidonic acid, commonly termed "essential fatty acids," are required nutrients for mammalian species(1). Essential fatty acids are also required for maximal growth by the chick(2,3) and cannot be synthesized *de novo* under ordinary conditions by either the hen or the chick(4,5,6). Feeding essential fatty acid-free diets to rats results in cessation of growth, increased basal metabolic rate and dermatitis. Bieri *et al*(7) fed chicks a fat-free diet for 12 weeks but did not observe extreme

TABLE I. Experimental Diets.

Ingredients	Basal proportions	10% soybean oil proportions
Sucrose	58.05	37.23
Hydrogenated coconut fat*	1.00	1.00
Soybean oil	—	8.80
Casein, methanol-extracted	28.00	28.00
Cellulose†	2.99	2.99
L-Arginine • HCl	1.50	1.50
DL-Methionine	.30	.30
Glycine	.80	.80
Mineral mixture, DTH-7‡	6.08	6.08
Vitamin mixture, DTH-6§	1.00	1.00
Choline Cl, 70%	.22	.22
Vitamin B_{12}, 0.1 mg/g	.05	.05
Ethoxyquin	.01	.01
Total	100.00	87.98

* *Hydrol*, Durkee's Famous Foods, Cleveland, Ohio.

† *Solka-Floc*, Brown Co., New York.

‡ Amounts furnished per kg of basal diet: $CaHPO_4$, 17.0; $CaCO_3$, 18.3; KH_2PO_4, 13.77; Na acetate, 8.42; $MgSO_4$, 2.50; $MnSO_4 \cdot H_2O$, 0.33; $FeSO_4 \cdot 7H_2O$, 0.33; ZnO, 0.075; $CoCl_2 \cdot 6H_2O$, 0.0017; KI, 0.0027; $NaMoO_4 \cdot 2H_2O$, 0.0083; $CuSO_4 \cdot 5H_2O$, 0.0167; Na_2SeO_3, 0.0001.

§ Amounts furnished per kg of basal diet: Biotin, 0.2 mg; menadione sodium bisulfite, 1.03 mg; pyridoxine • HCl, 4.5 mg; folic acid, 4.0 mg; thiamine • HCl, 10.0 mg; riboflavin, 10.0 mg; d-Ca pantothenate, 40.0 mg; niacin, 50.0 mg; inositol, 250 mg; Vit. D_3, 975 ICU; Vit. E, 66 IU; Vit. A, 5,000 IU; BHT, 22 mg.

symptoms of a deficiency disease similar to those seen in the rat, although feather depigmentation and some scaliness of the skin of the fat-deficient chicks was noted. Chicks fed the fat-free diet did show evidence of suppressed sexual development when compared to control chicks. Marion and Edwards(8) found that chicks fed diets low in linoleic acid had heavier livers and higher levels of liver lipid than chicks fed diets containing corn oil.

The present report describes a respiratory disease syndrome of unknown etiology which was encountered in chicks fed diets very low in essential fatty acids. This condition was not observed in chicks receiving dietary soybean oil.

Experimental. Chicks were fed one of 2 purified diets, shown in Table I. The basal diet consisted chiefly of alcohol-extracted casein and sucrose, and also contained 1% of hydrogenated coconut fat to insure adequate absorption of the fat-soluble vitamins. Hydrogenated coconut fat contains no linoleic acid. This diet was considered to be deficient in essential fatty acids. A second diet contained 10% of soybean oil, so added as to maintain a constant ratio of calories to protein, minerals and vitamins.

Progeny of hens fed a diet consisting chiefly of soybean oil meal, sucrose and either 1% or 20% of hydrogenated coconut fat, and low in unsaturated fatty acids, were fed the linoleic acid deficient basal diet (Exp. 1, 2 and 3). Chicks fed the diet containing 10% of soybean oil (in Exp. 1) were obtained from the same source, while those fed this diet in Exp. 2 and 3 were hatched from eggs of hens receiving diets containing 5% of safflower oil. All chicks were fed the basal diet for one week and then were fed the experimental diets. In each experiment chicks receiving both diets were raised five weeks in thermostatically-controlled electrically-heated, battery-type brooders. At the end of 5 weeks they were placed in battery-type growing units. At all times the chicks receiving both diets were in close proximity to each other and presumably had equal exposure to infectious diseases.

Results and discussion. Chicks in Exp. 1 were raised from one to 10 weeks of age without excessive mortality. At 10 weeks of age, the chicks receiving the diet low in unsaturated fat were observed to be suffering from respiratory distress, and some chicks died in the ensuing 3 weeks. The respiratory signs became so severe that the experiment was terminated. After the chick laboratories were thoroughly cleaned and fumigated with formaldehyde and potassium permanganate, week-old chicks were fed the diets of Exp. 2 and 3. Chicks fed the basal diet again developed respiratory signs and the experiments were terminated when the chicks were 11 weeks of age.

After the appearance of the respiratory signs, mortality (Table II) was confined largely to the chicks receiving the basal diet. All chicks that died were autopsied. Upon termination of the experiments, all chicks were killed and examined for respiratory lesions. Total incidence of such lesions is indicated in Table II. The figures include

the lesions found in chicks that died before the end of the experiment. Lesions were found in chicks receiving the diet deficient in linoleic acid, but none were observed in chicks receiving 10% of soybean oil. Both sexes were equally susceptible.

On gross examination, the lungs of the afflicted birds were of normal color and shape; however, hard cores could be palpated in the mesobronchi. Dissection revealed a grossly thickened mesobronchus impacted with an inspissated, pale white exudate (Fig. 1). The exudate also could be found in secondary bronchi. In advanced cases, the lung parenchyma immediately adjacent to the affected meso- and secondary-bronchi was consolidated. The borders of the consolidated areas were very discrete. Apparently death occurred when exudate filled the air passages to the extent that respiration became impossible. In some advanced cases, exudate was found in the air sacs and occasionally in the trachea. Significant gross lesions were not found in other organs.

The most severe microscopic alterations occurred in the mesobronchi. The lumen was filled with inflammatory exudate, *i.e.*, necrotic epithelial cells, mucus, and abundant heterophils. Although sloughing occurred occasionally, the epithelium of the mesobronchus was usually intact. The area of subepithelial, loose connective tissue was markedly thickened (0.5 to 1.0 mm) due to an accumulation of macrophages, some lymphoid cells, and a few neutrophils. Edema and hemorrhage were occasionally seen. The smooth muscle layer was disrupted by macrophage infiltration and cellular reaction extended outward to involve the adjacent lung parenchyma. The secondary bronchi showed similar alterations. Inflammatory exudate accumulated in the lumina of nearby tertiary bronchi and was closely surrounded by giant cells and macrophages, forming small granulomata. The intervening lung parenchyma was consolidated with extensive macrophage infiltration.

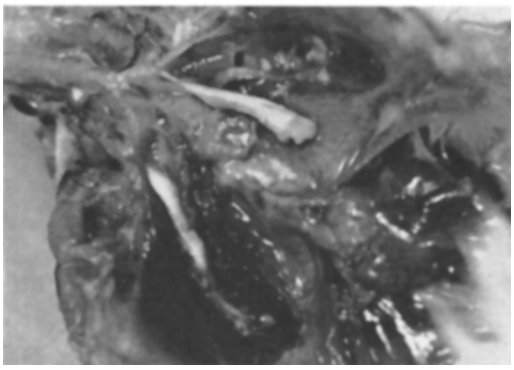

FIG. 1. Lungs showing lesions of respiratory disease from a chick fed a linoleic acid deficient diet. Both mesobronchi have been cut to demonstrate the caseated exudate. The exudate has been displaced in the right mesobronchus to show exudate present in the secondary bronchi.

TABLE II. Effect of Soybean Oil in the Diet on Mortality and Incidence of Lung Lesions.

Treatment		Exp No.	Body wt, g	Mortality	Total gross lung lesions*
				10-13 wk	
Basal	♂	1	2039†	4/12‡	11/12‡
	♀		1693	5/8	7/8
10% soybean oil	♂	1	2887	1/14	0/14
	♀		2346	0/13	0/13
				7-11 wk	
Basal	♂	2	1644§	2/9	8/9
	♀		1300	1/11	7/11
10% soybean oil	♂	2	2213	0/7	0/7
	♀		1706	0/10	0/10
				7-11 wk	
Basal	♂	3	1695§	5/13	11/13
	♀		1540	4/12	11/12
10% soybean oil	♂	3	2482	0/12	0/12
	♀		1965	1/12	0/12

* Thickening of mesobronchi with exudate in lumen.
† At 13 wk.
‡ Numerator is number dead or showing lesions; denominator is total number of chicks per group.
§ At 11 wk.

Since Bieri(7) showed that chicks fed diets low in fat may be unable to absorb Vit. A, 4 livers of birds receiving each of the diets in Exp. 2 were analyzed for Vit. A content(9). Chicks receiving the low fat diet contained 5,700 to 8,200 IU of Vit. A per liver, while chicks receiving soybean oil contained 5,800 to 8,200 IU of Vit. A per liver. In both cases, Vit. A storage was more than adequate, showing that the respiratory syndrome was not a complication of Vit. A deficiency.

Ross and Adamson(10) suggested that chicks fed diets low in fat developed aspergillosis more readily than chicks receiving diets

containing corn oil. The pulmonary lesions in these experiments superficially resembled those of aspergillosis. However, in sections stained with Gridley's fungus stain(11), mycelia were found infrequently in the bronchial exudate and never invaded the lung parenchyma. Furthermore, fungi could not be consistently isolated from the lesions.

The respiratory lesions may possibly be associated with chronic respiratory disease, although the characteristic lymphofollicular reaction described by Jungherr *et al*(12) was observed infrequently. Isolation of pleuropneumonia-like organisms was not attempted. Although inflammatory exudate was found in the air sacs of some affected chicks, particularly in advanced stages, no air sac lesions were noted unless lesions were also present in the lungs. The inconsistent airsacculitis and the predisposition of the mesobronchi to inflammation would make this syndrome at best an unusual manifestation of chronic respiratory disease.

Nagai *et al*(13) reported that mice fed diets deficient in essential fatty acids had decreased resistance to bacterial infection. Cultures of the bronchial exudate from birds in our experiments showed a profusion of many species of bacteria. The deficiency of linoleic acid may have decreased resistance of the chick's bronchial epithelium sufficiently to allow infection by resident bacterial flora.

In a fourth experiment, chicks fed the diet low in linoleic acid and a diet containing 10% of soybean oil were killed at 6, 7½, 9½ and 11 weeks of age, autopsied, and the lungs sectioned to study the pathogenesis of this syndrome. At 6 weeks both deficient and supplemented chicks showed a slight, diffuse lymphoid hyperplasia of the mesobronchi. Degenerative changes occurred in the mesobronchial epithelium and in one case a small amount of inflammatory exudate was present in the lumen. No lesions were seen grossly.

Chicks receiving the diet containing soybean oil showed no gross lesions, and lungs were histologically normal at all subsequent samplings. Apparently complete regression of the initial lesion occurred in this group. In contrast, the severity of pulmonary lesions increased in the deficient chicks. At 7½ and 9½ weeks the chicks on the diet low in linoleic acid showed increased lymphoid hyperplasia of meso- and secondary-bronchi. Cellular reaction extended into the epithelium and peripherally through the smooth muscle layer involving, in a few instances, adjacent tertiary bronchi and lung parenchyma. Macrophages were seen in the more advanced lesions and some chicks had accumulations of inflammatory exudate in the mesobronchial lumen. Some gross thickening of the mesobronchi was detected in the 9½ week deficient chicks.

By 11 weeks gross thickenings and exudate accumulation were seen in the mesobronchi of deficient chicks. The histological picture was similar to that described previously for the advanced syndrome, although slightly less severe.

Whatever the cause of the respiratory syndrome observed in these experiments, there can be no doubt that soybean oil was fully effective as a protective agent. Although the basal diet was deficient in linoleic acid, and soybean oil added this nutrient to the diet, it cannot be definitely ascertained that linoleic acid is the protective factor. The inflammatory nature of the lesions suggests an infectious etiology, but exposure to infective agents was the same in deficient and control birds. The respiratory disease syndrome may be the result of a linoleic acid deficiency *per se*, resulting in decreased resistance of the pulmonary tissues to infection.

Summary. A respiratory disease syndrome characterized by formation of bronchial exudate and lung consolidation was observed in chicks fed diets very low in essential fatty acids. These lesions were not observed in chicks receiving similar diets containing 10% of soybean oil.

1. Aaes-Jørgensen, E., *Physiol. Rev.*, 1961, v41, 1.
2. Hopkins, D. T., Nesheim, M. C., Carew, L. B., Jr., Norris, L. C., *Proc. Cornell Nutr. Conf.*, 1960, p71.
3. Machlin, L. J., Gordon, R. S., *J. Nutr.*, 1961, v75, 157.
4. Murty, N. L., Williams, M. C., Reiser, R., *J. Nutr.*, 1960, v72, 451.
5. Machlin, L. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 819.

6. Reiser, R., *J. Nutr.*, 1950, v42, 325.
7. Bieri, J. G., Briggs, G. M., Spivey Fox, M. R., Pollard, C. J., Ortiz, L. O., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 237.
8. Marion, J. E., Edwards, H. M., Jr., *J. Nutr.*, 1962, v77, 23.
9. Ames, S. R., Risley, H. A., Harris, P. L., *Analyt. Chem.*, 1954, v26, 1378.
10. Ross, E., Adamson, L., *J. Nutr.*, 1961, v74, 329.
11. Gridley, M. F., *Am. J. Clin. Pathol.*, 1953, v23, 303.
12. Jungherr, E. L., Luginbuhl, R. E., Jacobs, R. E., *Proc. Am. Vet. Med. Assn.*, 1953, p303.
13. Nagai, H., Sudo, M., Akaishi, K., *Annales Paediatrici Japonici*, 1961, v7, 36.

Received June 27, 1963. P.S.E.B.M., 1963, v114.

Effects of Acid-Base Alterations on Cerebrospinal Fluid Production. (28593)

W. W. OPPELT, T. H. MAREN, E. S. OWENS AND D. P. RALL

Clinical Pharmacology and Eperimental Therapeutics Service, National Cancer Institute, National Institutes of Health, Bethesda, Md., and Departments of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville

Since the introduction of ventriculo-cisternal perfusion with inulin-containing buffer as a technique to measure cerebrospinal fluid (CSF) production rates(1) it has become possible accurately to determine the effect of various drugs and metabolic manipulations on CSF formation rate. Using this technique we have previously reported on the effects of parenteral carbonic anhydrase inhibitors and parenteral and intrathecal cardiac glycosides on this rate(2). In the dog, carbonic anhydrase inhibitors were found to decrease CSF formation rate by 40-50%, whereas ouabain had no effect(2). In the cat, however, a significant decrease of CSF production was noted with intrathecal ouabain(3). To investigate further the factors that control CSF production rates the effects of metabolic acidosis and alkalosis, and respiratory acidosis and alkalosis on CSF production were studied in dogs.

Methods. Mongrel dogs weighing from 8-14 kg were lightly anesthetized with intravenous pentobarbital. Their heads were fixed in a stereotaxic apparatus, and a 22-gauge needle was introduced into the right lateral ventricle. A 19-gauge needle was placed into the cisterna magna. Ventriculo-cisternal perfusion was then begun with a buffer* containing inulin-C^{14}, 1 μc/100 ml. Perfusion rates were kept constant for each experiment at 0.123 ml/minute. CSF production rates were calculated by the following formula: production $= r\ (C_i - C_o)/C_o$, where C_i and C_o refer to inulin concentration in the inflow and the outflow and r refers to the inflow rate in ml/minute. It can thus be seen that CSF formation rates were calculated as a factor of the dilution of inulin as it passes from the lateral ventricle to the cistern. Studies of Pappenheimer and Halsey(1) and of Rall *et al.*(4) have shown that less than 1% of such perfused inulin passes either into the blood or the extracellular space of brain. Therefore, the assumption is valid that the dilution of inulin occurring as it passes through the ventricular system is due almost exclusively to newly formed CSF. This method allows for relatively exact determination of CSF production rates and is independent of brain vascular volume.

Metabolic acidosis was produced by intravenous infusion of 0.1-0.3 N HCl at a rate of 3-5 meq/kg/hr. Metabolic alkalosis was caused by intravenous 0.5 N $NaHCO_3$ infused at a rate of about 5 meq/kg/hr. The dogs were put into respiratory acidosis by allowing them to breathe a 5-10% CO_2/oxygen mixture. A Jefferson ventilator was used to hyperventilate the dogs with air to produce respiratory alkalosis. Each experiment consisted of a 2-hour control period followed by a 4-5

* NaCl – 123.5 meq/l; KCl – 3 meq/l $CaCl_2$ – 1.3 meq/l; $MgCl_2$ – .8 meq/l; $NaHCO_3$ – 25 meq/l; NaH_2PO_4 – .5 meq/l; Na_2HPO_4 – .5 meq/l.