oxidized lard was in some way responsible for the syndrome of loss of hair, skin lesions, anorexia, emaciation and intestinal hemorrhages observed in our dogs. As our control dogs remained in good condition, we are convinced that this pathological condition is not due to an inadequacy in our basal ration.

Several mechanisms of action by which partially oxidized fats may bring about this syndrome may be mentioned. It might be a direct toxic effect upon the animal, though this seems unlikely. It might produce a greater need for one of the vitamins or call for a different balance between them. It is possible that the action of the oxygen on the fat destroyed some important grouping such as the unsaturated linkage shown by Burr and Burr[3] to be essential for health in the rat. The signs and symptoms presented by the dog do not resemble very closely those described for the rat, but there may be a marked species variation.

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Blood Volume in Normal Chicks and in Chicks with Nutritional Encephalomalacia.

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The cerebral lesions in the disorder of chicks described by Pappenheimer and Goettsch[1] as *nutritional encephalomalacia,* appear unquestionably to result from vascular disturbances. Oedema, hemorrhage and hyaline thrombosis of capillaries are the conspicuous initial features of the lesions and they must be due either to alteration of the vessel walls, or to a quantitative or qualitative change in the blood itself.

In attempting to analyze the problem from this point of view, it seemed of interest to determine first whether the disease producing Diet 108* leads to an alteration in the total volume of blood. This paper presents briefly experiments bearing on this phase of the subject.

Material and Technique. Eighty 2-day-old chicks were placed on Diet 108 and 20 controls of the same hatch on the natural foods Diet

[1] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

634 of Hogan, Hunter, and Kempster.[2]† Additional control material on Diet 634 was also available. The chicks were of 4 different breeds, White Leghorns, White Wyandottes, Rhode Island Reds and Barred Plymouth Rocks, but it may be stated here that no significant correlation was obtained between breed of chick and blood volume. Neither was any difference found in the susceptibility to this disease, the percentage incidence being approximately the same in all breeds.

The determinations of the plasma and blood volume were carried out by the dye method described by Graff and Clarke,[3] but with certain minor variations. A 1.3% solution of K oxalate was found to be optimal for chicken erythrocytes, giving neither crenation nor hemolysis. A 1% solution of brilliant vital red in 0.9 NaCl solution filtered and sterilized, was used for injection. It was convenient with chicks to inject a much greater volume of dye in proportion to body weight than it was in humans. This permitted the withdrawal of smaller samples as was necessary with young chicks. Our dilution factors were hence approximately 10 times those of Graff and Clarke. The syringe (Tuberculin-Becton-Dickenson & Co.) was calibrated, and a suitable correction made in the calculations.

The technique was as follows: Under ether anesthesia, the heart was punctured through the chest wall, and with the needle in the ventricle, an accurately measured amount of the dye solution injected. Later this was modified by opening the thorax and injecting directly into the heart. After 5 minutes (±10″), the chick being kept under light anesthesia, the heart was aspirated, and the blood added to small test tubes containing weighed amounts of 1.3% potassium oxalate. Tube A, containing approximately 0.1 cc. of oxalate, was used for hematocrit determinations. Tubes B and C, containing 2.0 cc. of oxalate, were weighed, centrifuged, and the diluted plasma used for duplicate readings in a Koenig-Martens

[2] Hogan, A. G., Hunter, J. E., and Kempster, H. L., *J. Biol. Chem.*, 1928, **77**, 431.

* Diet 108 has the following composition

	%
Skimmed Milk Powder (Merrel-Soule)	15.0
Casein (Merck's technical)	20.5
Cornstarch	20.0
Lard	21.0
Cod Liver Oil	2.0
Yeast (Fleischmann's bakers, dried)	5.0
Salt Mixture (McCollum 185)	6.5
Paper Pulp (Eastman)	10.0

† Diet 634 of Hogan, Hunter and Kempster:

	%
Whole Wheat	55.6
Whole Milk Powder	8.2
Casein	12.3
Alfalfa Meal	2.5
Butter Fat	4.2
NaCl	0.9
$CaCO_3$	1.3
Cod Liver Oil	3.0
Yeast	12.0

[3] Graff, S., and Clarke, H. T., *Arch. Int. Med.*, 1931, **48**, 808.

spectrophotometer. For further details as to the optical technique, see Graff and Clarke.[3]

Precision of the Methods Used. 1. *Sampling Time.* One of the recognized difficulties in the application of the dye method is to obtain samples at the precise time when mixing has been completed, and before there has been an appreciable loss of dye from the circulating blood. These time relationships have been studied in detail in human blood volume determinations by Graff, d'Esopo, and Tillman.[4] They have shown that mixing is completed within 4 to 7 minutes after injection of the dye. This is followed by gradual disappearance of the dye at the rate of 10 to 20% per hour. The curves obtained in chickens from samples secured by repeated sampling at short intervals resembled those in humans, save that the rate of loss of the dye was very much greater. Thus 3 curves showed losses of 17.5%, 19.5%, and 20% per 10 minutes after mixing. This difference in the rate of dye loss probably has no physiological significance since a much greater proportionate volume of dye was used in the chicks. The rate of loss is probably a function of the concentration.

Although this rapid loss of dye undoubtedly introduces inaccuracy as regards the absolute value of the figures obtained, it seems probable that samples obtained at exactly identical times after injection of the dye will yield comparable values. As has been stated, the samples were taken in precisely 5 minutes ($\pm 10''$) after dye injection.

2. *Divergence of Checks in Duplicate Determinations.* This indicates the errors in the dye method which are the combined result of errors in hematocrit determinations, specific gravity, and spectrophotometric estimations. These individual sources of error may be briefly discussed. From our data of 93 hematocrit determinations, the P. E. is calculated as 0.23. This implies a maximal deviation of about 0.53% in the cell volume values in our series.

3. *Specific Gravity.* In our calculations a specific gravity of 1.060 has been assumed. Actual determinations of heparinized chicken blood gave specific gravity values of 1.046, 1.045, 1.047, and 1.044, an average of approximately 1.045. This shows an error of approximately 1.5% in the specific gravity and a slightly lower error in the final calculation. Thus in one example, recalculation

[4] Graff, S., d'Esopo, D. H., and Tillman, A. J. B., *Arch. Int. Med.*, 1931, **48**, 821.

using the specific gravity figure 1.045 gives 55.4 cc. plasma per kilo, instead of 54.8 cc. This is, therefore, not an important source of error, if we assume that the specific gravity of the blood under the experimental conditions is reasonably constant.

4. The error in the spectrophotometric estimation is the usual one of approximately 2%. In the estimation of duplicate samples from 67 individuals, the P. E. was 2.51%.

5. *Error Due to Varying Weight of Intestinal Contents.* A possible source of error which, if ignored, would lead to differences in the final calculation, is the varying weight of the intestinal contents, which may, as is shown in the following table, amount to 10% of the body weight. The weight of the alimentary contents including those of crop and gizzard, was estimated by weighing the intestinal tract full, and after washing out the contents. The mean average weight expressed in percentage of body weight in 25 chicks on the experimental Diet 108, was 7.4%; in 17 chicks on the control Diet 23, 7.5%.

During the first few days there is considerable uniformity; later the individual variations are wide, but there is no constant difference between the 2 diets.

It would seem desirable in estimating the blood volume, to deduct the weight of the alimentary contents from the body weight. Unfortunately, the contents were not weighed in the earlier determinations of this series. We have, therefore, introduced into these calculations an arbitrary deduction of 7.5% of the body weight, as approximating the weight of the alimentary contents, and recalculated our blood volume estimations with this correction.

Blood and Plasma Volume in Normal and Affected Chicks. The determinations are recorded in Table I and Table II,, which show convincingly that there is no significant difference between the two groups. The data may be summarized as follows:

Diet	108	634
No. Chicks	31	38
Aver. Plasma Vol. (cc./kilo)	56.1	55.0
Aver. Plasma Volume (corrected for wt. of alimentary contents)	62.2	59.7
Aver. Blood Volume (cc./kilo)	83.4	82.9
Aver. Blood Volume (corrected for wt. of alimentary contents)	91.0	95.4

Since the P. E. in the methods is at best 2.5% and possibly somewhat higher, it is obvious that the slight difference between the two groups is not significant.

TABLE I.
Chicks on Diet 634 (Normal Controls Without Symptoms or Lesions).

Age	Breed	Wgt.	C/V%	Uncorrected Plasma cc./kilo	Uncorrected Blood cc./kilo	Wt. of Alimentary Contents	Corrected Plasma cc./kilo	Corrected Blood cc./kilo
1	1	38	33.5	57.0*	81.0			
1	1	33	31.8	56.0*	83.5			
1	1	30	28.6	60.5*	84.5			
1	1	34	32.5	48.0*	77.0			
3	1	55	31.9	55.5	81.0	E. 4.1	59.6	87.2
3	1	50	34.0	45.6	69.0	E. 3.7	49.0	74.5
3	1	45	32.4	55.0	81.5	E. 3.4	59.7	88.5
4	1	54	31.9	40.8	60.0	6.5	48.2	75.0
4	1	51	34.6	56.0	85.5	E. 3.8	65.0	92.5
4	1	38	28.9	51.6	73.0	E. 2.8	56.0	78.6
5	1	56	33.3	55.7	83.5	E. 4.1	60.0	89.9
5	1	48	36.1	50.2	81.0	3.5	54.5	87.0
5	1	53	33.9	52.5	80.0	4.5	57.5	87.0
6	1	61	41.4	52.5	89.6	4.5	56.8	97.0
10	1	70	34.6	49.5	76.0	6.5	55.5	84.6
11	1	75	36.7	54.5	86.0	7.5	60.5	96.0
11	1	93	38.4	49.0	79.5	9.5	54.5	88.0
12	1	78	33.8	57.0	86.0	3.5	62.0	93.6
15	1	105	33.8	56.5	85.0	7.0	60.5	97.0
19	1	93	41.0	63.0	106.0	E. 6.9	66.0	111.0
22	1	188	32.0	52.0	77.0	16.5	57.4	84.5
23	2	139	35.4	54.0	83.5	E. 10.5	58.5	91.5
26	1	204	33.7	45.0	68.0	11.5	47.7	72.0
27	1	195	36.0	48.6	76.0	19.0	54.0	84.5
27	1	96	30.4	72.5	104.0	E. 7.2	78.0	112.0
28	1	185	43.7	43.5	77.2	10.5	46.0	81.7
30	2	245	34.4	55.0	84.0	18.3	59.5	91.0
33	3	244	23.3	59.0	77.0	18.2	64.0	84.0
33	3	290	30.8	51.8	75.0	21.8	56.0	81.0
34	1	203	31.5	53.5	78.5	15.2	58.0	84.5
39	3	331	37.8	57.5	92.5	24.8	62.2	100.0
43	1	275	37.8	55.0	88.4	20.6	59.0	95.0
48	3	357	34.9	52.1	80.6	26.7	56.6	88.0
?	2	426	26.4	55.5	75.5	32.0	60.0	82.0
?	2	357.5	35.1	59.3†	91.2	E. 27.3	68.0	100.5
?	2	218	33.7	84.2‡	126.0	E. 16.4	91.0	137.0
?	2	293	28.7	56.6	71.2	E. 22.0	61.7	86.6
?	2	109	32.0	69.9	100.2	E. 8.2	76.0	111.5

* 1 day chicks, no alimentary contents.
† Sampling time long; . · . high value.
‡ Sampling time less than 5 minutes.
1 = White Leghorn; 2 = Rhode Island Red; 3 = Barred Plymouth Rock.
E = Estimated at 7.5% of body weight.

Relation of Blood Cells to Plasma in Normal and Affected Chicks. The data are given in Tables I and II. The average cell volume percentage in 50 chicks on control Diet 634, was 33.0% with a σ of 4.65. On the experimental Diet 108, with 43 chicks, the average was 34.0, and a σ of 5.03. If this second group is subdivided into those showing pronounced symptoms and lesions, and those showing no or only slight changes, we find in the former group (28

TABLE II.
Chicks on Diet 108.

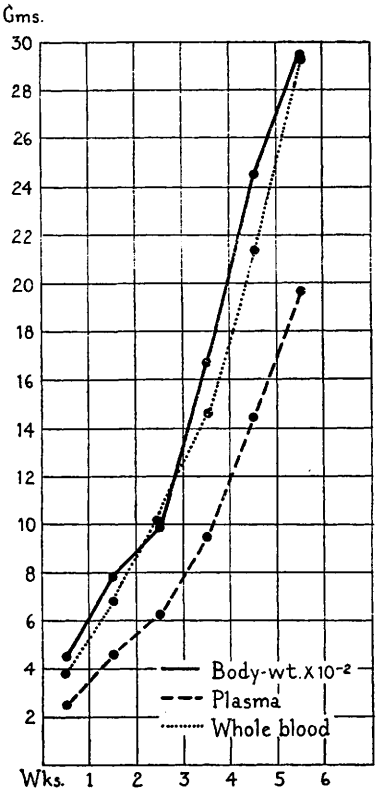
Age	Breed	Wt.	Symptoms	Lesions	C/V%	Uncorrected Plasma Blood		Wt. of Alimentary Contents	Corrected Plasma Blood	
						cc./ kilo	cc./ kilo		cc./ kilo	cc./ kilo
18	1	98	+++	++++	35.1	66.3	102.0	E. 7.4	74.5	115.0
19	1	76	+++	++	31.2	45.0*	65.0	E. 5.7	51.2	75.5
20	4	128	+++	—	34.6	57.8	88.0	E. 9.6	63.5	99.0
20	4	112	±	—	30.9	63.6	91.5	E. 8.4	69.0	99.0
21	1	99	++++	++++	38.0	50.7	81.7	10.5	56.5	91.2
22	1	88	+++	+++	41.0	74.6	126.0	E. 6.6	80.5	111.0
23	2	78	+++	+++	33.0	56.0	83.5	E. 5.8	61.0	91.0
24	2	85	++++	++++	33.6	68.0	93.0	E. 6.3	66.0	99.0
24	4	120	++++	+++	31.2	63.6	93.6	E. 9.0	68.5	99.5
25	2	97	+++	+++	35.6	61.0	94.7	E. 7.3	66.0	105.0
26	1	111	+++	+++	31.7	47.2	69.0	10.0	51.7	76.0
27	1	111	+++	++	34.1	39.2†	58.6	7.0	41.0	62.5
27	3	137	+++	+++	23.8	77.0	101.0	E. 8.3	83.0	108.5
27	4	91	+++	+++	33.2	66.0	99.0	E. 6.8	72.5	109.0
28	1	146	++++	++++	34.2	49.6	75.5	5.0	51.5	78.5
29	1	178	++++	++++	37.4	61.5‡	98.5	11.5	66.5	106.0
29	4	319	+	+	35.4	51.5	79.6	23.9	55.5	86.0
32	3	191	++	—	36.2	61.7§	97.0	14.3	67.0	104.0
32	2	129	+++	+++	24.4	59.8	79.0	9.7	65.0	86.0
35	1	199	+++	++++	39.4	48.2	79.5	19.0	53.5	88.0
35	1	263	+++	+	35.2	43.6	67.5	18.0	46.8	72.2
35	1	207	++++	++++	36.6	52.8	83.2	7.0	54.8	86.5
40	4	214	+++	+++	33.8	48.5	73.5	16.0	52.6	81.0
40	2	155	++	+++	39.0	60.5	99.0	11.6	63.5	104.0
40	2	194	—	+	43.9	48.9	87.2	E. 14.5	52.0	92.5
43	4	285	—	—	23.5	55.5	74.5	21.3	60.0	81.0
47	1	256	—	—	39.6	50.8	84.0	19.2	55.0	91.0
48	2	260	±	+++	52.0	48.0	100.0	19.5	52.0	108.0
49	2	247	+	+++	35.9	51.0	80.0	18.5	55.0	86.5
49	3	270	±	+	29.7	55.0	78.3	20.5	59.7	85.0
50	4	248	—	—	35.6	56.0	87.5	E. 18.6	58.2	90.5

* Moribund.
† Moribund. Heart beat very slow.
‡ High figure may be due to rapid loss of body weight from 192 to 172 gm. Recalc. for body weight of 192 gm.: 57 Pl/k. 91 Bld/k.
§ Stopped breathing immediately after injection. Heart stopped after second puncture.
1 = White Leghorn; 2 = Rhode Island Red; 3 = Barred Plymouth Rock; 4 = White Wyandotte.
E. = Estimated at 7.5% of body weight.

chicks), an average cell volume of 32.8%, with a σ of 5.13; in the latter (15 chicks), 34.6% with a σ of 4.98.

It is apparent from these figures that there is no significant difference in any of the groups; in other words, the disease is not accompanied by alterations in the cell plasma ratio.

Relation of Blood Volume to Age and Weight; Growth of the Blood as a Tissue. As is shown in Table III, our data included determinations on normal chicks from one day to 6 weeks or more



Growth of Plasma in Relation to Growth of Body Weight.

FIG. 1.

in age. There is thus afforded opportunity to study the growth of the plasma and whole blood in relation to the body weight and age during the early growth period. In Fig. 1 is shown (1) the weight of the chicks during the first 6 weeks, the points on the curve representing the average weight of the chicks killed in each 7-day period; (2) the average volume of the plasma, and (3) of the whole blood compiled from the same group of chicks.

The data upon which the graph is constructed are briefly summarized in the following:

TABLE III.

Week	No. Chicks	Aver. Wt. gm.	Plasma cc.	Whole Blood cc.	Wt. of Blood/Body Weight
1	14	46	2.6	3.9	.09
2	4	79	4.6	6.9	.09
3	2	99	6.2	10.3	.11
4	6	168	9.5	14.7	.09
5	4	246	14.5	21.5	.09
6	8	296	19.8	29.6	.10

It is obvious that within the age limits of the experiment, there is an extraordinarily close correspondence between the growth of the blood and plasma, and that of the body weight as a whole. This is borne out by the constancy in the relation between blood weight (calculated by multiplying volume by specific gravity factor 1.06), and the body weight.

Conclusions. 1. The disease described as nutritional encephalomalacia of chicks is not associated with significant alterations in cell plasma ratio, plasma or blood volume. 2. During the early growth period of the chick, the growth of the plasma and blood follow closely the growth of body weight. The blood and plasma volume per kilo, aside from individual variations, remain constant throughout the early growth period.

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The Central Nervous System in Relation to the Digestive Functions.

J. H. FERGUSON. (Introduced by J. F. Fulton.)

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In this communication a comparison is made between certain secretory, motor and vascular phenomena produced by pilocarpine administered into the cerebral ventricles of monkeys, and the corresponding responses to the drug when given subcutaneously.

A series of 15 green monkeys *(Lasiopyga callithricus)* has been used. On 6 of them, fractional gastric analyses have been made following the administration by stomach tube of 100 cc. of a farina test-meal. Several experiments have been conducted on each animal to demonstrate the action of the drug pilocarpine (hydrochloride), when administered at various times in the course of digestion of the test-meal. The drug has been given in varying doses subcutaneously and by the intraventricular route.

The intraventricular injection of pilocarpine, in doses of 5-10 mg. per kilo of body weight, invariably caused a sudden and complete cessation of the gastric secretion of free HCl, at whatever stage in the digestion the drug was given. The samples obtained after pilocarpine injection consistently failed to show any free HCl acid.

The total acidity curve usually paralleled the 'free acid' curve but at a higher level. After intraventricular injection of pilocar-