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POULTRY SCIENCE

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Effect of Charcoal and Length of Storage on the Calcifying Property of Cod Liver, Sardine (Pilchard), and Concentrated Cod Liver Oils

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(Received for publication November 7, 1936)

IT IS commonly believed different forms and amounts of vitamin D impregnated into feed are not destroyed by exposure to air, especially if the feed is stored for reasonable periods of time. Support for this belief is shown by experiments of Hart et al. (1925), who observed cod liver oil mixed with gound grains stored in galvanized iron cans equipped with loosely fitting covers at room temperature (about 70°F.) for three or six months retained its calcifying property when fed to chicks. Subsequent work conducted at the Ohio Experiment Station (1926) revealed the vitamin A content of cod liver oil was wholly or partly destroyed depending upon the condition of storage, while the content of vitamin D was not appreciably attenuated, although the amount of oil eaten (2 percent in a ration of grain, casein, and minerals) probably supplied greater quantities of antirachitic vitamin than are necessary to produce a noticeable decrease in potency after the brief period of storage employed, that is, one of four months' duration. Payne (1930) also found no loss in vitamin D potency of cod liver oil mixed with feed when stored in burlap bags for one year. Later Holmes and associates (1930) noted that no loss of *either* vitamin A or D potency occurred if cod liver oil mixed in a ration of grains, meat scraps, fish meal, dried skimmilk, alfalfa, bone meal, and salt was stored in grain sacks for 2, 4, 7 or 10 months.

Contrary to the above, Dunn (1924) observed cod liver oil mixed with granulated starch stored in jars in the dark six months at 50°F. and then fed in a ration of white corn, salts, and skimmilk to young chicks did not prevent rickets, while unmixed oil under similar conditions prevented the disease. Owing to the use of white corn, the birds may possibly have lacked vitamin A in addition to D, and the conclusion drawn by Dunn was probably not entirely valid, particularly since a lack of this vitamin upon the production of vitamin D deficiency in chicks was not theretofore clearly known or appreciated. Norris, Heuser, and Wilgus (1929) also discovered a difference between

oil freshly mixed in feed and that stored in burlap bags at room temperature for 4, 8, or 12 months, the quantity of vitamin activity destroyed varying with the length of storage.

From the evidence presented by these investigations, it may be concluded that loss of vitamin D potency of cod liver oil depends largely upon the (1) length and conditions of storage, (2) amount of vitamin fed, (3) type of ration employed, and (4) criteria used for interpretation of results. Accordingly, it seemed quite desirable in view of the contradictory communications reported and because of the increasing use and importance of other common sources of the vitamin for poultry, to reopen and renew the study, relating effect of storage to vitamin stability, and if possible to obtain some evidence relative to feeding cod liver oil mixed with charcoal under certain definite conditions of storage. It was somewhat surprising to learn from the literature that no attempt has heretofore been made to relate vitamin stability to length and conditions of storage with substances other than cod liver oil. Charcoal characterized industrially as an adsorbent, decolorizer, or "purifier" of various substances, either in the form of their solutions or the natural state whether solid, gas, or liquid, also enjoys a wide-spread use in present poultry practice as a base for incorporating vitamin-containing oils in feed. Due to its physical properties and chemical composition charcoal (carbon) is a non-polar carrier (holding oils in preference to water), and this fact forms the basis for our investigation of its utility.

With this in view, we planned to study among other things the possibility that digestive juices possessed by growing chicks are likely to be unable to assimilate vitamin D in the form of cod liver oil adsorbed on charcoal because of the difficulty of obtaining all the oil held in the capillary spaces of the adsorbing agent, with the result that only part of the vitamin would be available to the animal for the purpose of calcification and growth. Inferentially such a study appeared attractive in view of the experimental results reported by Lachat, Dutcher, and Honeywell (1930), which showed that the vitamin A and D of cod liver oil was adsorbed on "activated" silica gel so tenaciously that they were not utilized when the silica gel with adsorbed cod liver oil was eaten by rats. We realized in this connection that important differences in physical properties and chemical composition exist between silica gel and charcoal but *ipso facto* may possibly warrant further investigation of the latter's properties and uses.

EFFECT OF CHARCOAL ON CALCIFYING PROPERTY OF COD LIVER OIL

As a preliminary to studying the effect upon calcification and growth resulting from the feeding of vitamin D adsorbed on charcoal in commercial rations, day-old S.C. White Leghorn chicks divided into 8 lots of about 30 birds each (lots 1, 1c and p consisted of 15 birds each) were housed in electrically-heated battery brooders equipped with raised wire grids of coarse mesh and fed the A.O.A.C. vitamin-D deficient ration and distilled water. Charcoal prepared as a composite from 5 different samples of poultry feeding grades obtained commercially (mineral content: Ca 1.73 percent, P 0.03 percent), and/or cod liver oil, were added to the basal diet, preventive A.O.A.C. technic being employed throughout. After four weeks of feeding, surviving birds were sacrificed by breaking the neck, and the calcifying property of the different supplements judged by ash content of the left tibia determined by our usual procedure (Lachat 1935).

The essential data are presented in Table 1. A statistical (Fisher, 1932) examination of these data by sexes was made and showed

that no significant difference in ash content existed in any of the lots, the ash results therefore being given as averages. Birds weighed collectively at the start of experiment and individually at weekly intervals thereafter, displayed an extent and rate of growth positively correlated with the relative amount of vitamin consumed, as evident by inspection of the weights recorded in Table 1. Cod liver oil of poor quality for poultry was obtained commercially and comparable rations containing similar amounts of vitamin (with or without charcoal) tested for their calcifying property, the birds of lot p which received a good quality cod liver oil serving as positive controls. The graded amounts of oil given both in charcoal (the amount mixed being 3 parts charcoal to 1 of oil by weight) and in corn meal without charcoal, according to previously described technic (Lachat and Halvorson, 1936), were intentionally low in order that intermediate degrees of calcification accompanied by a retarded rate of growth would be produced. Statistical analysis of data showed no significant differences in ash content existed between comparable lots, for example—between lots 1 and 1c, 2 and 2c, 3 and 3c, 4 and 4c. Calcium and phosphorus and their ratio in the rations were determined and found to be insignificant as factors in the experiment, the variation of either element or both, being much too small to affect significantly the results obtained. It will be evident from these observations accordingly, that addition of charcoal had no demonstrable effect upon calcification or extent of growth as revealed by ash content and body weight

TABLE 1.—*Effect of charcoal on the calcifying property of cod liver oil by A.O.A.C. method*

Lot No.	Supplement to basal ration	No. birds surviving	Av. initial wt.	Av. final wt.		Av. ash content	Ration Ca	Mineral P	Analysis Ca-P ratio
				males	females				
			g.	g.	g.	%	%	%	
1	none	13	36	132	120	27.9	0.89	0.68	1.3
1c	2.64% charcoal*	13	37	133	111	28.8	0.85	0.66	1.3
2	0.38% cod liver oil†	29	36	168	140	32.2	0.91	0.69	1.3
2c	1.14% charcoal*+0.38% c.l.o.†	28	37	149	151	31.9	0.94	0.66	1.4
3	0.63% cod liver oil†	27	37	201	181	37.1	0.89	0.67	1.3
3c	1.89% charcoal*+0.63% c.l.o.†	26	37	206	181	36.6	0.91	0.65	1.4
4	0.88% cod liver oil†	26	36	217	201	42.6	0.91	0.65	1.4
4c	2.64% charcoal*+0.88% c.l.o.†	27	37	219	193	43.2	0.87	0.66	1.3
P	0.25% cod liver oil	13	37	244	227	45.5	0.82	0.67	1.2

* Substituted for an equivalent amount of corn meal in the ration.
† Previously bioassayed by A.O.A.C. method and shown to be rather poor as a source of vitamin D.

respectively at four weeks of age, whether the charcoal was eaten by the birds in the absence or presence of specific levels of a poor grade cod liver oil.

EFFECT OF CHARCOAL AND LENGTH OF STORAGE ON CALCIFYING PROPERTY OF COD LIVER, SARDINE, AND CONCENTRATED COD *LIVER OILS*

In these experiments of a practical nature in comparison to the exact method of bioassay described above, the birds were divided into 9 lots of approximately 20-25 birds each at the start (experiment 1), 6 months (experiment 2), and 12 months (experiment 3), and subsequently offered the different rations with distilled water for eight weeks. An ash determination of the left tibia was made, the alcohol extraction being somewhat longer and the ashing tem-

perature slightly higher than that used on four-week-old birds, while representative right tibiae were obtained from birds comprising the different lots at conclusion of the several experiments, and forwarded to Doctor F. D. Baird, nutrition laboratory of the National Oil Products Company, for silver nitrate staining and photographs of the prepared sections.

The basal ration (mineral content: Ca 1.95 percent, P 1.02 percent) was a mixture of 8 large batches of feed obtained commercially,* which were for the most part representative of the poultry growing mashes of commerce, with the sole exception they lacked the usual charcoal and cod liver oil additions. The following vitamin and charcoal supplements were added (September, 1934) in amounts indicated below:

Lot No.	*Supplement*
5	1.50% charcoal
6	0.50% poor quality cod liver oil*
6c	0.50% poor quality cod liver oil* + 1.50% charcoal
7	0.13% "concentrated" cod liver oil†
7c	0.13% "concentrated" cod liver oil† + 0.39% charcoal
8	0.50% sardine (pilchard) oil**
8c	0.50% sardine (pilchard) oil** + 1.50% charcoal
9	0.50% good quality cod liver oil††
9c	0.50% good quality cod liver oil†† + 1.50% charcoal

*Prepared from 4 oils previously bioassayed individually by A.O.A.C. method and found deficient in vitamin D when eaten by chicks at the 0.50% level.

†Prepared from 4 oils previously bioassayed individually by A.O.A.C. method and found to vary in vitamin D potency at the 0.13% level.

**Prepared from 4 oils previously bioassayed individually by A.O.A.C. method and found to vary in vitamin D potency at the 0.50% level.

††Prepared from 4 oils previously bioassayed individually by A.O.A.C. method and found adequate in vitamin D potency at the 0.50% level.

It will be manifest that each lot of chicks having access to the different vitamin supplements was balanced by a comparable lot fed a like supplement with three times its weight of charcoal, while lot 5 received charcoal but no vitamin D, and served as a negative control. Rations thoroughly triturated with charcoal, oil, or oil admixed on charcoal, were then stored in a dark unheated room similar to those of feed warehouses, in 100-pound cotton bags arranged separately in groups in order that each bag received an equivalent exposure to air and temperature.

Birds of the various lots were given the prepared diet with added charcoal, oil, or oil on charcoal, when it was newly mixed and in other experiments after storage of 6 or 12 months. Feed consumption records obtained by observing special precautions were recorded from the time chicks were started until the weekly weighing period 8 weeks later, and the birds then destroyed within 48 hours to obtain individual specimens for bone ash analysis. Results of several feeding trials are presented by Table 2.

The birds of lot 5 in each experimental period showed a lower ash content than that of those in the other lots, but no evidence of the severe rickets-like symptoms associated with vitamin D deprivation that are apparent when the A.O.A.C. ration is fed to chicks. Rate and extent of growth in this lot in experiment 1 were also below that attained by birds in the other lots and were somewhat greater than that in experiments 2 and 3 after the feed had been stored for 6 and 12 months respectively. With some individual exceptions the birds of the various lots exhibited better growth in experiments 1 and 2 whose rate of gain were somewhat analogous, than did those in experiment 3, after the feed had been stored 12 months. While rate of gain closely followed the amount of feed consumed, the

*Our thanks are extended to the following firms for kindly supplying the feed used in this experiment: Jameson Hevener Company, Minnesota Farm Bureau Service Company, Gopher Grain Company, all of St. Paul, and Pillsbury Flour Mills, Dickinson Feed and Seed Company, Washburn Crosby Company, Inc., Maney Bros. Mill and Elevator Company, Northrup King & Company, all of Minneapolis, and also to the latter for mixing the basal ration employed.

intake by birds, excepting the ones in lot 5 whose ration was unsupplemented with vitamin, was fairly large, but dropped off slightly in experiment 3 when this is compared with experiment 1 and 2. It is also evident, excepting lot 5, that the bone ash content for the various lots either in the presence or absence of charcoal was remarkably uniform, exhibiting no perceptible differences in either sex between any lots whether experiments 1, 2, or 3. With one exception (lot 6c, experiment 3) ash content was greater, and often significantly so statistically, for females than for males.

The incidence of perosis ("slipped tendon"), evident only rarely if at all in previous experiments, and mortality were quite low throughout the various feeding periods, and in none except possibly experiment 3 could the mortality be attributed solely to dietary origin. The birds of lot 5 frequently showed marked incidence of rickets (or osteoporosis) as revealed by their soft, swollen joints, inclination to squat, and post-mortem pathology. During experiment 3, these birds displayed some evidence of lacking vitamin D in the early portion of the feeding period, but this was not greatly marked in comparison with those of the same lot in experiment 1, when the feed was newly-mixed, or in experiment 2, after it had been stored six months. In all lots they had a noticeable tendency to defecate through the bars of the brooder cages into their drinking troughs, undoubtedly due to some deficiency existing in the diet, which could be corrected by the ingestion of excretory products resulting from the method of digestion of the feed, or from the physiological process of catabolism, although we have not conducted any experiments to determine the nature or action of this factor or factors. Post-mortem of birds in lot 5 were made at the conclusion of, and macroscopic appearance of birds in the other lots noted for vitamin A deficiency symptoms during, each feeding trial. These showed no discernible evidence of the pustule-like lesions in the mouth, pharynx or oesophagus, or on the surface of the heart, liver or spleen, no accumulation of urates in the kidneys and renal tubules, no symptoms of vitamin-A deficiency described by Elvehjem and Neu (1932), no cessation of growth as evident by the data in Table 2, and no observable development of xerophthalmia.

Samples of the various rations were obtained after one year's storage and cursory rancidity tests made, but they revealed no detectable decomposition of the different fish oils employed. Coincidentally at this time the feed, literally alive with insect life, was avidly consumed by the birds, although their body size was slightly inferior to that attained by chicks in experiments 1 and 2 on the same dietary regimen without the inclusion of insects. This extraneous living material (mostly of the species *tribolium conjusum duval*) was undoubtedly effective in altering the mineral content of the various rations as shown by the determinations made at that time, but did not apparently affect significantly the vitamin D activity of the different supplements eaten.

Photographs of silver nitrate stained sections of right tibiae near the average in ash content are shown by plate 1. An ash content of the opposite tibiae corresponding to the bones employed for these photographs was taken from the chick protocols, and these with the bird number, are as follows: Lot 5, those which received a supplement of 1.50 percent charcoal without vitamin addition: 3632—38.5 percent, 860—38.3 percent, 5629—39.3 percent; lot 6, 0.50 percent poor quality cod liver oil as a supplement: 3647—51.0 percent, 4039—50.4 percent, 5631—50.6 percent; lot 6c, 0.50 percent poor quality cod liver oil + 1.50 percent charcoal: 3674—49.4 percent, 4047—50.2 percent, 5679—48.8 percent; lot 7, 0.13 percent concentrated cod liver oil: 3661—49.9 percent, 4081—49.4 percent, 5699—48.5 percent; lot 7c, 0.13 per-

TABLE 2.—*Effect of charcoal and length of storage on calcifying property of cod liver, sardine, and concentrated cod liver oils.*

Lot No.	Supplement added to basal ration	No. birds	Av. Initial weight	Av. final weight		Av. feed consumed	Av. bone ash content		Mortality	Ration mineral analysis		
				males	females		males	females		Calcium	Phosphorus	Ca-P Ratio
			grams	grams	grams	kilograms	percent	percent		percent	percent	
	Experiment 1. Results when vitamin and charcoal supplements were newly mixed											
5	1.50% charcoal	21	38	407	291	1.07	36.1	37.4	none	2.03	1.01	2.1
6	0.50% poor quality cod liver oil	20	38	690	598	1.70	49.9	51.3**	none	2.00	1.01	2.0
6c	0.50% poor quality c.l.o.+1.50% charcoal	20	39	670	543	1.63	49.1	50.5**	none	1.98	1.01	2.0
7	0.13% concentrated cod liver oil	19	38	650	583	1.60	49.7	50.5	1	2.03	1.01	2.0
7c	0.13% conc. c.l.o.+0.39% charcoal	19	39	709	624	1.71	49.7	50.8**	none	1.99	1.00	2.0
8	0.50% sardine (pilchard) oil	15	37	710	634	1.78	50.0	50.4	1	1.97	1.01	2.0
8c	0.50% sardine oil+1.50% charcoal	15	37	707	594	1.77	49.9	51.4	none	1.96	1.01	1.9
9	0.50% good quality cod liver oil	14	36	781	602	1.86	49.9	51.4**	none	1.95	1.01	1.9
9c	0.50% good quality c.l.o.+1.50% charcoal	15	37	642	657	1.81	50.8	51.4	none	1.96	1.01	1.9
	Experiment 2. Results after feed was stored 6 months											
5	1.50% charcoal	27	39	321	268	0.96	37.6	38.6	1*	2.02	1.04	1.9
6	0.50% poor quality cod liver oil	23	38	701	594	1.63	50.2	51.2**	none	2.03	1.02	2.0
6c††	0.50% poor quality c.l.o.+1.50% charcoal	24	40	731	579	1.74	49.3	50.8**	1	2.00	1.00	2.0
7	0.13% concentrated cod liver oil	23	40	731	654	1.78	49.2	50.3	none	2.01	1.03	2.0
7c	0.13% conc. c.l.o.+0.39% charcoal	24	40	731	609	1.73	48.5	49.8	none	2.02	1.04	1.9
8	0.50% sardine oil	22	39	711	594	1.71	48.7	49.6	none	2.03	1.05	1.9
8c	0.50% sardine oil+1.50% charcoal	21	38	748	569	1.79	48.2	48.9	none	2.08	1.00	2.1
9	0.50% good quality cod liver oil	24	40	726	612	1.70	50.0	51.0**	none	2.02	1.05	1.9
9c	0.50% good quality c.l.o.+1.50% charcoal	22	38	805	602	1.87	50.0	50.6	none	2.07	1.01	2.0
	Experiment 3. Results after feed was stored 12 months											
5	1.50% charcoal	27	35	295	262	0.98	39.1	40.4	5†	2.19	1.14	1.9
6***	0.50% poor quality cod liver oil	25	37	675	574	1.70	47.4	50.0**	none	2.03	1.09	1.9
6c	0.50% poor quality c.l.o.+1.50% charcoal	25	36	614	544	1.70	48.8	47.8	1	2.15	1.12	1.9
7	0.13% concentrated cod liver oil	25	33	635	587	1.73	48.8	50.9**	none	2.00	1.10	1.8
7c	0.13% conc. c.l.o.+0.39% charcoal	25	32	659	568	1.77	49.2	51.3**	none	2.04	1.09	1.9
8	0.50% sardine oil	24	37	597	540	1.65	49.0	50.6	1*	2.09	1.04	2.0
8c	0.50% sardine oil+1.50% charcoal	25	36	548	526	1.49	48.3	49.6	2*	2.01	1.03	2.0
9	0.50% good quality cod liver oil	24	35	667	565	1.79	50.6	51.5**	1	2.13	1.09	2.0
9c	0.50% good quality c.l.o.+1.50% charcoal	24	39	700	582	1.77	50.2	51.8**	none	2.11	1.09	1.9

* Owing to deficient supply of vitamin D.
** Significantly higher statistically than average ash content of males.
*** One bird showed incidence of perosis, calcification being slightly below normal.
† Four deaths owing to deficient supply of vitamin D.
†† One bird showed incidence of perosis, calcification being normal.

cent concentrated cod liver oil + 0.39 percent charcoal: 3709—50.2 percent, 4100—50.1 percent, 5714—50.7 percent; lot 8, 0.50 percent sardine (pilchard) oil: 3715—49.7 percent, 4219—48.9 percent, 5739—50.1 percent; lot 8c, 0.50 percent sardine oil + 1.50 percent charcoal: 3740—51.1 percent, 4250—48.7 percent, 5772—47.0 percent; lot 9, 0.50 percent good somewhat deficient in calcification, but even these comparative ones displayed no marked differences at all for the three experiments.

DISCUSSION OF RESULTS

It is clear from inspection of Table 1 that charcoal when newly mixed with cod liver oil exerted no demonstrable detrimental effect whatsoever on the vitamin D

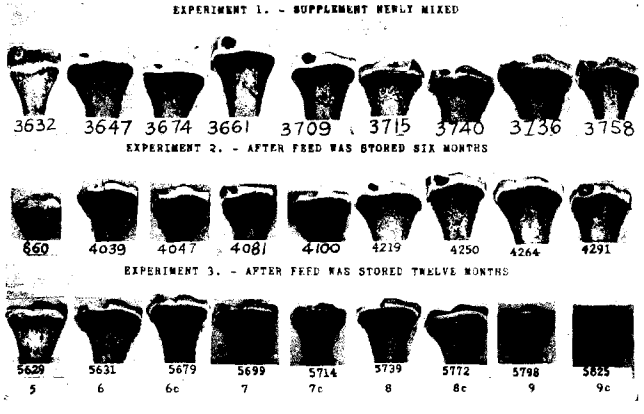

PLATE 1. Photographs of $AgNO_3$ stained sections of chick tibiae arranged by lot number (shown from top to bottom for each experiment).

quality cod liver oil: 3736—49.6 percent, 4264—50.2 percent, 5798—51.4 percent; lot 9c, 0.50 percent good quality cod liver oil + 1.50 percent charcoal: 3758—51.8 percent, 4291—50.5 percent, 5825—52.0 percent. These results should be compared with the average of ash content for each lot in the different experiments as shown by Table 2. From comparison of the individual photographs of plate 1 it will be evident that very small differences in internal calcification of the bony trabeculae are apparent for the different feeding periods, with exception of the photographs of chick bones in lot 5 which received no vitamin D and were activity of the oil, when A.O.A.C. method of feeding and the criteria of bone ash content and body size at four weeks of age, were used to determine its utilization by the chick for calcification and growth. It is apparent from data in Table 2 and the supplementary silver nitrate stained sections of bones shown by plate 1, that charcoal also has no demonstrable effect on the vitamin D activity of poor or good quality cod liver, sardine, or concentrated cod liver oils, either when newly-mixed or after six or twelve months of storage with admixed oil in a commercial growing mash, as observed by the criteria of bone ash content

and body weight of chicks at eight weeks of age.

During the growing period (eight weeks in duration) the level of vitamin eaten in the form of the four different kinds of fish oils had little influence on total feed utilization, that is, feed required to induce unit gains in weight. Since the birds were not in individual compartments the effect of sex on utilization of feed was impossible to measure, the average intake per chick being computed from the amount consumed by each lot. That cessation of growth of the vitamin-D deficient animals in lot 5 cannot be attributed primarily to failure of appetite, unpalatability or monotonousness of the ration, is evident by consideration of the feed consumption records.

There is some evidence that a cod liver oil found to be a poor source of vitamin D by A.O.A.C. method of bioassay may give apparently good results when given as a supplement to a commercial feeding mash. It is clear that the condition produced is only relative in its nature, and that an exact procedure for bioassay under controlled laboratory methods is necessary to ascertain with any degree of accuracy the vitamin content of a particular product.

CONCLUSIONS

1. When charcoal in conjunction with a "poor quality" cod liver oil was eaten by White Leghorn chicks according to A.O.A.C. method of bioassay, the charcoal exerted no appreciable effect either on the calcifying or growth-promoting property of the oil.

2. When charcoal in conjunction with "poor" or "good quality" cod liver, sardine (pilchard), or concentrated cod liver oils, was fed to chicks for eight weeks in an all-mash chick growing ration which produced subnormal calcification and growth when supplemented with charcoal but no vitamin, neither the oils nor the charcoal showed any demonstrable effect on the calcifying or growth-promoting property of the ration, either when they were newly-mixed with it or after six or twelve months of storage, as observed by the criteria of normal bone ash content and satisfactory body size attained at eight weeks of age.

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The Comparative Growth Rates of Turkeys, Ducks, Geese and Pheasants*

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EXTENSIVE studies have been made of the growth rates of various species of animals, and several attempts have been made to deduce laws of growth from these data. Before general laws of growth can be established it is essential that a large body of data should be collected and carefully classified and analyzed. One of the objectives of any growth study, therefore, is to add to this body of data.

Knowledge of the rates of growth of different species of animals is of value for at least two reasons. (1) Species may differ in the efficiency of transformation of food into flesh. (2) The efficiencies of transformation may vary at different ages within species.

It is generally accepted that environmental conditions affect rates of growth even though environmental influences may be overcome at "maturity." Therefore it would be highly desirable that environmental influences be as nearly identical as possible for the animals whose growth rates are to be compared. This has not been possible in some of the comparative studies which have been made in the past for the reason that the data were accumulated at various times and from a number of widely different geographical locations. There are other difficulties which prevent the attainment of identical or ideal environmental conditions for all the species of animals which may be under consideration. A few of such difficulties are:

1. Differences in food habits.
2. Differences in the season of the year in which birth or hatching normally occurs.
3. Environmental conditions which may be normal or ideal for one species may not be normal for another.

In the study reported herewith an attempt was made to rear a few of the domesticated avian species, viz.: turkeys, ducks, and geese in the same environment. A few Ring-necked pheasants were also reared for comparative purposes at a different time.

Though there has been little work on the growth of other species of domestic fowl it is generally known that ducks attain a relatively large size early in life, being commonly sold as "green" ducks at an age of 10 to 12 weeks and a weight of five to six pounds, while chickens of a comparable age weigh only two to two and one-half pounds.

There are two questions which these data partially help to answer. The first is whether or not all species of birds grow in a similar manner, and if not, in what respects their growth rates differ? The second is, at what age should the birds be sold to realize the most economical meat production?

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PARTIAL REVIEW OF LITERATURE

There is a rather extensive body of literature dealing with the growth of the chicken under various experimental conditions. Mitchell, Card, and Hamilton (2,3) have made a careful technical study of the growth of Single Comb White Leghorn and White Plymouth Rock chickens. They fitted fourth degree polynomial equations to their data by the method of least squares, admitting that such an equation was purely arbitrary as far as biological significance was concerned. They contend that equations based on certain assumptions concerning the laws of growth are not satisfactory in describing the growth from its beginning to its completion, and that to apply these equations to certain segments of the growth curve is, in the light of our present knowledge, to a certain extent empirical.

Brody (1), while admitting that there are some inconsistencies yet to be explained, contends that his exponential equations fit the data satisfactorily. He states that the curve of growth may be divided into two principal segments: first a segment of increasing slope in which the velocity of growth tends to be proportional to the growth already made; second, a segment of decreasing slope during which the velocity of growth tends to be proportional to the growth yet to be made to reach maturity. In mammals the junction between these two segments occurs at puberty. Brody has not applied his equations to any species of domestic fowl except chickens.

Henderson and Penquite (4) have compared the embryonic growth of chickens, turkeys, ducks, and geese. They found that chicken and duck embryos grew at about the same rate, the ducks being heavier at hatching time because they grew longer. Goose embryos grew somewhat faster than those of ducks and chickens.

The authors are not aware of any published data on the growth of pheasants beyond the age of ten or twelve weeks.

EXPERIMENTAL

Object

The object of this experiment was to compare the growth rates of various species of domestic fowl under as nearly similar environmental conditions as possible. It was also intended to compare the efficiency of gains of the various species from the standpoint of feed consumption.

Procedure

In 1931 three lots of White Pekin ducks, one lot of Toulouse geese, and one lot of Bronze turkeys were grown. The birds were weighed at weekly intervals to the fifteenth week, and at bi-weekly intervals thereafter. The geese and turkeys were weighed until they reached the age of 31 weeks, at which time the turkeys were sold and the experiment discontinued. Two lots of ducks were sold as "green" ducks at the age of twelve weeks, and one lot was continued until the birds were 27 weeks of age. Feed consumption was obtained for the ducks and geese. The sex of the ducks and geese was not determined. Lot 3 of the ducks was hatched the same week as the turkeys and geese, but since there was no significant difference between the three lots of ducks with respect to growth the data from all three lots were combined.

In 1934 one lot of White Holland turkeys was weighed at bi-weekly intervals to 36 weeks of age, and a small lot of Ring-necked pheasants was weighed at bi-weekly intervals to the age of 32 weeks.

The ration fed to the ducks, geese and turkeys in 1931 consisted of the following mixture:*

50 lbs. ground yellow corn
15 lbs. wheat bran
16 lbs. wheat middlings

* The ration was fed dry to the turkeys, and mixed with water to the consistency of a thick

15 lbs. dry skimmilk
3 lbs. steamed bone meal
1 lb. salt

Water and gravel were available ad libitum.

The turkeys and pheasants reared in 1934 were fed the following feed:

45 lbs. ground yellow corn
16 lbs. wheat middlings
15 lbs. wheat bran
5 lbs. meat and bone meal
15 lbs. dried milk
3 lbs. ground oyster shell
1 lb. salt

Analysis of Data

The exponential equations given by Brody (1) were fitted to the data by the graphical method, as the amount of data was deemed insufficient to warrant more exact methods of fitting the curves.

RESULTS AND DISCUSSIONS

I. The growth of the various species plotted on coördinate paper.

The growth curves of the various species are shown in Figure 1. Though all the curves are of the sigmoid type characteristic of growth in general, there are some striking differences. The increase in weight of the ducks and geese is very rapid early in life and falls off quite rapidly when the birds are eight to ten weeks of age. At about the twenty-first week the geese again begin to make large gains in weight, while the ducks do not. The latter period of rapid gains of the geese probably represents fattening.

On the other hand the turkeys and chickens increase in weight more slowly during the first few weeks, but their growth appears to be sustained for a longer period. The growth curves of the turkeys, chickens, and pheasants are quite similar in general shape, though the pheasants reach their mature weight much more quickly than the other species. It is interesting to note that for a time the pheasant males actually exceed the White Plymouth Rock females in weight. As has been pointed out previously, the White Plymouth Rocks were from a rather small strain.

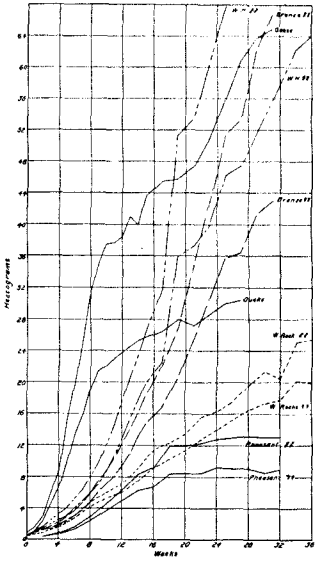

FIG. 1. The growth curves of Toulouse geese, White Pekin ducks, Bronze and White Holland turkeys, Ring-necked pheasants, and White Plymouth Rock chickens, plotted on coördinate paper.

Waters (5) found that Single Comb White Leghorn and Light Brahma chickens both reach physical maturity at about 40 weeks of age. Unfortunately, none of the birds whose weights were recorded in this experiment, except the pheasants, were weighed long enough to determine the age at which physical maturity was reached. The chickens were still gaining slightly at

36 weeks and would probably have reached physical maturity at about 40 weeks. The pheasant were apparently physically mature at about 24-26 weeks. The turkeys, ducks and geese were still gaining when they were last weighed, though the ducks were gaining quite slowly.

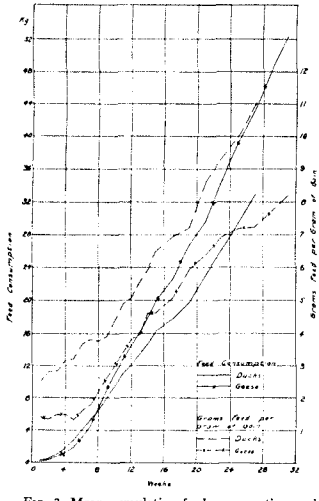

FIG. 2. Mean cumulative feed consumption and the grams of feed required to produce a gram of gain in Toulouse geese and White Pekin ducks.

II. Feed consumption and economy of gain of ducks and geese.

Feed consumption for the ducks and geese was recorded at weekly intervals until the birds were 15 weeks of age and at bi-weekly intervals thereafter. The mean cumulative feed consumption and the grams of feed required to produce a gram of gain are shown in Figure 2.

Two facts are brought out in this study. First, the geese are considerably more efficient than the ducks in economy of gains, especially during the first six to eight weeks. Second, the ducks make economical gains only during the first nine weeks of their life, after which the grams of feed required to produce a gram of gain increase quite rapidly. On the other hand, the geese are quite efficient in converting feed into meat for 12 to 13 weeks. The efficiency of the geese remains relatively constant at about one and one-fourth to one and three-fourths grams of feed to produce a gram of gain for the first seven weeks, after which time the grams of feed required to produce a gram of gain increases at a fairly regular rate.

The ducks and geese were running on a grass range, and there is no way to estimate how much green feed they ate. The remarkable economy of gain of the geese during the first seven weeks may be due to their having obtained considerable nutrients from green feed. The feed consumption figures for the ducks and geese are not strictly comparable with each other nor with the average feed consumption of chickens. They are offered as an indication of the amount of feed other than green feed which ducks and geese may be expected to consume under range conditions.

III. The growth of the various species plotted on a semi-logarithmic graph.

The equations fitted to the data in this study are those discussed and recommended by Brody. The first is Brody's equation 4, $W = A^{kt}$, which is only applicable to the phase of growth preceding the major inflection, according to Brody. The second is equation 14, $W = A - Be^{-kt}$, which fits the data following the major inflection. By eliminating the constant, B, equation 14 may be changed to equation 16,

$$\frac{W}{A} = 1 - e^{-k(t-t_0)}$$

The latter equation is useful in determining the "equivalence of age" of various ani-

mals. In this study these three equations will be designated by the same numbers that Brody used.

TURKEYS

In Figure 3 the growth of Bronze and White Holland turkeys, plotted on semi-logarithmic paper, is shown. The value of k, as shown in Figure 3 and succeeding figures, is the same constant used by Brody in describing the growth of various animals, except that in this study one week has been used as the unit value of time. Brody used one day as the unit of time in describing the growth of the rat.

In the White Hollands the growth rate appears to be constant between the ages of two and seven weeks (six and eleven weeks from the beginning of incubation). The value of k for this period is 0.28 for the males and 0.26 for the females. On the other hand the Bronze poults grew at a constant rate for the first three weeks after hatching, at which time there is a definite change in growth rate in the curve. The White Hollands were not weighed between the ages of two and five weeks, and if the weights between these ages were available it is possible that the differences between the two breeds at this stage of growth might disappear.

The White Hollands grew at the rate of 19 percent per week from the seventh to the fifteenth week, at which time there was a very marked decline in the growth rate. From 19 weeks to 32 weeks, at which time the males were sold, the value for k was 0.04. During this period the females grew slightly more rapidly, k having the value of 0.045. The males grew more rapidly than the females only during the first seven weeks, but the advantage they obtained during this period was sufficient to account for the difference in mature weight of the sexes.

There appear to be four periods of growth from hatching time to the end of the experiment in the Bronze, with a slight change in rate at three weeks, a very definite decline in the rate of growth at eleven weeks and another at 24 weeks. The males grew

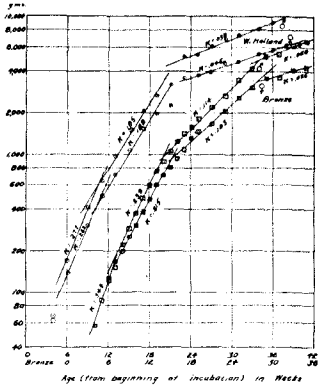

FIG. 3. The course of growth in Bronze and White Holland turkeys.

at a slightly greater rate during each of the first three periods, but the difference in rate was marked only during the period from three to eleven weeks.

In Figure 4 is shown the course of growth in Bronze and White Holland turkeys with respect to the growth yet to be made to reach the mature weight. The constants of Brody's equation 14 ($W = A—Be^{-kt}$) have been evaluated from the data and are shown in the figure. This equation, according to Brody, only fits the data from the inflection of the growth curve to maturity. Therefore, the point of inflection of the curve may be determined by applying this equation to the data and noting the age when it begins to fit the data satisfactorily.

Two very interesting things may be noted from Figure 4. First, though the

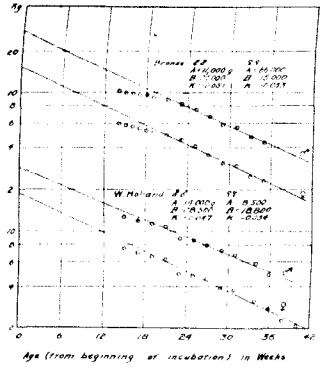

FIG. 4. The course of growth in Bronze and White Holland turkeys with respect to the weight yet to be attained to reach mature weight.

breeds and sexes differ widely in mature weight the value of k is very similar for all. This seems to indicate that the decline in rate of growth with respect to the weight yet to be attained is practically constant regardless of sex or breed. Second, the equation fits the data on the Bronze turkeys from the twenty-first week (seventeenth week from hatching) to the end of the experiment very well. While the data on the White Hollands are more erratic, they appear to fit very well from the nineteenth week to the end of the experiment. This might indicate that the White Hollands were slightly earlier maturing, but since the number of individuals are smaller, the data on the White Hollands are not as dependable as those on the Bronze.

Comparing Figure 4 with Figure 3, it will be noted that in Figure 3 there apparently is a rather definite change in the rate of growth of the Bronze at about the twenty-seventh week, while there is a pronounced decline between the nineteenth and twenty-third week in the case of the White Hollands, with the weights for the twenty-first week falling far below either the preceding or the succeeding curve. These changes in rate are not at all apparent when equation 14 is fitted to the data. Apparently equation 14 fits the data more closely than equation 4 during the latter part of the growth curve of turkeys. This is in agreement with Brody's findings for other animals.

PHEASANTS

The growth of a small group of Ring-necked pheasants is shown in Figure 5. Apparently pheasants grow quite rapidly for about nine weeks, their rate of growth during this period being quite comparable to that of turkeys. However, their growth rate declines quite rapidly, and they nearly reach their adult weight by the time they are 18 to 21 weeks of age. The maximum average weight recorded for the males was

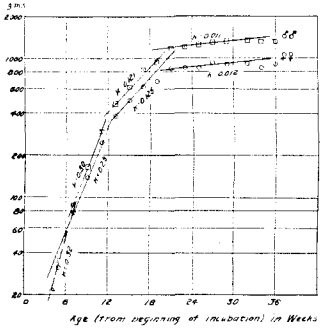

FIG. 5. The course of growth in Ring-necked pheasants.

at the age of 32 weeks from the beginning of incubation, and for the females the maximum was at 27 weeks.

These birds were not weighed until they were two weeks of age. At a later date a lot of 19 pheasant chicks were weighed at

hatching time, and the mean weight was found to be 21.9 grams. This point is marked with a triangle on Figure 5, and the dotted line represents the rate of growth for the first four weeks, assuming that this hatching weight is approximately the same as that of the birds used for the growth study.

In Figure 6 is shown the growth of pheasant chicks with respect to the growth yet to be made to attain mature weight. The points are rather erratic, probably because the numbers are quite small. Only six males and ten females remained alive at the end of the sixth week, so the data on the birds that died were discarded.

These data fit a straight line fairly well for the fifteenth week (twelfth week from hatching) to the end of the experiment. The value of k was found to be —0.17. It will be noted that the rate of increase in weight with respect to the weight yet to be attained

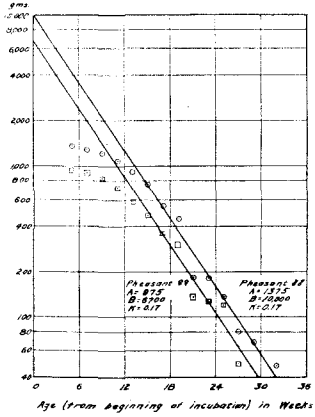

FIG. 6. The course of growth in Ring-necked pheasants with respect to the weight yet to be attained to reach mature weight.

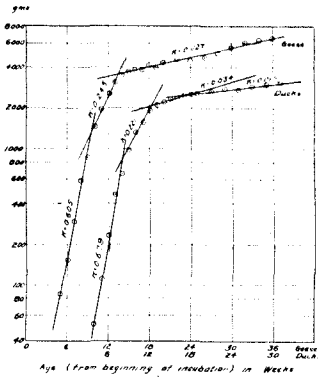

FIG. 7. The course of growth in Toulouse geese and White Pekin ducks.

declines much more rapidly in the case of the pheasants than it does in the case of the turkeys, the value of k for the latter being approximately —0.05.

DUCKS AND GEESE

In Figure 7 is shown the growth curves for White Pekin ducks and Toulouse geese. Both ducks and geese make a very rapid gain during the first four weeks following hatching, the ducks growing slightly more rapidly than the geese during this period. The value of k for this period is 0.60 for the geese and 0.68 for the ducks, compared to 0.35 for Bronze turkeys grown at about the same time, and 0.32 for pheasants.

The first decline in the rate of growth occurs between the fourth and fifth week following hatching for both the ducks and geese. The value of k drops from 0.60 to 0.24 in case of the geese and from 0.68 to 0.22 in case of the ducks. This second period of growth lasts from the fifth week to the eighth week, inclusive, at which time there

is another sudden and decided decline in the growth rate.

The growth of the geese apparently fits a straight line fairly well from the eighth week to the end of the experiment, while

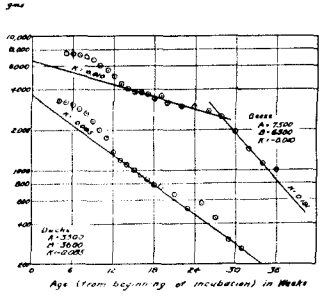

FIG. 8. The course of growth in White Pekin ducks and Toulouse geese with respect to the weight yet to be attained to reach maturity.

the ducks grew at a constant rate from the eighth to about the thirteenth or fourteenth week, following which there was another decline in the growth rate of the latter.

As contrasted with turkeys, the rate growth of ducks and geese is initially very rapid, but quickly declines to a very low figure. It is apparent that the growth curves of ducks and geese are very similar, and this is brought out even more strikingly in Figure 7 than it is in Figure 1.

The course of growth in ducks and geese with respect to the growth yet to be made to reach mature weight is shown in Figure 8, together with the constants required to fit equation 14 to the data. The data fit the equation nicely in the case of the ducks from the twelfth week (eighth week from hatching) to the end of the experiment. The point of inflection on the growth curve appears to be at the twelfth week, while in the case of the geese it is about the thirteenth or fourteenth week (eighth or ninth week after hatching).

It is obvious that one equation will not fit the data in the case of the geese. From the fourteenth to the twenty-eighth week there is a satisfactory fit, and then a very decided change in rate occurs. It is known that geese fatten readily, and the latter period probably represents fattening and not growth. Brody has stated that equation 14 does not apply to fattening animals.

The phenomenal early growth of ducks and geese and the fact that they reach the point of inflection on the growth curve very early in life is quite interesting. In the wild state these species are migratory, usually nesting in the far north and migrating southward in the fall. Their growing season is short, and apparently it is necessary that they complete a large share of their growth very quickly, compared with non-migratory species such as the turkey or the chicken. It would be interesting to ascertain whether or not other migratory species behave in a similar manner.

CHICKENS

Data on the growth of White Plymouth Rock chickens are presented in Figures 9 and 10, for comparison with the other species studied. These data are taken from Mitchell, Card, and Hamilton (2). Most growth studies of chickens have been made on the Single Comb White Leghorn, and the above data represent one of the few growth studies made on a general purpose breed through the major portion of the period of growth. Mitchell and co-workers admit that the birds on which they kept data were from a rather small strain, so the growth obtained probably is not entirely representative of the capability for growth of the general purpose breeds. However, the number of individuals is quite large and the curve should be indicative of the general character of the growth curve of a breed of

chickens which is more commonly maintained for meat production than as an egg laying breed.

The data fit equation 4 quite well, the ages at which the rate of growth changes being six to eight weeks and sixteen weeks after hatching, there being another change in rate in the curve of the females at twenty-six weeks. The general form of the curve and magnitude of the percentage growth rates are quite similar to those obtained in the case of the turkeys, except that the early periods of rapid growth are more prolonged in the latter. The first two periods of growth are almost identical with those of pheasants, and the values of k are very similar. In fact, at 17 weeks (14 weeks from hatching) the pheasants were nearly as large as the chickens, and the only apparent reason that the latter surpass the former in adult weight by such a wide margin is that they maintain a fair rate of gain after this age while the pheasants grow much less rapidly and soon reach their mature weight.

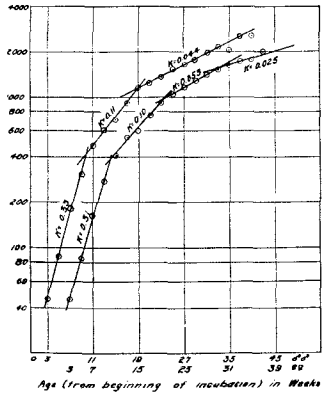

FIG. 9. The course of growth curve in White Plymouth Rock chicks.

The data fit equation 14 quite well beginning at the fifteenth week in the case of the females, but there is a change in the rate in the curve of the males at 29 weeks which cannot be explained easily. The inflection in the curve appears to be about the fifteenth week (twelfth week after hatching). This is intermediate between the age found for turkeys and that found for ducks and geese, and is the same as the age of pheasants at the inflection.

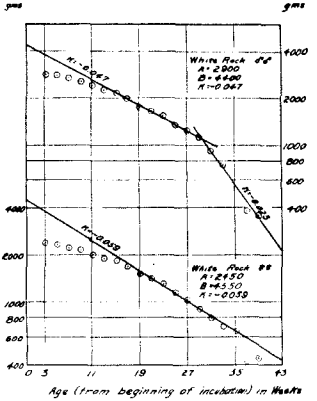

FIG. 10. The course of growth in White Plymouth Rock chickens with respect to the weight attained to reach maturity.

THE RELATION OF SEXUAL MATURITY TO THE AGE AT INFLECTION OF THE GROWTH CURVE

Brody has stated that the major inflection in the growth curve of animals occurs at puberty. Apparently this is not true of the different species of birds considered in this study, if we may assume that the beginning of egg production corresponds to puberty. The age at sexual maturity of

the White Plymouth Rocks whose growth is discussed above was not stated, but it is assumed that it can not have been as early as 12 weeks. Neither does the age at sexual maturity of pheasants, turkeys, ducks, and geese correspond to the age at the major inflection of the growth curve, for these species usually do not begin to lay until the late winter or spring following the year in which they are hatched. Strains of chicks that are early maturing sexually begin to lay some time before they reach their maximum adult weight, but many birds certainly do not begin to lay until after they are physically mature.

SUMMARY

Comparative studies have been made of the growth rates of turkeys, ducks, and geese hatched at approximately the same time and reared on the same ration. The growth rates of the above birds have also been compared with those of another group of turkeys and a small group of Ring-necked pheasants grown at a later date and the growth rates of a large group of White Plymouth Rock chickens whose growth was reported by Mitchell, Card, and Hamilton (2).

The growth rates of chickens, turkeys and pheasants during the first few weeks following hatching were of practically the same magnitude, the value of k ranging from 0.26 to 0.34. The rates of growth of these species were also quite similar during the other period of constant growth rate, the only marked differences between the species being that each growth period was somewhat more prolonged for the turkeys than for the chickens and pheasants. The growth rates of the latter were almost identical with those of chickens to the age of 19 weeks from the beginning of incubation, after which the pheasants grew very little, while the chickens continued to grow for some time.

The growth rates of the ducks and geese were very similar, and were strikingly different from those of the other species studied. The rate of growth during the first few weeks following hatching was practically double that of the turkeys, chickens, and pheasants, while the decline in rate of growth was much more pronounced and occurred at a much earlier age.

It is interesting to note that the form of the growth curve was practically the same for two migratory species (ducks and geese) and markedly different from the form of the growth curve of a non-migratory species (turkeys) grown at the same time and on a similar ration. Very rapid early growth and quick attainment of the greater portion of the mature weight may be characteristic of migratory species of birds, while non-migratory species grow at a more moderate rate for a longer period of time. This is a tentative hypothesis which should be verified by obtaining growth rates for other species of birds, both migratory and non-migratory.

The amount of feed consumed by the ducks and geese in comparison to the gains produced was not radically different from the amount normally consumed by chickens in comparison to their gains over a comparable period of time. The only exception to this statement occurred during the first few weeks of the life of the geese, during which time they apparently exhibited a remarkable economy of gain. This high efficiency of gain was probably due to the fact that the geese consumed much green feed during this period, but it was not possible to determine how much green feed they ate. Ducks and geese grew very rapidly during the first eight to ten weeks of their life, but consumed proportionately large amounts of feed.

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The Anti-hemorrhagic Vitamin

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A. PRELIMINARY INDICATIONS OF EXISTENCE

INDICATIONS of the existence of a dietary disease in which chickens suffer from hemorrhagic tendencies and delayed blood clotting may be seen in a number of papers describing results obtained in experiments dealing with various phases of poultry nutrition.

During work on the sterol metabolism of chicks, Dam (1929, 1930) observed that the animals often exhibited internal, subcutaneous, and intra-muscular hemorrhages. These symptoms were accompanied by ulcerative changes in the horny stratum of the gizzard. In a subsequent paper (1934), he showed that the disease was not caused by lack of vitamins A, D, B_1, B_2, C and of "fat" or cholesterol. The suggestion was made that, since a diet containing certain common feedstuffs did not produce the disease, a new dietary factor must be involved.

McFarlane, Graham, and Richardson (1931) in work on the fat-soluble vitamin requirements of the chick observed hemorrhages and loss of blood clotting power when ether-extracted fish meal or ether-extracted meat powder was used. The unextracted supplements did not allow the development of hemorrhages. Similar results were mentioned by McFarlane, Graham and Hall (1931).

A syndrome consisting of bleeding from pin feathers, hemorrhages in muscles, under the skin and in the abdomen together with dark erosion spots on the gizzard lining and low hemoglobin blood levels in hemorrhagic individuals was reported by Holst and Halbrook (1933). The disease was cured by feeding fresh cabbage. The opinion was advanced that growing chicks may suffer from this scurvy-like disease because of an absence of vitamin C in the diet or that under certain conditions they may be unable to synthesize this vitamin in sufficient amounts.

Dam and Schönheyder (1934) readily produced the hemorrhagic syndrome in chicks by the use of a diet containing vitamin A—free casein, dried yeast, sucrose, salts, and cod liver oil. Hemorrhagic symptoms were noted as early as 11 days when newly-hatched chicks were fed only this diet. Lesions and ulcerations of the gizzard lining were again observed. Ascorbic acid (vitamin C) given by mouth or subcutaneously did not affect the disease. Similar results in regard to the negative effect of vitamin C have been found by Halbrook (1935) who also noted that the hemorrhages were prevented by dehydrated alfalfa or an ether extract of alfalfa.

Although the above reports demonstrated the existence of a new dietary disease of chicks, they did not provide the evidence which would permit recognition of a new member in the group of vitamins.

B. PROOF OF EXISTENCE AS A NEW DIETARY FACTOR

As in the case of other dietary requisites, proof of existence of a vitamin as a separate entity depends upon its isolation of such or upon purification to such an extent that other or complicating factors can be assumed to be absent or shown to be ineffec-

tive in the case of the deficiency in question.

Signal progress in the isolation of a substance possessing marked anti-hemorrhagic activity has been recently achieved. Dam (1935a, b) reported that tbe factor was found present in the unsaponifiable, non-sterol fraction of hog liver fat. It was stable to moderate heating. Dam suggested for this factor the designation vitamin K.

Almquist and Stokstad (1935a, b) showed that the anti-hemorrhagic factor was localized in the non-saponifiable fraction of alfalfa lipids. It was stable to heating in air at 120°C. for 24 hours. Chlorophyll and sterol fractions from alfalfa were impotent. Neither carotene nor xanthophyll were effective as anti-hemorrhagic agents. The active factor possessed no appreciable basicity.

Almquist (1936a) concentrated the anti-hemorrhagic factor by extraction of dried alfalfa with hexane, preliminary adsorption with activated magnesium oxide and carbon to remove the green and a portion of the red and yellow pigments, and separation of solid inert material by concentration and cooling both in hexane and in methyl alcohol. Addition of water to the final solution of the factor in methyl alcohol caused the separation of a reddish oil very rich in the anti-hemorrhagic vitamin. This oil was adequate at a level of 2 milligrams per kilogram of diet by the preventive method of assay. The concentrate contained a small proportion of sterols which were removed by digitonin without affecting the potency. It also contained a negligible quantity of saponifiable material. Saponification procedures were abandoned because it was found that the factor was alkali-labile. Residual carotenoids were removed by treatment with activated magnesium oxide. The material not adsorbed had the appearance of a light yellow, viscous oil when free from the solvent and prevented hemorrhagic symptoms when fed at a level of 2 milligrams per kilogram of diet. By careful addition of bromine, the reddish oil could be bleached without great destruction of the factor.

Dam and Schönheyder (1936) concentrated the anti-hemorrhagic factor by extracting dried alfalfa with acetone, taking up in petroleum ether, partitioning with 90 percent methyl alcohol (during which the factor remains for the most part in the petroleum ether), transferring the concentrate to absolute alcohol and removing inert solids by cooling and filtering. Adsorption reagents (calcium carbonate and sugar) were found effective in further concentration. The most active concentrate had the appearance of a viscous oil. Since these workers used entirely the curative technique in their assays, it is difficult to compare the potencies of the products obtained by the two methods but the fact remains that very small quantities of the concentrates are required. These workers also abandoned saponification because of destruction of the anti-hemorrhagic factor.

Almquist (1936b) by distillation of his concentrate under a high vacuum (molecular distillation) increased its potency approximately four fold. A first distillate fraction consisting of a colorless oil obtained at 50 to 70°C. and a pressure of 10^{-6}mm. of mercury proved to be inactive. A second distillate obtained at a temperature range of 120 to 145°C. was adequate at a level of ½ mg. per kilogram of diet. A non-volatile residue fraction containing most of the pigments gave no evidence of activity. The active distillate still had the appearance of a yellow viscous oil.

Almquist and Stokstad (1935a, b) reported that the vitamin was produced in ether-extracted fish meal which was subsequently moistened and allowed to putrefy slightly. Similar results were obtained with rice bran. When the putrefied fish meal was dried and re-extracted with ether an active

extract was obtained. Almquist and Stokstad (1936a) found that the factor was present in the droppings of chicks which had received none of the factor in their diets. Such results suggest that the vitamin may be readily synthesized by action of microorganisms. It was also found that the vitamin can be transferred from the diet of the hen to the chick. The anti-hemorrhagic vitamin was present in egg yolk but not to any appreciable extent in egg albumen or the livers of young chicks given normal diets containing 5 percent of dried alfalfa. Dam and Schönheyder (1936) reported similar results in regard to normal young chicken liver.

That the anti-hemorrhagic vitamin is not identical with vitamin E was concluded by Dam (1935a, b) who obtained very little evidence of anti-hemorrhagic activity in wheat germ oil. Almquist and Stokstad (unpublished data, 1935) found that a sample of wheat germ oil known to be highly potent in vitamin E was without effect on the hemorrhagic disease at a 5 percent level.

Recently Goettsch and Pappenheimer (1936) discovered that their factor which prevents nutritional encephalomalacia in young chicks is contained in the non-saponifiable fraction of vegetable oils. Alfalfa leaf meal at a 10 percent level was powerless to prevent this form of paralysis and brain lesion. This fact clearly shows that the anti-encephalomalacic factor is not the anti-hemorrhagic vitamin. Moreover, there appears to be no relation between the two diseases in respect to symptoms or pathology.

The work reviewed in the above section seems sufficient to establish beyond question the existence of a new vitamin. For those who prefer alphabetical designations for vitamins, the early suggestion of Dam that the anti-hemmorrhagic vitamin be named K (koagulations vitamin) is worthy of adoption.

The syndrome by which its absence in the diet is recognized has recently been simplified by the elimination of gizzard lesions. Almquist and Stokstad (1936b, c) have shown that the gizzard disorder is a separate deficiency disease which is caused by the lack of a fat-soluble factor clearly distinguishable from vitamins A, D, E, F, K and the anti-encephalomalacic factor.

C. FUNCTIONAL PROPERTIES

Both Holst and Halbrook (1933) and Dam and Schönheyder (1934) have referred to the hemorrhagic syndrome as resembling scurvy. As pointed out by Almquist and Stokstad (1935) the primary feature of the disease is the prolonged blood clotting time. Other portions of the syndrome, that is, hemorrhages and anemia are probably entirely secondary.

Schönheyder (1935, 1936) has made a particular study of blood composition in this disease. In deficient blood there were no marked abnormalities in regard to pH, fibrinogen, calcium, thrombokinase or antiprothrombin. Normal and deficient plasma showed enormous differences in response to the same clotting agent (embryonic tissue or lung tissue juice). In normal plasma a component was present which accelerated the clotting of plasma from sick animals. Normal clotting power was rapidly restored when a source of the anti-hemorrhagic vitamin was fed to deficient birds, however, the vitamin itself possessed no activity as a clotting agent.

Dam, Schönheyder and Tage-Hansen (1936) discovered that the prothrombin precipitate from normal chicken plasma contained the vitamin and was active as a clotting agent when added to deficient plasma. Washing the prothrombin fraction with fat solvents did not affect its clotting power or remove the vitamin indicating that the vitamin was held in rather firm combination in the prothrombin fraction. A cor-

responding precipitate from the plasma of deficient chicks was inactive. It may be noted that the resemblance of this dietary blood disease to hemophilia is very striking.

Hemorrhages are the most marked external symptoms. In the writer's experience these may be found on practically any portion of the chick. Somewhat more frequently they develop in locations exposed to injury or contact with the cages so that to some extent they may be initiated by traumatic stimuli. There is much indication, however, that hemorrhages may be completely spontaneous particularly when they are internal or located on protected body surfaces such as on the inside of the leg. In the examination of several hundreds of chicks given the basal diet plus an adequate level of purified anti-hemorrhagic vitamin concentrate the writer has never observed the slightest evidence of hemorrhage, or even bruise, although such chicks were maintained under exactly the same conditions as others with a high incidence of hemorrhage. Except in the case of animals with hemorrhages, an anemic condition has not been distinctly or consistently produced by an anti-hemorrhagic avitaminosis.

Leg weakness, poor growth and encephalomalacia were encountered by Dam and Schönheyder (1934) and Schönheyder (1935) on some of their diets. On the other hand, the diets used by Almquist and Stokstad (1935) and Almquist (1936a, b) did not lead to these complications. The diet used by the latter contained ether-extracted fish meal 17.5 parts, ether-extracted brewer's yeast 7.5 parts, ground polished rice 73 parts, salt plus small amounts of ferrous and cupric sulphate 1 part and cod liver oil 1 part. Growth on this diet is rapid and chicks appear to be vigorous and normal in development. Hemorrhages may develop very suddenly with early fatal conclusion. With care to exclude all extraneous sources of the anti-hemorrhagic vitamin, a nearly complete incidence of external hemorrhages may be induced frequently amounting to 75 percent or more of the chicks within two weeks from hatching. In chicks with a low reserve of the vitamin, external hemorrhages have been observed as early as 5 days from hatching.

The vitamin is clearly not a growth factor, in fact, the syndrome appears to develop at an earlier date in more rapidly growing chicks or with diets that promote rapid growth (Almquist and Stokstad, 1935b).

Deficiency of this vitamin is extremely unlikely in commercial poultry rearing since it is common practice to include several percent of dried alfalfa in practical feeds and to supply additional fresh greens. Almquist and Stokstad (1935b) found that ½ percent of dried alfalfa was sufficient to supply the needs of the chick for the anti-hemorrhagic vitamin. Recognition of the anti-hemorrhagic vitamin is of the greatest importance in the perfection of synthetic diets for the study of all phases of poultry nutrition.

D. PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of the anti-hemorrhagic vitamin are at present incompletely known. It is probable that these will be considerably elaborated in the near future. Such properties as seem to be definitely established are listed in Table 1.

E. METHODS OF DETERMINATION

As in the case of many similar factors, the methods for determination by biological assay have been the first to be employed and have been of two categories, namely, preventive and curative. Both have been found capable of producing early and highly significant results.

The preventive method may be said to be of greatest practical value as it establishes

the daily quantity or dosage of an anti-hemorrhagic vitamin supplement which will meet the requirements of the chick. This has been the method adhered to by Almquist and Stokstad. The curative technique has been employed by these workers chiefly for qualitative testing.

week of age. Hemoglobin level was normal in all groups. Hemorrhages and prolonged clotting time were noted in all groups except one which received only 20 percent more anti-hemorrhagic vitamin than the least deficient group. That the levels of the anti-hemorrhagic vitamin for meeting the

TABLE 1.—*Physical and Chemical Properties of the Anti-hemorrhagic Vitamin*

Property	*Remarks*	*Authority*
Form	Most active concentrates have been viscous oils	Almquist (1936a, b) Dam and Schönheyder (1936)
Color	Colorless	Almquist (1936c)
Solubility	Very soluble in hexane, petroleum ether, ether, acetone and anhydrous alcohols. Insoluble in water or 50% alcohol	Almquist (1936a, b) Dam and Schönheyder (1936)
Volatility	Volatile appreciably at 120–140°C. and 10^{-6} mm. of mercury	Almquist (1936b)
Basicity or acidity	None or very slight	Almquist and Stokstad (1935a,b)
Treatment		
Light	Comparatively stable to visible light Rapidly destroyed by sunlight	Almquist (1936a) Almquist (1936c)
Heat	Stable at 120°C. in air Stable at 120°C. in CO_2 for 24 hours Stable at 100°C. in air	Almquist and Stokstad (1935a, b) Almquist (1936a) Dam (1935a, b)
Alkaline hydrolysis	Very unstable Very unstable Unstable in atmosphere of nitrogen	Almquist (1936a) Dam and Schönheyder (1936) Almquist (1936c)
Bromine	Rapidly destroyed at room temperature	Almquist (1936c)
Perbenzoic acid	Destroyed at room temperature	Almquist (1936c)
Phenyl isocyanate 3:5 dinitro benzoyl chloride 2:4 dinitro phenyl hydrazine	No effect on potency	Almquist (1936c)

Almquist and Stokstad (1935b) reported that chicks did not show bleeding tendencies by the wing puncture method when maintained upon diets containing a level of an anti-hemorrhagic vitamin supplement which was minimal for the prevention of hemorrhages. The same workers (unpublished data, 1936) found in a study of groups of chicks given graded levels of the anti-hemorrhagic vitamin that deficient groups showed prolonged blood clotting time at one requirements for prevention of hemorrhage and for maintenance of normal blood clotting time are quite close seems to be indicated.

Schönheyder (1936) has worked out a complicated assay technique using entirely the curative procedure and blood clotting time. This has been exclusively employed by Dam and Schönheyder in their later

work (1936). That this technique is based upon the most fundamental characteristic of the disease cannot be questioned. As employed by these workers, however, the method involves the determination of clotting power of the blood in one animal, by the use of a clotting agent and measurement of clotting time and the effect of the curative supplement on the restoration of normal blood clotting power in one of two other animals, thereby also involving the assumption that all the experimental animals were physiologically identical. Somewhat more time is consumed in the rearing of chicks to a suitable age and condition for testing than is necessary by the preventive method. If the quantity of vitamin K supplement fed is supra-optimal, additional tests must be performed with lower dosages, as in the preventive method.

Schönheyder (1936) has defined a unit of vitamin K as "that amount which is required per gram of the animal on three successive days in order to render the clotting power of the blood normal (the whole treatment of an animal weighing 333 grams will require 1,000 units)." Almquist (1936b) has stated that the requirement of the chick for the anti-hemorrhagic vitamin is not more than 0.5 microgram per gram of diet.

It is of interest, before concluding, to review several papers which present somewhat different views on the hemorrhagic disease. Cook and Scott (1935a, b) have stated that a condition, the main symptoms of which were (1) severe anemia, (2) increased clotting time of blood, (3) distortion of the blood picture with appearance of immature red cells and great increase in white cells, and (4) numerous hemorrhages, was noted in chicks on a diet of yellow corn 64, wheat 20, "functionally-low-sulphur fish meal" 10, dried yeast 1, calcium carbonate 1.1, tri-calcium phosphate 0.4 and sardine or cod liver oil 1. When the above fish meal was replaced by "functionally-high-sulphur fish meal" the symptoms did not occur. The symptoms were attributed to the presence of nitrogeneous bases which in the case of the latter fish meal were apparently detoxified by the sulphur compounds. The term "functionally" was not further explained. It may be seen that the diet was deficient in the anti-hemorrhagic vitamin. It had no definite provision for a margin of safety in regard to content of the minerals, copper and iron, required to insure against nutritional anemia. Furthermore, the diet may easily have been suboptimal in content of certain water-soluble vitamins required by chickens. In the writer's experience, less than 3 percent of a pure dried brewer's yeast will not provide adequate amounts of these vitamins in such diets.

In a later paper, Scott and Cook (1936) presented further results in regard to a distorted blood picture, hemorrhages and anemia, when fish meal and nitrogenous bases were fed. In only one case where fish meal was used did the diet contain an adequate supply of the anti-hemorrhagic vitamin. Nitrogenous bases were fed at relatively enormous levels. Explicit information on mortality, size and age of birds and grade of fish meal were not provided. In general, the diets used could easily have been inadequate in a number of respects already mentioned. It is doubtful if the data given on blood cell counts and percent hemoglobin indicate any notable departures from the normal range of blood composition of poultry. That the blood cell counts of normal chickens are extremely variable has been shown by Biely and Palmer (1935), Cook and Dearstyne (1934), Forkner (1929) and others. In view of this extreme variability, data on blood cell composition cannot be interpreted without statistical examination to determine whether or not certain differences may be regarded as sig-

nificantly greater than the errors of such differences.[1]

Scott and Cook have re-interpreted some data of Almquist and Stokstad (1935b) in such manner as to fit in with the theory of toxemia induced by nitrogenous bases. The data of Almquist and Stokstad, however, do not furnish a sound basis for such finer re-interpretation. The trends pointed to by Scott and Cook could equally well have been attributed to variation in individual birds.

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[1] Since the preparation of this manuscript the writer and Mr. E. L. R. Stokstad have repeated some of the work by Scott and Cook (1936) finding no evidence of intoxication due to nitrogenous bases in fish meal. All blood characteristics were normal except in the absence of vitamin K, where prolonged blood clotting time and hemorrhage were noted.

The Pigment of Egg Shell Membranes

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THE shell membranes of white-shelled eggs usually show a distinct pink tinge, sometimes sufficiently intense as to create a pinkish coloration when the egg is candled. This coloration, when extreme, has probably led to some confusion in the commercial grading of eggs by candling, since it lends the egg an appearance of having a "pink white," a type of storage deterioration which results when laying hens have access to malvaceous plants or products of these plants. (Lorenz and Almquist, 1934.)

The pigment found in the mineral portion of egg shell has been identified as ooporphyrin (or protoporphyrin as Fischer (1923) has renamed it), but, so far as we know from a search of the literature, no attempt has been made to determine the chemical nature of the membrane pigment.

In order to isolate this pigment in sufficient quantity for study, shells and their attached membranes from about 15 dozen White Leghorn eggs were decalcified in dilute hydrochloric acid. The membranes were washed, dried in vacuum, and extracted with hot absolute ethyl alcohol in a Soxhlet extractor. Evaporation of this extract left a red residue which was then taken up in about 25 c.c. of diethyl ether and filtered.

The absorption spectrum, in the visible region, of this ether solution was determined with a grating spectrograph and with a Bausch and Lomb spectrophotometer.* Spectrophotometric measurements were also made of dilute ammonia and hydrochloric acid solutions of the extracted material.

In the following table, a comparison is made between the absorption bands of the membrane pigment and those of two typical porphyrins, hematoporphyrin and protoporphyrin, a designation which includes ooporphyrin and Kammerer's porphyrin. These last two compounds have been proven identical by Fischer (1923). The wave lengths of the positions of maximum absorption are given in mμ (1 mμ = 10^{-7} cm.).

A close over-all agreement is evident between the spectrum of the membrane pigment and that of the porphyrins. Bands I and IV of the membrane pigment in ether solution are weak in comparison with II and III, and were not detected with the grating spectrograph. The weakness or total disappearance of these two bands may be associated with a similar effect observed by Kajdi (1925) who found that, in hematoporphyrin solutions containing certain unidentified alcohol soluble impurities, these same two bands, at 495 mμ and 625 mμ, which were quite strong in pure hematoporphyrin solutions, disappeared.

Porphyrins occur in a wide variety of natural sources, from which they can either be obtained directly or derived by chemical degradation. The porphyrins, as a class, are compounds made up of a conjugated ring of four pyrrole groups, the difference between the various porphyrins being in the substituted groups on the pyrrole rings. As a consequence of this, practically all of the porphyrins show quite similar absorption spectra in the visible region. A natural assumption would be that we are dealing with

* We are indebted to Dr. H. F. Blum for the use of the spectrophotometer.

TABLE 1.—*Position of Absorption Maxima*

	Hematoporphyrin Kajdi (1925)	Protoporphyrin		Membrane Pigment
		Ooporphyrin Fischer (1923, 1925)	Kammerer's Porphyrin Fischer and Schneller (1923)	
Neutral Ether Solution	I 625	632	632	630 (weak)
	II 570	576	575	570 569**
	III 530	537	533	528 528**
	IV 495	503	498	490 (weak)
Dilute HCL Solution	I a 596	602	602	596
	II a 552	558	557	552
Dilute Alkaline Solution	I b 622	626	624	610
	II b 577	576	598–567 (582)	584
	III b 547	538	554–535 (544)	540
	IV b 507	504	518–499 (508)	505

** These two values were obtained by Dr. J. W. Givens with the grating spectrograph; all of the other values for the membrane pigment were determined with the spectrophotometer.

protoporphyrin, which Fischer (1923) has identified as the porphyrin pigment occurring in all pigmented egg shells.

SUMMARY

A spectroscopic analysis of the pigment of the egg shell membrane offers adequate evidence of its identity with a natural porphyrin. The spectra of the two correspond in neutral, acid and alkaline solution.

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Further Studies on Vitamin G in Chick Nutrition*

WITH SPECIAL REFERENCE TO FLAVINS

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THE recent nutritional literature contains a number of reports (Elvehjem and Koehn, 1935; Lepkovsky and Jukes, 1935; Lepkovsky, Jukes, and Krause, 1936; Ansbacher, Supplee, and Bender, 1936) which show that vitamin G, as originally postulated, contains more than one factor which is required by the chick. Hauge and Carrick (1926) were the first to present evidence that the chick required a thermostable factor present in yeast in addition to vitamin B_1. These observations were confirmed by Norris and co-workers (1930, 1933) and Bethke et al. (1931) who also reported the occurrence of a leg disorder in a variable percentage of the chicks fed a ration low in vitamin G. The addition of autoclaved yeast, liver, alfalfa leaf meal, and certain milk products to these rations increased growth and prevented the leg disorder.

Ringrose and associates (1931) reported the occurrence of a pellagra-like syndrome in chicks fed a corn, wheat middlings, purified casein, mineral, and cod liver oil ration. A certain percent of the chicks on this ration also developed the leg disorder previously referred to. Kline et al. (1932-33) described similar lesions in chicks fed a corn, wheat middlings, commercial casein, minerals, and cod liver oil ration, which had been heated for 144 hours at 100°C. or for 24 hours at 120°C. Ringrose and associates (1931) found that autoclaved yeast and a milk vitamin concentrate were effective in preventing the leg disorder and pellagra-like lesions. Kline et al. (1932-33) also reported that skimmilk powder, autoclaved yeast, and liver extract (Lily 343) prevented the pellagrous symptoms. That this factor was present in the filtrate from liver extract from which the flavins had been removed by adsorption on fuller's earth was shown by further studies of Elvehjem and Koehn (1935), Stare (1935), and Lepkovsky and Jukes (1935). The factor was also found to be present in an extract of alfalfa and rice bran by Lepkovsky and Jukes (1935) and in a rice polish and flavin-free milk vitamin concentrate by Ansbacher and associates (1936).

The relation of flavins to vitamin G has been investigated by many workers (György, 1935; Chick, Copping, and Edgar, 1935; Stare, 1935; Elvehjem and Koehn, 1935; Booker, Blodgett, and Page, 1934; Otter, Orent, and McCollum, 1935; Lepkovsky and Jukes, 1935; Ansbacher, Supplee, and Bender, 1936; and Richardson and Hogan, 1936; and others). The results of these investigations show that flavins are an integral part of vitamin G, as originally considered, and that they are essential in the nutrition of the rat. The function of flavins in chick nutrition has not been extensively investigated. The observation of Lepkovsky and Jukes (1935) that the fuller's earth adsorbate of liver extract caused increased growth when added to an unheated corn meal, wheat middlings, purified

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casein, mineral ration is the only report that has come to our attention indicating definitely that flavins are a necessary component in poultry nutrition.

The present paper reports a series of studies undertaken to obtain further information on the growth and leg-disorder factor or factors as originally reported by Norris and co-workers (1930) and Bethke et al. (1931). When the earlier studies herein reported were initiated in 1930, very little was known about the chemistry of vitamin G and many conflicting results had been published concerning the solubility and heat stability of this factor. While the investigations herein reported were in progress, numerous reports dealing with the complexity and chemical behavior of vitamin G appeared. Some of these findings were made use of in the later experiments. As previously indicated, our investigations were primarily directed to study the factor or factors present in yeast, liver, milk, and so forth, which augmented growth and prevented the occurrence of the leg disorder in chicks fed an untreated ration of yellow corn, wheat, wheat bran, casein, minerals, and cod liver oil.

EXPERIMENTAL

White Leghorn chicks, hatched from eggs obtained from the Station's stock, were used throughout. The chicks were transferred to heated brooders equipped with wire floors on the day following the twenty-first day of incubation and put on the experimental rations, unless otherwise stated. Individual weights and notations were taken every week. The experiments were usually of 8 weeks duration, although in a few instances the experiments were not terminated until the tenth week.

The basal experimental ration consisted of ground yellow corn, 58; ground wheat, 20; wheat bran, 5; Argentine casein, 12; steamed bone meal, 3; salt, 1; and cod liver oil, 1. All substitutions of ingredients in the basal ration were made on the basis of weight; whereas, all additions, unless otherwise stated, were made by adjusting the casein and corn in the basal ration so as to maintain approximately the same level of total protein. The experimental rations were mixed fresh every 7 to 14 days.

Effect of Modifications of the Basal Ration

Early in the investigations we observed, as did Ringrose, Norris, and Heuser (1931), that the rate of growth and the percentage of chicks that exhibited the leg disorder was influenced by the casein in the basal ration. This led us to make further comparisons of different caseins and to study the effect of certain modifications of and additions to the basal ration. A smaller incidence of the leg disorder occurred and a markedly greater growth was obtained with the untreated domestic casein than with the untreated Argentine product, as shown by the results summarized in Table 1. Less growth and a larger percentage of the leg disorder occurred when the domestic casein was washed with acidulated water, but further purification of the Argentine product did not significantly affect growth or the occurrence of the leg disorder. Growth on the Labco casein ration was somewhat less and the mortality was greater than on the ration containing the untreated Argentine product; whereas no great difference in the incidence of the leg disorder was observed. Similar variations in the growth promoting values of caseins have been reported by van der Horn, Branion, and Graham (1935); Lepkovsky and Jukes (1935); and Ansbacher and associates (1936).

The results also show that wheat middlings supply more of the growth factor than ground wheat, which can probably be attributed to the larger amount of germ in the middlings than in the wheat; since it

was noted that wheat germ contained the growth- and leg-disorder factor present in liver and autoclaved yeast.

It was also noted that a small percentage of the chicks on the rations containing ground wheat or rice polish and Argentine or Labco casein showed encrustations at the corners of the mouth, a granulation about the eyes, sore vents and feet, and frequently a bowel disorder—similar to the pellagra-like conditions described by Ringrose and associates (1931) and Kline et al. (1932-33). These lesions were not observed on the rations containing domestic casein, liver, autoclaved yeast or wheat germ.

TABLE 1.—*The Effect of Different Caseins and Certain Modifications of the Basal Ration on Growth and the Incidence of the Leg Disorder*

Expt. No.	Modifications of ration	Average weight 8 weeks	Chicks showing leg disorder	Chicks* with leg disorder recovered	Mortality	Pellagra-like lesions**
		gm.	percent	percent	percent	
1	Domestic casein (1)	354.7	10.0	0.0	5.0	None
	Argentine casein (1)	195.8	35.0	0.0	0.0	None
2	Domestic casein (2)	429.8	5.0	100.0	0.0	None
	Domestic casein (2), washed	263.3	35.0	0.0	10.0	Few cases
	Argentine casein (2)	161.3	65.0	0.0	15.0	Few cases
	Argentine casein (2), washed	151.7	40.0	12.5	20.0	Several cases
	Argentine casein (2) plus 3 percent liver meal	600.0	5.0	100.0	0.0	None
	Argentine casein (2), washed plus 3 percent liver meal	580.8	0.0	0.0	5.0	None
3	Argentine casein (2)	171.8	60.0	8.3	15.0	Several cases
	Labco casein	150.8	50.0	10.0	35.0	Several cases
	Argentine casein (2) with rice polish replacing wheat	168.6	35.0	0.0	20.0	Several cases
	Argentine casein (2) with wheat germ replacing wheat	570.3	5.0	100.0	10.0	None
4	Argentine casein (2)	180.4	65.0	15.3	12.0	Few cases
	Argentine casein (3) with wheat middlings replacing wheat	245.0	70.0	7.1	16.0	None

Twenty chicks were started in each lot in Experiments 1, 2, and 3; and 25 chicks in Experiment 4. The Labco casein was obtained from the Casein Company of America.
* The percent is based on the total number of chicks that exhibited the leg disorder.
** Has reference to mouth, feet, and eye lesions. (see text).

Effect of pH Control in Autoclaving

Several investigators (Williams, Waterman, and Gurin, 1929; Guha, 1931) had reported that vitamin G, as determined on rats, was stable in an acid but not in an alkaline heat-treated medium. In order to determine the effect of pH in autoclaving on the chick growth and leg disorder factor or factors, dried pork liver and dried bakers' yeast were made into a thick paste with

distilled water and sufficient hydrochloric acid or sodium hydroxide was added to bring the pH of the medium to approximately 4.3 and 10.7 and the products heated for 6 hours at 15-17 pounds pressure. The autoclaved materials were then dried in a hot air oven, ground, and the treated liver fed at 2 and 5 percent and the heated yeast at 5 and 10 percent of the basal ration. Comparable groups of chicks were fed the unsupplemented basal ration and the basal ration plus 2 percent and 5 percent of untreated dried liver and dried yeast, respectively.

TABLE 2.—*The Effect of pH Control in Autoclaving on Vitamin G*

Lot No.	Additions to the basal ration	Weighted average 10 weeks	Chicks showing leg disorder	Chicks* with leg disorder recovered	Mortality
		gm.	percent	percent	percent
1	None	248.3± 8.7	68.0	5.9	4.0
2	2 percent dried pork liver	741.4± 9.6	0.0	0.0	0.0
3	2 percent acid autoclaved liver	711.8±14.3	0.0	0.0	12.0
4	5 percent acid autoclaved liver	743.0± 9.6	0.0	0.0	0.0
5	2 percent alkaline autoclaved liver	276.2±12.4	88.0	31.8	12.0
6	5 percent alkaline autoclaved liver	259.4±10.5	92.0	20.9	12.0
7	5 percent dried yeast	744.8± 9.2	4.0	100.0	8.0
8	5 percent acid autoclaved yeast	725.1±11.4	0.0	0.0	4.0
9	10 percent acid autoclaved yeast	699.1±11.9	0.0	0.0	8.0
10	5 percent alkaline autoclaved yeast	498.8±15.3	80.0	90.0	8.0
11	10 percent alkaline autoclaved yeast	635.9±15.0	4.0	0.0	12.0

Twenty-five chicks were started in each lot.
* The percent is based on the total number of chicks that exhibited the leg disorder.

The results, presented in Table 2, show that the factor or factors were stable to autoclaving in an acid medium and that at an alkaline reaction the factor in liver was totally inactivated; whereas only partial destruction occurred in case of the yeast. Similar differences in the stability of vitamin G in alkaline-treated liver and yeast has been referred to by Guha (1931). We are of the opinion that this difference in the stability of the factor in liver and yeast was due to the greater buffer effect of the yeast. Hydrogen ion determinations on the products after autoclaving supported such a hypothesis. Vitamin G studies on the autoclaved samples with rats gave results similar to those obtained with chicks.

Solubility in Alcohol

Our next step was to determine the solubility of the factor or factors in different concentrations of alcohol, because it had been reported that Vitamin G, as determined on rats, was soluble in the lower but not in the higher concentrations of cold ethyl alcohol. It was also thought desirable to repeat, in part, the experiment on autoclaved acid and alkaline liver and determine whether the neutralization of the alkaline autoclaved product before drying and feeding would give a different response. Dried pork liver was used because it had been found to be an excellent source of the growth- and leg-disorder factor or factors.

The alcoholic fractions were prepared by treating 1.5 kilos of dried liver with several volumes of either 20, 70, or 95 percent ethyl alcohol, stirring for 2 to 3 hours, allowing to stand over night, filtering, and again repeating the procedure for a total of four extractions. The alcoholic extracts were

concentrated to approximately 750-1,000 c.c., filtered to remove the precipitate, which was added to the respective liver residues, and then shaken with ether to remove the fats and other extraneous matter. The ether insoluble fraction was made up to 1,500 c.c. with water for incorporation in the basal ration. The insoluble residues were dried and added to the basal ration on the basis of 2 percent of dried untreated liver.

The autoclaved liver samples were prepared as in the previous experiment, except that part of the alkaline-autoclaved fraction was treated with hydrochloric acid to bring the pH of the mixture to 6.2 before drying and feeding.

The data, presented in Table 3, show that the growth- and leg-disorder factor or factors in liver was stable to autoclaving at an acid reaction and completely destroyed when the medium was alkaline. The results in this respect are in complete accord with those of the previous experiment. Neutralization of the alkaline liver after autoclaving gave similar results as the unneutralized alkaline sample. The results with the alcoholic fractions clearly show that the factor or factors were insoluble in cold 95 percent alcohol and completely soluble in 20 percent alcohol; whereas at a 70 percent concentration, only partial solubility occurred.

The different alcoholic fractions as well as the untreated dried pork liver were assayed for their vitamin G content on rats. The results of the rat experiments were in complete agreement with the results obtained on chicks—namely: the 20 percent alcoholic extract and 95 percent alcoholic residue were found to be approximately as potent in vitamin G as the untreated liver, while the 20 percent residue and 95 percent extract were practically devoid of the factor. The results with the 70 percent fractions showed that vitamin G was about equally divided between the alcohol soluble and insoluble fractions.

TABLE 3.—*The Solubility of Vitamin G in Dried Pork Liver in Different Concentrations of Cold Alcohol and Further Observations on the Effect of pH Control in Autoclaving*

Lot No.	Additions to the basal ration	Weighted average 8 weeks	Chicks showing leg disorder	Chicks* with leg disorder recovered	Mortality
		gm.	percent	percent	percent
1	None	171.5± 3.3	60.0	8.3	15.0
2	1 percent dried pork liver	478.4±19.2	10.0	100.0	5.0
3	2 percent dried pork liver	561.5±10.0	0.0	0.0	0.0
4	20 percent alcoholic liver residue†	205.0± 7.5	60.0	25.0	25.0
5	70 percent alcoholic liver residue†	520.4±13.1	5.0	100.0	5.0
6	95 percent alcoholic liver residue†	554.4±12.2	0.0	0.0	0.0
7	20 percent alcoholic extract of liver†	504.1±12.8	0.0	0.0	10.0
8	70 percent alcoholic extract of liver†	388.9±10.5	15.0	33.3	5.0
9	95 percent alcoholic extract of liver†	203.4± 4.7	55.0	9.1	25.0
10	2 percent alkaline autoclaved liver	189.4± 7.5	44.4	0.0	30.0
11	2 percent alkaline autoclaved liver (neutralized)	196.5± 6.4	47.0	0.0	30.0
12	2 percent acid autoclaved liver	560.5±20.1	0.0	0.0	5.0

Twenty chicks were started in each lot.
† The liver extracts and residues were fed on a basis equivalent to 2 percent untreated dried pork liver.
* The percent is based on the total number of chicks that exhibited the leg disorder.

Adsorption Experiments

The initial trial consisted of treating 1,800 c.c. (equivalent to 900 grams of dried pork liver) of a concentrated 20 percent

cold alcohol extract of dried liver with two separate portions of 250 and 200 grams of fuller's earth and thoroughly shaking for two hours after each treatment. The earth was removed by filtration, washed with distilled water, dried, ground, and fed at a 1 percent level—equivalent to 2 percent of the original dried liver. The filtrate was concentrated to 900 c.c. and incorporated in the basal ration at the rate of 50 c.c. per kilo of feed, or the equivalent of 5 percent of the dried liver. The 20 percent alcohol extract was prepared as previously described, except that the dried liver was defatted by means of ether prior to extraction with alcohol. The residue remaining after extraction was dried and fed on a basis equivalent to 2 percent of untreated dried liver, in comparison with the other fractions.

TABLE 4.—*The Adsorption of Vitamin G from Liver Extract*

Lot No.	Additions to the basal ration	Weighted average 8 weeks	Chicks showing leg disorder	Chicks* with leg disorder recovered	Mortality
		gm.	percent	percent	percent
1	None	168.4± 8.4	31.6	16.6	35.0
2	2 percent dried pork liver	565.8± 9.8	0.0	0.0	5.0
3	†20 percent alcoholic extract of liver	561.3±10.2	0.0	0.0	5.0
4	†20 percent alcoholic liver residue	206.8±12.4	68.0	15.4	25.0
5	††Fuller's earth adsorbate of alcohnlic extract	401.1±14.7	40.0	100.0	5.0
6	††Filtrate from the treated alcoholic extract	382.3±10.1	47.0	75.0	5.0

Twenty chicks were started in each lot.
† Fed on the basis of 2 percent untreated dried pork liver.
†† Fed on the basis of 5 percent untreated dried pork liver.
* The percent is based on the total number of chicks that exhibited the leg disorder.

It is apparent from the results in Table 4 that the growth- and leg-disorder-preventing factor or factors were soluble in 20 percent cold alcohol and that at least partial adsorption of these factors occurred on the fuller's earth. The positive response noted in case of the filtrate either indicated incomplete adsorption or that some other essential factor was present in the unadsorbed fraction.

We had also found that the growth- and leg-disorder factor or factors could be extracted from dried whey with hot alcoholic solutions. About the same time European investigators reported that fuller's earth at proper pH concentration adsorbed vitamin G, which upon elution and further purification was identified as flavin. We employed the procedure of treating a concentrated extract of dried whey, free from alcohol, with fuller's earth at an acid reaction and eluding with a pyridine-methyl alcohol-water mixture. The eluate was further purified by concentrating under vacuum and treating twice with several volumes of acetone. The concentrated eluate and the filtrate from the fuller's earth were incorporated in the basal ration on a basis equivalent to 10 percent dried whey. The untreated whey extract was fed at a level of 7.5 percent of the dried powder. The fractions were fed to groups of 15 chicks each. The results are recorded in Figure 1.

The whey extract and the fuller's earth eluate caused increased growth; whereas the filtrate from the treated whey extract was without effect. No evidence of the leg disorder was apparent in the whey extract or fuller's earth adsorbate groups, while on the basal ration and the basal plus the fuller's earth filtrate, 36 and 66 percent of the chicks, respectively, showed the leg disorder. These results suggested that flavin

was the factor primarily concerned in the results secured.

Effect of Lactoflavin

To obtain further data on the effect of flavins, a pure preparation of lactoflavin was fed in doses of 40 and 80 micrograms per bird every other day to two groups of 8 chicks each. The supplemental feeding was begun after the chicks had been on the unsupplemented basal ration for 2 weeks. The lactoflavin caused a marked increase in growth, as shown in Figure 2. None of the flavin-fed chicks showed the leg disorder; whereas 5 out of the 8 chicks on the unsupplemented basal ration were affected. The results of these investigations, which are in accord with the reports of Lepkovsky and Jukes (1935-1936), show that flavins are essential in the nutrition of the chick and that they are probably associated with the leg disorder referred to. The results indicate also that the increased growth and well being resulting from the use of dried milks, dried whey, liver, yeast, and so forth, in the chick ration are, in a large part, due to the flavins present in these products.

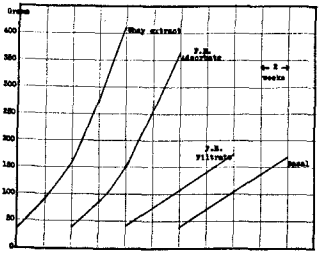

FIG. 1. The adsorption of vitamin G on fuller's earth. The extract of whey was fed on the basis of 7.5 percent of the original powder and the fuller's earth adsorbed fraction and the filtrate on a basis of 10.0 percent of the dried powder.

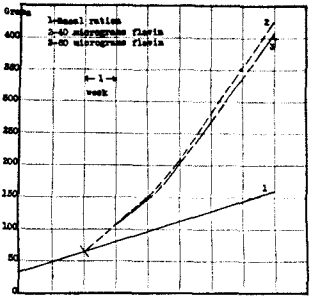

FIG. 2. The effect of lactoflavin. The quantities of lactoflavin indicated were fed individually every other day.

SUMMARY

1. Studies with chicks showed that caseins vary in their growth-promoting values and that wheat middlings contain more of the growth factor (vitamin G) than ground wheat, probably because of a greater germ content.

2. Autoclaving dried pork liver and yeast at an acid reaction for 6 hours at 15-17 pounds pressure did not destroy the growth- and leg-disorder factor (vitamin G); whereas at an alkaline reaction the factor was totally inactivated in liver and partially destroyed in yeast.

3. It was found that the growth- and leg-disorder factor (vitamin G) of dried pork liver was soluble in cold 20 percent ethyl alcohol and insoluble in cold 95 percent ethyl alcohol. Partial solubility of the factor was noted in 70 percent cold ethyl alcohol.

4. Adsorption experiments showed that the growth- and leg-disorder factor (vitamin G) was adsorbed from an extract of dried liver and dried whey by fuller's earth, indicating that flavins were the principal factor involved, which was confirmed by feeding pure lactoflavin.

5. The results show that flavins are essential in chick nutrition and they also suggest that the beneficial results commonly noted in case of feeding milk, yeast, liver, and so forth, to poultry are, in a large part, due to the flavin content of these products.

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The Inheritance of Shank Color in Chickens

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PREVIOUS WORK

SEVERAL hypotheses have been advanced for the inheritance of shank color in chickens. According to a number of investigators, the following genes are concerned in shank color inheritance: (1) E, e, for extension and non-extension of melanic pigment to plumage; (2) I, i, for inhibition and non-inhibition of melanic pigment of plumage; (3) C, c, for color and lack of color; (4) B, b, sex-linked for barring and non-barring; (5) D, d, sex-linked for inhibition and non-inhibition of melanic pigment in the shanks.

Dunn (1925) and Dunn and Jull (1927) concluded that light shank color was dominant to dark and was due to the action of the sex-linked D gene. Jull stated that it remained to be determined whether the D and d genes controlled dark and light shank colors in all cases. Warren (1928) reported that yellow shank (Y) was dominant to blue (y) and was sex-linked. Knox (1935) stated that since the crosses of Bateson and Punnett (1911), Dunn (1925), Dunn and Jull (1927), and Warren (1928) involved a sex-linked plumage factor, usually that for barring which was known to influence shank color, that these crosses could not be considered critical tests for the presence of a sex-linked inhibitor of shank color. The progeny from the crosses of Knox (1935), Jersey Black Giant ♂ x Rhode Island Red ♀ ♀ and the reciprocal, were black-plumaged and black-shanked. He concluded that melanic pigment in the shanks (E) was dominant to yellow (e), contrary to the conclusions of Warren (1928). MacArthur (1933) designated the shank color genotype of the Rhode Island Red as DD while Knox (1935) designated it as ee.

While the test of the linkage relations between the barring gene and the so-called specific gene for shank color has been attempted, results thus far can not be considered conclusive since in most cases these genes were not adequately differentiated.

PROCEDURE

The following crosses were made:

(1) Black Langshan ♂ x White Leghorn ♀ ♀,

(2) Black Langshan ♂ x White Orpington ♀ ♀,

(3) Black Langshan ♂ x Brown Leghorn ♀ ♀.

F_2 progeny of three crosses were secured. The progeny from these crosses were classified for shank and plumage color at hatching time and at maturity. In this study light shanks are referred to as those that are predominantly white or yellow, but they may show slight traces of melanic pigment particularly on the toes and front of shank. Dark shanks include blue, bluish-black, and black. All tables except Table 4 have listed only light and dark shanks. At hatching time shank color was classified as dark and light; however, the dark shanks were usually black and the light shanks were either white, slaty-white, or yellow.

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RESULTS

Black Langshan ♂ x White Leghorn ♀ ♀. At hatching time the F_1 progeny from this cross were white-plumaged and light-shanked. At about two months of age the females began to develop blue pigment in the shanks, and this color was retained to maturity. The shanks of the males remained white to maturity. These results can be explained on the assumption that the gene for extension of melanic pigment to plumage (E) likewise extends melanic pigment to the shanks of chicks if not inhibited by I or B. Since the parental White Leghorns carried I, the chicks were not dark-shanked. As the females carried the d gene, they developed dark shanks at maturity. The males remained white-shanked because of the action of B. It was impossible to determine whether the P_1 females carried B and D or B and d until data on the F_2 were secured, since the results in the F_1 progeny would not have been altered by the presence of the D gene. The genotypes proposed for the parents were: EECCii(bd)(bd) for the male; EECCII(Bd)(—) or EECCII(BD)(—) for the females.

TABLE 1.—*F_2 Progeny from Black Langshan Male × White Leghorn Females as Classified at Maturity*

	White Plumage		Black Plumage		Barred Plumage	
	Shank Color		Shank Color		Shank Color	
	Dark	Light	Dark	Light	Dark	Light
Observed	36	48	23	0	0	21
Expected	42	42	23	0	0	21
P	.19		1.0		1.0	
Observed	84		23		21	
Expected	96		16		16	
P	.04–.05					

The F_2 Progeny from Black Langshan ♂ x White Leghorn ♀ ♀ consisted of 84 white-downed, light-shanked chicks, 37 black-downed, black-shanked, and 7 black-downed, light-shanked chicks. The down color of some of the black chicks contained varying amounts of white on the head and body; these at maturity were found to carry the barring gene. The observed shank colors of both the white- and black-downed chicks were as expected. This expectation was based on the assumption that down color and shank color are highly correlated. In case of the black-downed chicks which carried B this correlation was not always maintained.

At maturity 36 of the 84 white-plumaged individuals were blue-shanked, Table 1. These results did not deviate significantly from the expected 1:1 ratio for shank color. The 23 black-plumaged birds at maturity were all dark-shanked, while the 21 barred ones were all light-shanked. The observed ratios of 84 white, 23 black, and 21 barred-plumage adult birds did not deviate significantly from the expected 6:1:1 ratio. The fact that all the black-plumaged individuals were dark-shanked seems to indicate that the P_1 White Leghorn females carried only B and not D; however, the numbers involved might not have been large enough to preclude the possibility of a very close linkage between B and D.

Chicks from the cross, *Black Langshan ♂ x White Orpington ♀ ♀*, had black shanks, and black plumage. At maturity, the males had predominantly white shanks and black plumage. In every instance, however, the shanks of the males were not entirely white. There was a slight amount of pigment in the toes of some individuals. At maturity the shanks of some of the females were black, while the shanks of others were blue to bluish-black. The results can be explained by assuming the presence of a D gene in the male progeny whose action became effective at about six weeks to two months of age, resulting in light shanks at

maturity. Since the males of this cross were black in plumage color at maturity with light shanks, it is certain that the barring gene was not responsible for the ligbt shank color. While some of these males at maturity developed traces of silver in plumage and others gold, yet all were light-shanked, it is unlikely that these factors influenced the shank color of these individuals. The results of shank and plumage colors at hatching and at maturity were as expected, based on the following genotypes for the parents: Black Langshan ♂, EECCii(bd)(bd), White Orpington ♀ ♀, eeccii(bD)(—).

The F_2 progeny from *Black Langshan ♂ x White Orpington ♀ ♀* consisted of 30 chicks with black plumage and black shanks, and 27 chicks with white plumage and white shanks. At maturity 16 of the 30 black-plumaged progeny had dark shanks; 14 had light shanks. Of the 27 white-plumaged progeny at hatching, 20 of this number at maturity carried the columbian pattern eeCC, while 7 were solid white in plumage color. These 20 columbian-patterned offspring comprised 14 light-shanked and 6 dark-shanked birds. Of the 7 white-plumaged birds at maturity, 4 were light-shanked and 3 were dark-shanked.

The observed shank colors at hatching and at maturity for the black-plumaged individuals did not deviate significantly from the expected 1:1 ratio, nor did the observed shank colors for the white-plumaged individuals. All chicks had the same shank color as down color. At maturity the observed shank colors of the black-plumaged and the white-plumaged individuals did not deviate significantly from the expected 1:1 ratio, Table 2. The observed shank colors of the columbian-patterned progeny likewise did not deviate significantly from the expected 1:1 ratio.

The observed down colors did not deviate significantly from the expected 9:7 ratio. The down colors concerned were black and white. The observed plumage colors at maturity deviated significantly from the expected 9:3:4 ratio, Table 2. The plumage colors concerned at maturity were black, columbian-patterned, and white. At hatching time only black and white down colors were concerned. The white-downed group at maturity were classified as white-plumaged and columbian-patterned individuals. All individuals which had melanic pigment confined either to the wing or neck feathers or to other parts of the plumage were classified as columbian-patterned. Some of these birds, classified as columbian were buff or gold in color. It is possible that there was an error in the classification of some of the gold or buff birds, since the presence of slight traces of melanic pigment in such birds is not beyond expectation.

TABLE 2.—*F_2 Progeny from Black Langshan Male × White Orpington Females Classified at Maturity*

	Black Plumage		White Plumage		Columbian	
	Shank Color		Shank Color		Shank Color	
	Dark	Light	Dark	Light	Dark	Light
Observed	16	14	3	4	6	14
Expected	15	15	3.5	3.5	10	10
P	.72		.70		.07	
Observed	30		7		20	
Expected	32.1		14.2		10.7	
P			.01			

The presence of the columbian-patterned offspring (eeCCii) with blue shanks is proof that the gene for blue shanks (d) expresses itself independently of E and I. The white-plumaged progeny included birds with both light and dark shank color. This further indicates that D and its allele act independently of the effects of plumage genes on shank color. The D gene in this cross

accounts for all the white-shanked individuals. This is not in agreement with Knox (1935) who attributed the light shank color of progeny from crosses of Jersey Black Giant and Rhode Island Red to the effects of the e gene.

Results from the cross, *Black Langshan ♂ x Brown Leghorn ♀ ♀*, were similar to those obtained from the cross, Black Langshan ♂ x White Orpington ♀ ♀; that is, the F_1 black-plumaged females comprised both blue- and black-shanked individuals, while the shanks of the F_1 males were predominantly white. These results show that the Brown Leghorn carries the D gene. This is in agreement with the work of Jull (1932, unpublished), as reported by Knox (1935). The genotypes of the parents were: EECCii(bd)(bd) (Black Langshan), eeCCii(bD)(—) (Brown Leghorn).

TABLE 3.—*Progeny from F_1 Male (Black Langshan × White Orpington) F_1 Females (Black Langshan × White Leghorn) Classified at Maturity*

	White Plumage		Black Plumage	
	Shank Color		Shank Color	
	Dark	Light	Dark	Light
Observed	15	12	10	9
Expected	13.5	13.5	9.5	9.5
P	.57		.81	
Observed	27		19	
Expected	23		23	
P	.23			

When an F_1 ♂ (Black Langshan x White Orpington) was crossed with F_1 ♀ ♀ (Black Langshan x White Leghorn), both white-downed and black-downed chicks were produced. The 27 white-downed chicks had light shanks. The 19 black-downed chicks had black or dark shanks. At maturity 15 of the 27 white-plumaged individuals had dark shanks and 12 had light shanks, Table 3. Ten of the 19 black-plumaged individuals had black shanks; 9 had light shanks. The observed shank colors at maturity did not deviate significantly from the expected 1:1 ratio. Neither did the observed plumage colors deviate significantly from the expected 1:1 ratio.

Results secured from the P_1 crosses demonstrated that the Black Langshan ♂, White Orpington ♀ ♀, and White Leghorn ♀ ♀ were of the following genotypes respectively: EECCii (bd)(bd), eeccii(bD)(—), and EECCII(Bd)(—), or EECCII (BD)(—). The genotypes of the F_1 parents of this cross, EeCcii(bD)(bd) for the male, EECCIi (bd) (—) for the females are adequately explained by the genotypes assigned to the above P_1 birds. Results from this cross also indicate that D acts independently of the plumage genes.

DISCUSSION

The results of this study indicate that the action of E and I on shank color are confined to the outer epidermal layer; while the action of D and its allele are confined to the lower strata of the shank, and further that the d gene, as so called in this study may not be the same as the d gene of the White Silkie whose action is known to be effective at hatching time.

Knox (1935) pointed out that female segregates from a Black Minorca ♂ x White Leghorn ♀ ♀ were blue-shanked because the I gene diluted the melanic pigment in the shanks. His explanation might imply that the E gene extends melanic pigment to the lower layers of the shank, even though its effects are inhibited in the stratum corneum. That the E gene extends melanic pigment only to the stratum corneum was indicated by the appearance of black-plumaged segregates that have light shanks which show traces of epidermal pigment on the front on shanks and toes. These individuals carry the E, C, and D genes.

The D gene inhibits melanic pigment in the lower strata of the shanks only, leaving the E gene free to extend pigment to the stratum corneum. The American Standard of Perfection (1930) describes the Black Leghorn as a black-plumaged, yellow-shanked breed which may show traces of melanic pigment in the shanks. It is suggested that this breed carries the E, C, and D genes.

A critical test for the action of the d gene independent of the effects of the E gene was revealed in the F_2 progeny from the Black Langshan x White Orpington cross. Blue-shanked segregates having the columbian plumage pattern eeCC were secured. These individuals carried the d gene which accounted for the blue shank color.

Not all black-plumaged individuals which carry the D gene have traces of epidermal melanic pigment in the shank. This would seem to indicate that the E gene is variable in its expression to the stratum corneum of the shank. Further evidence of this effect was obtained from the crosses, Black Langshan ♂ x Brown Leghorn ♀ ♀, and Black Langshan ♂ x White Orpington ♀ ♀. Some of the F_1 black-plumaged females from both crosses had black shanks, while others had blue shanks. These females were of the genotype Ee(d—), hence only black-shanked females were expected. On careful examination, the black-shanked individuals were found to have varying amounts of pigment in the stratum corneum. In the blue-shanked birds, the stratum corneum was devoid of pigment. These results can be explained only by assuming that the E gene, at least in the heterozygous form, is variable in its action. More evidence concerning the apparent variability of expression of the E gene is given by the American Standard

TABLE 4.—*Genotypes and Phenotypes for Shank and Plumage Color at Hatching and at Maturity*

	Age Classified	Shank Color	Plumage Color
EECCii (db) (db)	At *Hatching*	*Black*	*Black*
	At Maturity	Black**	Black
EECCii (Db) (db)	At Hatching	Black	Black
	At Maturity	Light*	Black
EECCii (dB) (db)	At Hatching	Light to black	Black with varying amounts of white
	At Maturity	Light	Barred
EECCII 1. (DB) (db) 2. (Db) (db) 3. (dB) (db) 4. (db) (db)	At *Hatching*	Light	White
	At Maturity	1. Light 2. Light 3. Light 4. Blue	White
eeCCii (db) (db)	At Hatching	Light	White
	At Maturity	Blue	Columbian pattern
eeccii (db) (db)	At Hatching	Light	White
	At Maturity	Blue	White

* Dd individuals may show slight traces of melanic pigment on toes and front of shank.
** Ee individuals may have dark blue shanks.

of Perfection (1930). Standard-bred, black-plumaged breeds supposedly homozygous for E may have shank colors varying from dark-slate to black. The Standard of Perfection lists dark-slate shank color for the Black Australop and Black Minorca breeds, while black shank color is listed for the Jersey Black Giant, Black Java, and others. It is suggested that hormones or any factors affecting metabolism may be responsible for the variability of expression of the E gene, or other genes where variability in expression is known to occur. Knox (1935) suggested that hormones might influence shank color.

Knox (1935) suggested the genotype WWDDbbEE for dark-shanked birds belonging to the black plumage group. He stated, however, that this genotype was not demonstrated in his crosses. The genotype 26—Poultry Science P17 10-12-15 Schomer WWEECC (db) (db) is suggested for birds belonging to the above group. He also suggested that the genotype of the White Langshan was bbEEiicc. This breed has white plumage and blue shanks at maturity. Since this suggested genotype does not include the d gene, he evidently attributed the blue shanks to the interaction of E and c. From the crosses in the course of this study, it would seem more logical to attribute the blue shanks of the White Langshan to the action of the d gene rather than the interaction of E and c.

CONCLUSIONS

The D gene is a sex-linked gene for shank color which produces predominantly light shanks.

Blue shank color is caused by a specific gene d. This gene is sex-linked and therefore segregates independently of the autosomal plumage genes I, C, and E. The action of this gene is independent of the separate actions or the interactions of C, E, or I. The action of this gene is delayed until the bird is about two months old. The data indicate that the d gene as so called in this study may not be the same as the d gene of the White Silkie whose action becomes effective at hatching time.

The unrestricted action of the E gene extends melanic pigment to the down and shanks of chicks and to the stratum corneum of adult shanks.

The E gene, at least in heterozygous form, is variable in its action on shank color. Birds of the genotype Ee(d—) may vary in shank color from black to blue.

The unrestricted action of E in combination with D results at maturity in light shanks, which show traces of melanic pigment. The action of e in combination with D results in perfectly white or yellow shanks.

The I gene inhibits the action of the E gene in the shanks and down of chicks, also in adult plumage and in the stratum corneum of adult shanks.

The B gene almost completely inhibits melanic shank color.

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Pullorum Disease in Ducklings*

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PULLORUM disease in ducklings has been described by Lerche (1929), Miessner (1930), and Stenius (1932) (1932A) in Europe and by Hendrickson and Hilbert (1931) in United States. Of these writers, Lerche gives the most complete description in his report of two outbreaks occurring near Breslau. Stenius reported what he believed to be the first outbreak among ducklings in Finland. Miessner mentions that he has isolated *Salmonella pullorum* from ducklings in Germany. Since, however, he is one of the few investigators that believes *S. pullorum* and *S. gallinarum* are identical, it is not known whether he was dealing with pullorum disease or fowl typhoid. Hendrickson and Hilbert's report is on the occurrence of an outbreak in ducklings on a Long Island (New York) farm. It is of interest that Lerche, Stenius, and Hendrickson and Hilbert all mention infected chicks as a probable source of the disease in ducklings.

This is a report of an outbreak in a small flock of Mallard ducklings which occurred simultaneously with an outbreak in Barred Rock chickens reared on the same ranch in California. The report includes the results of studies on the causative organisms and attempts to transmit the disease to chicks and ducklings with them.

HISTORY

On April 16, 1935, five four-weeks-old Barred Rock chicks and a one-week-old Mallard duckling were brought to the California State Department of Agriculture Poultry Pathological Laboratory at Petaluma for diagnosis. The owner reported a loss of 93 chicks from a flock of 380 during the first four weeks. Seven ducklings had died from a flock of 35 during the first week. A tentative diagnosis of pullorum disease in both the ducklings and chicks was made on the basis of isolation of pure cultures from the hearts and livers of gram negative, non-motile organisms which produced acid and gas in 1 percent dextrose, and mannitol broth, and no acid or gas in lactose, maltose, or sucrose broth. Agglutination tests using antigens prepared from the isolated cultures and *S. pullorum* antiserum were also positive to the complete titer of the serum. The autopsy of the ducklings failed to reveal distinctive lesions.

The chicks and ducklings were purchased from the same hatchery, but there was three weeks difference in their ages, and they were hatched in separate incubators. The chicks were, however, brooded in a pen directly connected with the quarters occupied by the ducklings. Although there had been no previous laboratory confirmation, the chicks were suffering from a pullorum disease-like illness at the time the ducklings were purchased, and there was ample opportunity for mechanical transmission from the chicks to the ducklings. Since no other evidence concerning the method of transmission was obtained, this appears to be the logical one.

* University of California and Calif. State Dept. of Agriculture coöperating.

Two visits were made to the ranch during the seven weeks following the diagnosis. A number of chicks died during this period and many others failed to grow normally. Three additional ducklings died during the 7 weeks, making a total mortality of 10 (28.57 percent).

At the age of 8 weeks, the 25 duckling survivors were bled and the serum tested by the tube method for the presence of *S. pullorum* agglutinins. All of the tests were negative in dilutions 1-25, 1-50, 1-100, and 1-200, of the blood sera.

CULTURAL STUDIES

One culture (P40) isolated from the heart blood of a duckling, and one (P39) isolated from the liver of a chick were selected for detailed studies. These cultures were typical of the ones obtained from the two species, but were somewhat atypical to the well described smooth type of *S. pullorum*. They produced larger colonies on agar plates, and a more abundant growth on agar slants with a peculiar wrinkled appearance after 48 to 72 hours incubation. After 72 hours the broth cultures developed a pellicle which spread over the surface as a thin, but unstable membrane which broke up with ease and settled to the bottom of the tube. The broth remained fairly turbid after several days of incubation.

The organisms have been checked at intervals since isolation and these characteristics have remained unchanged. The wrinkling of the growth on agar has been more marked when the organisms are grown on freshly prepared meat infusion agar, than when grown on commercial meat extract agar. No attempt to segregate "normal" types has been made.

The staining characteristics of young meat extract agar cultures have been consistently typical of *S. pullorum*. True motility has not been observed either by the hanging drop method, or by the semi-solid agar technic described by Tittsler and Sandholzer (1935).

Nitrates were reduced to nitrites and tests for indol production have been negative. Litmus milk became slightly acid in 48 hours but reverted to neutral after 96 hours and then became slightly acid after 21 days. Plain milk changed from a pH of 6.8 to a pH of 6.0 for the chick culture (P39) and to a pH of 6.2 for the duckling culture (P40) after 30 days of incubation.

The following carbohydrates and alcohols were fermented with acid and gas production by the end of a four weeks incubation period: dextrose, galactose, levulose, xylose, mannose, mannite, sorbite, and rhamnose. No visible changes were noted in lactose, maltose, sucrose, dextrin, inulin, dulcite, salicin, and raffinose. Very slight acidity was noted in arabinose and glycerol after 3 weeks incubation, but no gas was observed in either. A mineral mixture liquid medium suggested by Scott (1930) plus 0.5 to 1.0 per cent carbohydrates and alcohols was used for the tests. Bromthymol-blue was added as an indicator. These studies were made within 2 months after the original cultures were isolated, and a known laboratory strain of *S. pullorum* was inoculated in all the media as a control. This control culture gave the same reactions, in all the media except arabinose, which was attacked with acid and gas production in 48 hours.

After 9 months the cultures were studied again, with results identical to those reported above. During the latter studies all fermentation tests were made with peptone water as a base medium and Andrade's indicator was used to determine acid production.

After one year, P40, the duckling strain, produced acid and gas in arabinose medium. By selection of individual colonies on dilution plates, both non-arabinose fermenters and arabinose fermenters were obtained. At this time the cultures were checked with

Jordan's tartrate agar recommended by Mallman (1931), and with the cysteine-gelatin medium used by Hinshaw and Rettger (1936) for differentiation of *S. pullorum* and *S. gallinarum*. Typical reactions for *S. pullorum* for these media were obtained.

Antigens prepared from meat extract agar cultures showed no tendency to self agglutinate. Both the chick and duckling strains of antigen reacted to complete titres (1-1280 and 1-640) with two immune sera prepared by inoculation of chickens with old laboratory strains of *S. pullorum* (P15 and P133). These same sera absorbed with P39 and P40 antigens were found to be freed completely of their homologous agglutinins. Conversely, the sera absorbed with their homologous antigens were not agglutinated when tested with P39 and P40 antigens. Reciprocal absorption tests using anti-serum prepared by immunizing rabbits with P40 also resulted in complete absorption of the agglutinins for the test organisms as well as for the control organisms. The technic suggested by Edwards and Rettger (1927) was used in the agglutinin absorption studies.

PATHOGENICITY STUDIES

Chicks:—The pathogenicity of the cultures was tested on day-old Barred Rock cross-bred chicks from pullorum disease free stock. Two lots of five chicks were each given orally 0.5 c.c. of a 24-hour broth culture of the organism concerned. At the same time a drop of each of the cultures was put into the eye and nostril. In 48 hours after the original inoculation 5 c.c. of 72-hour old broth culture was added to the drinking water of each lot. The two lots were kept in separate brooders and three controls were also kept in a third brooder.

Three chicks from each of the inoculated groups died with typical pullorum disease by the end of the 12 days that they were kept under observation. Five of these were dead within one week and the sixth, inoculated with P39 culture, died on the twelfth day.

Pure cultures of organisms that were typical of those inoculated were recovered from all the dead chicks and from two of the remaining ones, that were killed on the twelfth day. These two were from the lot given the P40 (duckling) culture. They were all visibly sick at the time of killing.

The control chicks remained healthy and failed to yield cultures of *S. pullorum* at the time of autopsy at 12 days of age.

Ducklings:—Two unsuccessful attempts were made to infect ducklings with the cultures P39 and P40. In the first of these, 12 cross-bred Mallard-Pekin ducklings 11 days old were used. Four were kept as controls, four fed 1 c.c. each of a saline suspension of a 24-hour old agar culture of P39, and four fed 1 c.c. each of a similar suspension of P40. A pipet was used for feeding the cultures. The suspensions had a turbidity equal to a number 3 tube of McFarland's nephelometer. In addition to giving each bird the oral dosage, one drop of the suspension was placed in each eye.

One from each of the groups was killed at the end of two weeks, and at this time each of the ducklings in the two test lots was either given 2 c.c. orally or 1 c.c. intraperitoneally of the respective culture for the group. They were then held for an additional two to three weeks and killed for autopsy. At no time during the period of observation were any symptoms of disease noted. Attempts to recover the injected organisms from the heart's blood, liver, spleen, pancreas, and bone marrow were futile, and the agglutination tests were negative.

The second attempt was made with 10 Muscovy ducklings 36 hours old. These were divided into 5 lots of two each and treated as follows: Two were kept as untreated controls, and two each were inocu-

lated with strains P39, P40, P15, and P133, respectively. The last two strains were stock cultures which have been typical of *S. pullorum* and pathogenic for chicks when tested at various times. In each of the treated lots one duckling was given 0.5 c.c. of the respective culture subcutaneously and the other was fed one cubic centimeter of a saline suspension of a turbidity equivalent to McFarland nephelometer tube 3.0, prepared from a 24-hour-old agar slant culture.

The various groups were brooded separately for the duration of 14 days which they were left under observation. At the end of 4 days, 5 c.c. of a 95-hour-old broth culture of each organism was placed in the drinking water of the respective groups. This was repeated in another 4 days with 5 c.c. of a 24-hour-old culture.

No indications of illness occurred, and at the time of killing, the temperatures were normal. All of the ducklings more than tripled their hatching weight in the 14-day interval.

Blood samples taken at the time of killing were tested by means of the tube agglutination test using a stock *S. pullorum* antigen. With the exception of one duckling, all the tests were negative in the dilutions used (1-10, 1-20, 1-40, 1-80). The exception (1412) gave a complete reaction in 1-10 and a partial reaction in 1-20. This duckling had received 0.5 c.c. of the strain P40 subcutaneously. Duplicate tests with an antigen prepared from this strain gave identical results.

Bacteriological examinations consisted in taking cultures from the heart's blood, liver, spleen, bile, and intestines of each specimen. Turkey meat and liver infusion agar, and brilliant green extract agar were used as cultural media. Attempts to isolate the injected organism from these organs were without success.

The results obtained in these two attempts to infect ducklings are in keeping with Beller's (1926) findings that ducklings could not be artificially infected with *S. pullorum*. No mention is made by Lerche (1929), Stenius (1932), Miessner (1930), or Hendrickson and Hilbert (1931) of attempts made by them to infect ducklings with the organisms isolated from natural outbreaks.

It is possible that the negative results obtained by the writers were due in the first case to the age of the ducklings used. It is also possible that Mallards are more susceptible than Mallard-Pekin crosses or Muscovys. Pure strains of Mallards were not available at the time the experiments were made.

SUMMARY

An outbreak of pullorum disease in week-old Mallard ducklings is reported. Pullorum disease was diagnosed in four-week old Barred Plymouth Rock chicks from the same ranch simultaneously and circumstantial evidence is presented that the disease was transmitted to the ducklings from the chicks.

Complete studies of the strains of *S. pullorum* isolated from the ducklings and chicks are included in the report. These strains differed from the laboratory strains with which they were compared in the production of a wrinkled growth on agar, the formation of a slight pellicle on meat extract broth and in their failure, when first isolated, to produce acid and gas in arabinose medium. After one year, the duckling strain began producing acid and gas in arabinose broth.

Reciprocal agglutinin-absorption tests made with 2 tested laboratory strains as controls showed the strains to be antigenically identical to *S. pullorum*.

Both the duckling and chick strains produced typical pullorum disease in chicks but failed to infect one lot of two-day-old Muscovy and one lot of 11-day-old Mallard-Pekin cross-bred ducklings.

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News and Notes

Mr. W. P. Albright (B.S., N.Car., 1929, M.S., Kansas, 1930) has been appointed extension specialist at Purdue University. He was previously engaged in extension, teaching and experiment station work as well as county agent work in Oklahoma.

Mr. Richard F. Brueckner (B.S., Purdue, 1933) who has been in charge of the grading work in a large Indiana plant, has been appointed to supervise the Indiana U.S. Egg Grading work which is being handled by the Poultry Department of the Agricultural Experiment Station.

The Maryland State Legislature has appropriated $125,000 for a poultry research laboratory building and a new poultry plant. Provision has also been made for increasing the personnel of the poultry department.

Some Responses of the Immature Female Fowl to Injections of Mare Gonadotropic Hormone and Oestrin

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AN INCREASE in the weight of the ovary and oviduct of immature female fowl after injection of pregnant mare's serum was reported by Asmundson and Wolfe (1935). Breneman (1936) also found that there was a marked increase in the weight of the gonads (ovaries and testes) of newly hatched chicks; mare's serum and follicle stimulating hormone giving maximum response while there was some increase when luteinizing hormone and pregnancy urine were injected. Domm and Van Dyke (1932) had previously reported that there was an increase in the weight of the ovary after injection of extracts of the anterior pituitary into immature female fowl while Evans and Simpson (1934) have reported that the ovary of the pigeon increases in weight after injection of pregnant mare's serum.

The present paper deals with certain experiments which show in some detail what responses are obtained with varying amounts of mare gonadotropic hormone when injected into immature female fowl (Gallus gallus domesticus) at different ages and for varying periods of time.

MATERIALS AND METHODS

For the experiments reported in this paper crossbred chickens (from Rhode Island Red males mated to Barred Plymouth Rock females) and also White Leghorn chickens were used. The Leghorns were used for Experiment 8 only.

The pregnant mare's serum, kindly supplied and assayed by Dr. H. H. Cole, contained about 75 rat units of gonadotropic hormone per c.c. The pregnant mare's serum extract was prepared by a method described by Catchpole and Lyons (1934). The serum was diluted with a five-fold volume of acetone, allowed to stand over night in a refrigerator, and then filtered through a Buchner funnel. The still wet precipitate was then extracted with an aqueous solution containing 60 percent acetone and 4 percent ammonia for 16 hours at room temperature. The solution was separated from the solid residue by centrifuging, after which the extracted material was precipitated from the solution by bringing the concentration of acetone up to 80 percent. The light precipitate which was formed was allowed to settle over night in the refrigerator, and was then filtered, washed several times with acetone, then with ether, and finally dried in a slightly warmed vacuum desiccator. The usual form of assay showed the potency to have been satisfactorily carried through in the extraction procedure.

Oestrin was prepared from pregnant mare's urine by a method suggested by the work of Leonard, Hisaw, and Fevold

(1932). The urine was made acid to litmus with hydrochloric acid and concentrated on a steam bath to one-fifth of its original volume. Four volumes of 95 percent alcohol, containing 20 c.c. of concentrated hydrochloric acid per liter, were then added to the urine concentrate and the resulting solution was refluxed for about four hours. The solution was then poured from the solid residue and neutralized with concentrated sodium hydroxide. The alcohol was distilled off and the viscous residue taken up in absolute alcohol. This absolute alcohol solution was poured off from any undissolved solid material, distilled down, and the thick gummy residue remaining in the distillation flask was extracted repeatedly with acetone. The acetone extract was concentrated down to an almost solid mass and then extracted with ether until the ether layer showed no color. This ether extract was concentrated and the residue taken up in about 50 percent alcohol solution. All of the above described distillations were carried out under reduced pressure in an atmosphere of carbon dioxide. The final alcohol solutions contained in the neighborhood of 4,000 rat units per c.c.

All birds were selected for size before the beginning of each experiment. The birds were selected in pairs or groups so that the weight of one uninjected bird corresponded to that of the injected bird (or birds, if several levels of hormone were used). In this way, two or more groups were usually made up for each experiment to ensure that the average weight of the uninjected birds and the injected birds on the various levels of hormone was about the same at the beginning of the injection period.

The birds were weighed and killed on the day following the last injection. The ovaries were dissected out immediately, weighed and fixed in Bouin's fluid. The oviduct or a part of it was also fixed in Bouin's fluid after it was measured and weighed.

Sections of the ovary were, in most cases, taken at intervals throughout the ovary while a few serial sections were also taken. Counts, when made, were based on all ova in three sections.

CHANGES IN EXTERNAL APPEARANCE

The average weights of the birds used in the various experiments are summarized in Table 1. Body weight, as measured by the average weight of the uninjected birds, fluctuated due to variations in the birds available. This was partly due to the fact that the birds used in the various experiments were hatched at different times. Thus the 30- and 40-day age groups were about the same in weight (except for the first group of 40-day-old birds which were small for their age) while the average weight of the 63- to 70-day-old crossbreds differed slightly in favor of the younger birds.

When injection was started at 10 days of age or less, the largest dose of pregnant mare's serum (2 c.c.) had a retarding effect on growth. Out of four chicks injected when hatched and daily thereafter with 2 c.c. of serum, only one survived to 10 days of age. The effect of the different amounts of serum on the weight of the survivors in this youngest group was slight. Ten day injections of 2 c.c. of mare's serum had a slight effect on the chicks killed when 15 days old. Twenty day injections (necropsy at 30 days) also apparently retarded growth. Mare's serum perhaps retarded growth in the second group of 40-day-old birds but except for this group apparently had little or no effect on the weight of the older birds even when injection was continued for 42 days. Some batches of oestrin used were toxic and retarded growth. It was usually possible to discontinue the use of such batches of oestrin and use instead preparations which did not retard growth. Never-

theless, it is evident that the weight of the birds injected with oestrin was retarded but since there did not appear to be a similar effect on the ovary and oviduct, the data for these birds are included.

tibia for some of these birds. The weight of the tibia was then calculated as a percentage of total body weight with the following results: average for three birds injected with 150 R.U. of serum with or without 500

TABLE 1.— *Summary of weights of birds, ovaries, and oviducts*

Exp. No.	Hormone injected daily	Injection period	Age when killed	No. of birds	Average weight of birds	Average weight of ovary	Average weight of oviduct
		days	days		gm.	mgm.	mgm.
1	Not injected		10	2	49	6	8
1	P.M.S.* 15 R.U.	10	10	2	50	16	23
1	P.M.S. 38 R.U.	10	10	2	45	27	27
1	P.M.S. 150 R.U.	10	10	1	58	37	—
2	Not injected		15	3	104	27	—
2	P.M.S. 38 R.U.	10	15	2	91	150	—
3	Not injected		30	4	247	73	33
3	P.M.S. 38 R.U.	5	30	3	253	112	35
3	P.M.S. 150 R.U.	5	30	4	231	149	75
4	Not injected		30	4	287	79	33
4	P.M.S. 150 R.U.	20	30	4	210	473	1586
5	Not injected		40	2	217	58	26
5	P.M.S. 15 R.U.	10	40	2	219	94	66
5	P.M.S. 38 R.U.	10	40	2	214	87	108
5	P.M.S. 150 R.U.	10	40	2	205	260	296
6	Not injected		40	3	269	117	148
6	P.M.S. 150 R.U.	10	40	4	229	341	473
6	P.M.S. 150 R.U. & Oestrin 1000 R.U.	10	40	4	225	283	950
7	Not injected		42	2	382	101	52
7	P.M.S. 150 R.U.	21	42	2	422	1172	7173
8	Not injected		63	2	698	179	215
8	P.M.S. 150 R.U.	42	63	1	680	680	6970
8	P.M.S. 150 R.U. & Oestrin 1000 R.U.	42	63	1	475	2470	10020
8	Oestrin	42	63	1	376	114	1600
9	Not injected		63	3	795	167	163
9	P.M.S. 38 R.U.	42	63	2	720	343	1890
9	P.M.S. 150 R.U.	42	63	3	825	4612	13060
9	P.M.S. 38 R.U. & Oestrin 200 R.U.	42	63	2	648	338	2810
9	P.M.S. 150 R.U. & Oestrin 500 R.U.	42	63	3	665	1094	12720
10	Not injected		70	2	733	201	99
10	P.M.S. 15 R.U.	10	70	2	708	313	195
10	P.M.S. 38 R.U.	10	70	2	738	346	1361
10	P.M.S. 150 R.U.	10	70	2	733	364	1153
11	Not injected		100	2	920	265	204
11	P.M.S. 15 R.U.	10	100	2	1020	239	189
11	P.M.S. 38 R.U.	10	100	2	885	384	408
11	P.M.S. 150 R.U.	10	100	2	948	599	349

* P.M.S. = Pregnant mare's serum.

Most of the pullets injected for 42 days with pregnant mare's serum (150 R.U.) or pregnant mare's serum (150 R.U.) and oestrin (500-1,000 R.U.) appeared to be broader across the back and shorter in the leg than the uninjected birds or the birds injected with less serum. Data were obtained on the length and weight of the right

R.U. of oestrin—0.729 percent; average for four birds injected with 38 R.U. of serum with or without 200 R.U. of oestrin—0.915 percent; average for three uninjected birds —1.054 percent. The difference between the first and last group is 0.325 ± 0.069 or 4.7 times the standard error and hence statistically significant. Hutt (1929) has reported

that there is a sex dimorphism in the appendicular skeleton of the fowl, the long bones of the leg and wing being shorter in the female. Hutt also found that the bones of the capon were longer than those of normal males. Similar results have been reported for other species. The results of Tandler and Keller (1910) are of particular interest since they found that castrated cows had longer bones than normal cows. Our results indicate that prolonged stimulation of the ovary may cause cessation of growth of the long bones. In this connection Evans (1935) has stated that "it is possible that function of the reproductive system hastens closure of the epiphyseal disks."

The comb and wattles were measured at the beginning and end of each injection period and at other intervals when injection was continued for more than 10 days. Only the measurements taken at necropsy will be considered here. The maximum length plus height was used as a measure of size. This test of comb growth devised by Gallagher and Koch (see p. 377 "Sex and Internal Secretions" edited by Allen) has since been used by many investigators. It is not an accurate measure of weight differences, since a 37 mm. comb (length plus height) weighed about 0.3 gm. whereas a 127 mm. comb weighed 12.1 gm., but is used here because only a few of the combs were weighed. All statements made in the following paragraph regarding increase in size of comb are based on comparisons with combs of uninjected birds of the same age and do not refer to the absolute increase in the size of the comb during the injection period.

The effect of pregnant mare's serum on the comb and wattles of pullets in the different groups varied with age, length of injection period and breed. There was no measurable change in the comb of 10- or 15-day-old crossbred pullets which had been injected for 10 days, nor in the size of the comb of crossbred pullets killed when 30 days old and previously injected for five days with pregnant mare's serum at a level of 150 R.U. When pullets were injected for 10 days and killed when 30 days old the size of the comb increased about 80 percent. There was an increase of 150 percent in the size of the combs of birds injected for 21 days and killed when 42 days old. The combs of pullets injected with the same amount of pregnant mare's serum (150 R.U.) for 10 days and killed when 40 days old increased about 20 percent in size, the response being less when a smaller amount of mare's serum was injected. Crossbred pullets injected for 42 days with 150 R.U. serum showed an increase in size of about 125 percent whereas the increase for Leghorn pullets was about 350 percent. The comb increased less in size when both mare's serum (150 R.U.) and oestrin (500-1,000 R.U.) were injected; when less serum (38 R.U.) was injected the comb was approximately doubled in size after 42 days' injection but oestrin (200 R.U.) appeared to have little effect in this case. The combs of the pullets injected for 10 days and killed when 70 days old increased slightly in size while the combs of the oldest group (100 days) did not increase in size except perhaps at the highest level of pregnant mare's serum (150 R.U.) The single Leghorn pullet injected with oestrin only, had a small, pale comb.

The enlarged combs of the crossbred pullets were erect and turgid resembling those of males, although it should be stated that mature females of similar breeding also have erect combs. The 63-day-old Leghorn pullet injected with serum had a lopped comb typical of a mature hen while the bird injected with serum and oestrin had an erect comb resembling that of a male. The fact that Leghorn pullets injected by Asmundson and Wolfe (1935) had combs typical of mature females perhaps indicates that the comb response depends, at least partly, on

the age of the birds. The greater size of the comb of the Leghorn pullets after injection as compared with that of the crossbreds undoubtedly represents a genetic difference since the mature Leghorn normally has a much larger comb than these crossbreds or birds of similar breeding. That the difference is not due to the hormone level is indicated by the results of Callow and Parkes (1935) who found that the comb of the Plymouth Rock capon gave a much smaller response to injections of androsterone than the comb of the Leghorn capon.

In general, there was less increase in the size of the wattles than in the comb, but the variation was similar. The pullets injected for 20 or more days showed particularly large development of the wattles. This was also true of the earlobes. Thus the Leghorn pullets, injected for 42 days with serum, had large enamel white earlobes, whereas the earlobes of the uninjected birds, and the bird injected with oestrin only, were small and pale colored.

The cloaca and the pelvis of the 63-day-old birds differed. The cloacae of the injected birds (150 R.U. of serum, 1,000 R.U. of oestrin or oestrin and serum) were noticeably larger and more vascular than those of the uninjected birds. The pubic bones of the former were farther apart, a response also reported for mature birds by Bates, Lahr, and Riddle (1935) after injection with gonadotropic hormone. The birds injected with less serum showed little change. Similar differences were observed in the 42-day-old group and to a lesser extent in others. The 63-day-old birds injected with 150 R.U. of pregnant mare's serum, or with 150 R.U. of serum and/or oestrin for 42 days were frequently observed to exhibit a desire to mate.

CHANGES IN THE OVARY

The data on the weight of the ovary and oviducts of injected birds are summarized in Table 1. The average weight of the ovaries of uninjected birds increased with age except in the case of the first group of 40-day-old birds which were smaller than other birds of about the same age.

In general, it will be observed that the weight of the ovary increased with an increase in the amount of pregnant mare's serum injected. However, the response of the ovary to a given amount of serum decreased with increasing age so that in the case of 100-day-old birds 15 rat units daily had no effect. The largest dose (150 R.U. daily per bird) increased the weight of the ovary six times in the case of the youngest birds injected for 10 days while in the case of the oldest group the weight was not quite doubled. Since the older birds were much larger than the younger birds, the amount of hormone injected per unit of body weight was much less in the case of the older birds. It is possible that the amount of hormone injected, particularly in the case of the older birds, was not sufficient to elicit a maximum response.

The length of the injection period has a definite effect on the weight response. The variation in the weight of the ovary was considerable in all groups but was especially marked in the older birds and the birds injected for a longer period of time. Thus the weight of the ovaries from the three birds injected for 42 days with 150 R.U. of serum ranged from 1,270 mgm. to 9,190 mgm. There is no indication of refractoriness with an increase in the length of the injection period since the ovaries of the crossbred pullets injected for 42 days were about four times the weight of those of birds injected for 21 days. If the average weights of the ovaries of injected birds are compared with those of the uninjected birds it will be seen that at 42 days (after 21 days of injection) they weighed 11 times and at 63 days (after 42 days of injection) they weighed about 27 times as much as the ovaries of the latter.

Simultaneous injection of oestrin with serum did not have a consistent effect on the

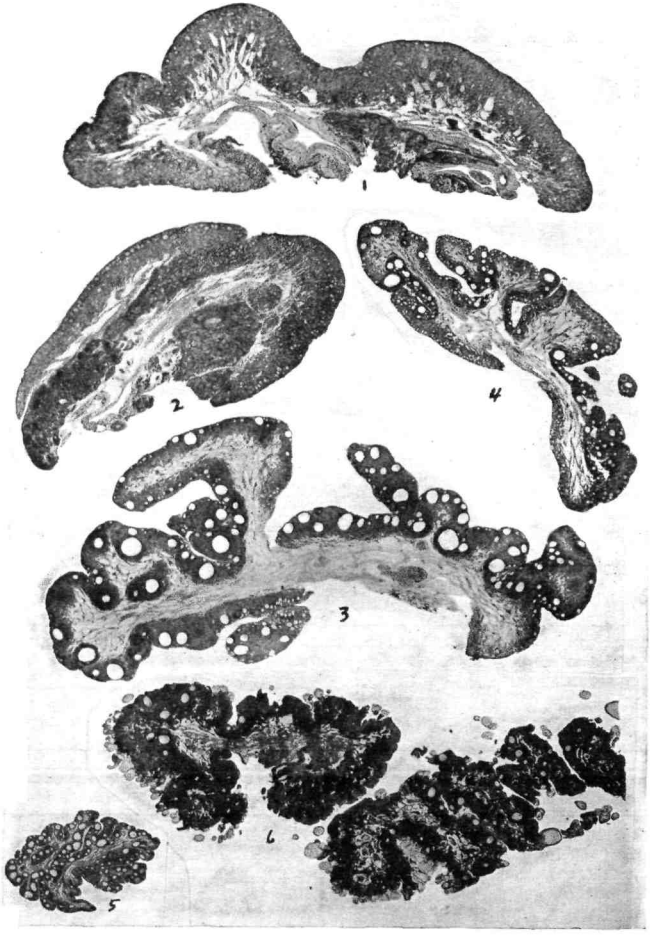
ovarian response and as found by Juhn, D'Amour, and Gustavson (1930), oestrin alone had little or no effect on the ovary.

The histological changes in the ovary are shown in Figures 1 to 9 inclusive. They may be summarized as follows: In general, the ovaries of injected birds are more vascular. There is an increase in the interstitial tissue of the ovary of birds injected for 10 days and killed when 10 or 15 days old but no increase in the size of the ova (Figures 1 and 2). There does not appear to be any marked stratification in these ovaries such as reported by Domm (1934a).

When older birds are injected, the amount of interstitial tissue is increased and the ova stimulated to grow more rapidly than those of the uninjected birds. Thus the average difference (0.01 mm.) between the size of the ova of 30-day-old birds injected with pregnant mare's serum for five days and the ova of uninjected birds was 4.6 times the probable error. For the 40-day-old birds injected with serum for 10 days the increase in the average diameter of the ova (0.03 mm.) over the controls was 42 times its probable error. As a result of the increase in the interstitial tissue the ova are pushed farther apart (compare Figures 2 and 3). The extreme development of the interstitial tissue occurred in birds injected for a period of 20 days or more. Many of the ova are virtually pushed beyond the periphery of the ovary (see Figures 5 to 8). The largest one of these was 5 mm. in diameter. Some appeared to be cystic, while all were more or less misshapen and were presumably incapable of normal development. The ova still within the ovary were apparently developing normally. Not all of the ovaries show the extreme development of ova grown beyond the periphery of the ovary, even after 42 days of injection with serum (Figure 8). There was, however, in all cases an increase in the size of the ova and an increase in interstitial tissue. This applied also to birds over 63 days old. Thus measurements on the ova of injected and uninjected Leghorn pullets (see Asmundson and Wolfe [1935] for data on the weights of these ovaries) killed when 117 days old show a statistically significant difference (7.4 times the probable error of the difference) in favor of the bird injected for 10 days. Nevertheless, the differences observed by us and by Asmundson and Wolfe (1935) in the ovaries of nearly mature injected and non-injected pullets are slight as compared with the differences between the ovaries of laying and non-laying birds. There was a comparatively large increase in the weight of ovaries of young birds but in no case observed by us does mare gonadotropic hormone appear to have induced rapid yolk formation such as occurs normally immediately prior to ovulation.

In many of the ovaries of these birds there were two or more ova within a single follicle. There were slightly more of these observed in the ovaries of 40-day-old birds injected with pregnant mare's serum and oestrin than in the ovaries of the other birds of the same age or about five double ova in the ovaries of injected birds to every three in the ovaries of uninjected birds, but the differences are not statistically significant since there is considerable variation within the groups. Two ova in a single follicle have been previously observed by Brambell (1925). Many are probably resorbed but it seems possible that some of them mature and after ovulation give rise to a part of the double-yolked eggs of the type where two yolks have all envelopes in common (Curtis 1915).

Extract of pregnant mare's serum was tested by injecting the preparation for five days at levels of 38 and 150 rat units daily with necropsy at 30 days. The average weight of the ovaries was: 38 R.U.—94 mgm. (3 birds): 150 R. U.—101 mgm. (4 birds). The response was thus less than when untreated serum was injected (see Table 1 for data on birds injected with un-



FIGS. 1-6 (See opposite page for explanation)

treated serum). Histologically the ovaries of the birds injected with the extract of mare's serum resembled those of birds injected with the untreated serum. There was thus no difference in the type of response although the changes that were apparent were, on the average, less marked than in the ovaries of the birds injected with untreated serum.

The ovary of the bird injected with oestrin did not differ from that of the control (see Figure 9).

CHANGES IN THE OVIDUCT

The weight of the oviduct increased after 10 days of injection with serum into day-old chicks. Five-day injections of 150 R.U. of serum or extract also increased the weight of the oviduct while less hormone apparently had no effect in so short a time. Ten-day injections into older birds also increased the weight of the oviduct except in the case of the oldest group injected with 15 rat units daily of mare's serum in which case there was no increase in the weight of either the ovary or the oviduct. Longer periods of injection resulted in a much greater increase in the weight of the oviduct (Table 1).

When both oestrin and serum were injected there was a significantly greater increase after 10 days' injection than when serum only was injected. This difference was obliterated at the higher levels of serum after 42 days' injection since the oviduct had in each case apparently attained approximately its maximum functional non-stimulated size. That the oviducts had reached their approximate maximum size is indicated by the average weight and by the range in the weight of the oviducts of the six crossbred birds injected with the maximum amount of mare gonadotropic hormone (150 units daily for 42 days) which was less than a gram above and below the average weight of about 13 grams shown in Table 1. Oestrin alone also caused marked growth of the oviduct.

None of the birds injected for five or 10 days had perforated oviducts. In the case of the birds injected for 20 days and killed when 30 days old, the oviduct was separated from the cloaca by only a thin membrane. The birds which were injected for 21 days and were killed when 42 days of age and all of the birds which were injected for 42 days and were killed when 63 days of age had perforated oviducts whereas ordinarily, according to Greenwood (1935), the oviduct usually does not become perforated until the birds are about 140 days old or older which agrees with unpublished results obtained by Palmer (1932). Perforation of the oviduct or, more precisely, of the vagina thus resulted from prolonged injection of oestrin or of pregnant mare's serum. Presumably the serum stimulated the ovary to secrete oestrin which in turn acted on the oviduct since there is no indication that serum had any effect on the oviduct when the weight of the ovary did not increase. Since domestic fowls may lay before they are 140 days old, it is evident that the vagina is sometimes perforated much earlier but there is no evidence to show that this occurs as early as 42 to 63 days of age.

Histological changes, similar to those ob-

FIG. 1. Ovary of 10-day-old crossbred female injected daily from time of hatching with 150 R.U. of mare gonadotropic hormone. Note absence of follicular response and increase in interstitial tissue as compared with Figure 2 (×18).

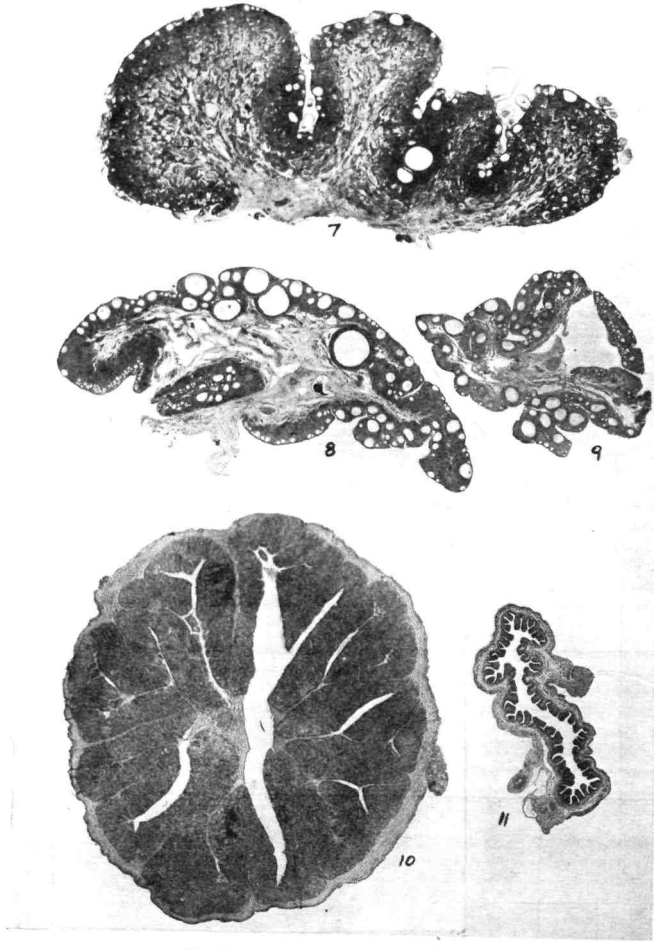
FIG. 2. Ovary of 10-day-old uninjected crossbred female (× 18).

FIG. 3. Ovary of 40-day-old crossbred female injected 10 days with 150 R.U. of mare gonadotropic hormone. Note increase in interstitial tissue and in size of follicles as compared with Figure 4 (× 9).

FIG. 4. Ovary of 40-day-old uninjected crossbred female (× 9).

FIG. 5. Ovary of 42-day-old uninjected crossbred female (× 4.5).

FIG. 6. Ovary of 42-day-old crossbred female injected 21 days with 150 R.U. of mare gonadotropic hormone. Note marked increase in interstitial tissue and growth of follicles (× 4.5).



FIGS. 7-11 (See opposite page for explanation)

served by Juhn, D'Amour, and Gustavson (1930) and by Raspopova (1935), followed the injection of pregnant mare's serum, pregnant mare's serum extract or oestrin. There was general growth of the oviduct with an increase in the muscles, connective tissue stroma and the epithelium. This growth of the oviduct was less in birds injected for five or 10 days than in those injected for 20 or more days. The oviducts of the latter had well developed tubular glands; the cells of the epithelium were crowded together giving them a high, columnar appearance. The nuclei of these cells were in the center of the cell but in some places were stratified, forming two apparent layers, the nucleus of one cell being lower or slightly further from the surface than that of the next cell. In contrast to this, the tubular glands were absent or poorly developed in the oviducts of the uninjected birds and the epithelium was low. The gross differences between the oviduct of an uninjected, 63-day-old Leghorn pullet and the oviduct of a pullet of the same age previously injected for 42 days with pregnant mare's serum are shown in Figures 10 and 11. In addition to the apparent differences in the growth of these oviducts, there are indications of secretion into the lumen of the oviduct of the injected bird (Figure 10) whereas there are no such indications in the case of the oviduct of the uninjected bird (Figure 11) nor were any indications of secretion observed in the case of any uninjected birds or of birds injected for only 10 or fewer days.

The bursa of Fabricius, according to Jolly (1915) reaches its maximum size when the fowl is about four months old after which it regresses until only a vestige remains when the bird reaches the age of one year. The bursa was largest in the 63- to 70-day-old birds (see Table 2) while some involution had apparently occurred by the time the birds were 100 days old. Injection of pregnant mare's serum or of oestrin was followed by regression of the bursa. Jolly and Pezard (1928) found that castration retards the involution of the bursa in the fowl while Riddle and Frey (1925) and Riddle (1928) have demonstrated a close parallelism between the growth and involution of the thymus and bursa in pigeons and doves. No effects that could be attributed to the involution of the bursa were observed which agrees with the results obtained by Riddle and Krizenecky (1931) and others that removal of the bursa and thymus has no apparent effect on the first generation of thymectomized birds.

DISCUSSION

It may be considered well established that there is an increase in the weight of the ovary of female fowl injected with pregnant mare's serum. This increase in weight occurs for birds varying widely in age as shown by the experiments of Asmundson and Wolfe (1935), Breneman (1936), and in this paper. However, the histological changes vary with age, there being only an interstitial response in the very young bird up to about 15 days of age while in older birds there is also a follicular response. The extra-follicular period in the growth of

FIG. 7. Ovary of 63-day-old White Leghorn female injected 42 days with 150 R.U. of mare gonadotropic hormone +1,000 R.U. of oestrin daily. Compare with Figures 6, 8, and 9 (× 8).

FIG. 8. Ovary of 63-day-oid White Leghorn female injected 42 days with 150 R.U. of mare gonadotropic hormone daily. There is a definite follicle response and also an increase in interstitial tissue though less marked than in Figures 6 and 7 (× 8).

FIG. 9. Ovary of 63-day-old uninjected White Leghorn female (× 8).

FIG. 10. Oviduct of 63-day-old White Leghorn female (from same bird as ovary in Figure 7). Note secretion which is collected mainly at the tips of the folds (× 16).

FIG. 11. Oviduct of 63-day-old uninjected White Leghorn female (from same bird as ovary in Figure 9) (× 16).

the oocyte in the ovary of the domestic fowl is terminated about four to six days after hatching according to Marza and Marza (1935) who have recently made observations on the growth of the avian ovum and have reviewed the literature on this subject. Injection of pregnant mare's serum before the termination of this period does not result in follicular growth. It is not certain that the termination of this period coincides with the beginning of follicular response although it is evident that no follicular response is obtained by ten day injection of serum into females of the heavier breeds if injection is started on or before the fifth day after hatching. Domm (1934b) has reported follicular growth in Brown Leghorns injected, for 15 to 21 days, with hebin starting on the second or fourth day after hatching. The birds were, therefore, slightly older when killed which, together with the difference in the hormone and the breed, probably accounts for the apparent difference in results. Schockaert (Allen: "Sex and Internal Secretions"), using a fresh saline emulsion of cattle anterior pituitary, has apparently obtained results with 31-day-old ducks that correspond to those obtained by us with chicks of a similar age. Similar results with rats to those obtained by us, that is only interstitial response in very young animals with a follicular response in older animals, have been reported by Saunders and Cole (1936) and Cole (1936) with mare gonadotropic hormone while Selye and Collip (1933), Selye, Collip, and Thomson (1935) and Smith, Engle, and Tyndale (1934) have shown that ovarian response of rats to other gonadotropic hormones varies with age.

TABLE 2.—*The average weights of the bursa Fabricii*

Expt. No.	Age when killed	Injection period	Uninjected birds		Pregnant mare's serum (150 rat units daily)		Pregnant mare's serum (150 rat units daily)				Oestrin 1000 rat units daily	
							500 rat units of oestrin daily		1000 rat units of oestrin daily			
	days	days	No. of birds	Aver. wt. gm.	No. of birds	Aver. wt. gm.	No. of birds	Aver. wt. gm.	No. of birds	Aver. wt. gm.	No. of birds	Aver. wt. gm.
1	10	10	2	.043	1	.069						
3	30	5	4	.90	4	.65						
4	30	20	4	1.12	4	.33						
6	40	10	3	.50	4	.30			4	.30		
7	42	21	2	1.59	2	.83						
8	63	42	2	3.70	1	.20			1	.10	1	.70
9	63	42	3	1.86	3	.38	3	.08				
10	70	10	2	2.2	2	1.8						
11	100	10	2	1.2	2	1.3						

Once the intra-follicular period has started and a follicle has formed around the oocyte, both interstitial and follicular growth was observed, that is there was a definite follicle response in the case of birds injected from 10 to 30 days of age. The rate of growth of the ovum normally remains slow for a long time and, while injection with mare gonadotropic hormone (serum) hastens the rate of growth, it still remains slow compared with that observed just prior to ovulation. Even if ovulation can be induced in the fowl by the injection of serum it can obviously not occur so soon after injection (72 hours) as reported for the rat, ewe, cow, and sow (Cole [1936]).

There are quantitative differences in the response of the ovary according to the hormone level and the length of the injection period. Prolonged injection causes excessive

interstitial growth and the formation of cystic follicles.

The length of the injection period is important when the secondary effects of injecting pregnant mare's serum are considered. Here again the effects of prolonged injection appear to be quantitatively rather than qualitatively different from the effects of short periods of injection. Thus the perforation of the oviduct and indications of secretory activity in the case of the 63-day-old birds at the end of a 42 day injection period, are merely the culmination of a process which can be seen in the initial stage in birds injected for five or 10 days. After prolonged injection of oestrin or of mare's serum, the presence of secretion in the oviduct makes it appear probable that the oviduct of these birds is capable of functioning in every way like that of the mature bird. The results appear to be comparable to those obtained with mammals by Allen and Doisy (1924). It should be observed that growth of the oviduct apparently occurs prior to induced growth of the ovum indicating that the ovary is capable of secreting oestrin before it responds with follicular growth. On the other hand, there was no apparent growth in the combs of very young birds. This does not necessarily indicate that a comb stimulating hormone was not secreted by the ovary of these young birds, but merely perhaps that the amount was below the threshold required to cause measurable growth in the comb.

The changes in the oviduct, the bursa, and the skeleton after prolonged injection with serum indicate that the changes are comparable to those which occur when birds mature. Presumably they result from the elaboration of hormones by the ovary. Theelin is known to affect the growth of the avian oviduct (Juhn, D'Amour and Gustavson *[1930]*, Raspopova *[1935]*). Our results indicate that this hormone is also responsible for the changes observed in the bursa and skeleton.

SUMMARY

Young Leghorn and crossbred (Rhode Island Red × Plymouth Rock) female chickens were injected for five to 42 days with pregnant mare's serum and/or oestrin and killed when 10 to 100 days old. The combs of Leghorns responded more quickly than those of crossbreds although the crossbreds had large, turgid combs after prolonged injection with pregnant mare's serum.

The ovaries of birds injected with pregnant mare's serum were enlarged. In the crossbred birds injected for 10 days and killed when 10 or 15 days old, there was an increase in interstitial tissue but no apparent effect on the follicles. The ovaries of the older females injected for five to 42 days and killed when 30 to 100 days old were hyperemic, had increased interstitial tissue and enlarged follicles. No refractoriness was observed with increasing length of injection period but there was less response to a given amount of hormone in the older than in the younger birds.

The oviduct was enlarged after injection with pregnant mare's serum or oestrin. Prolonged injection (21 to 42 days) caused perforation of the vagina and secretion by the glands of the duct in birds that were 42 and 63 days old at necropsy.

The bursa of Fabricius was reduced in size after injection with oestrin or pregnant mare's serum. The tibia tarsus was shorter in birds injected for 42 days with serum than in uninjected birds of the same age (63 days).

The changes in the oviduct, bursa and long bones of the legs were presumably caused by the secretory activity of the ovary.

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Annual Meeting

University of Wisconsin, Madison, Wisconsin
August 10-13

Program Committees:

Extension—J. C. Taylor, Chairman, New Jersey State University, New Brunswick, N.J.
G. E. Klein, Massachusetts State College, Amherst, Mass.

Teaching and Administration—R. M. Smith, Chairman, Univ. of Arkansas, Fayetteville, Ark.

Genetics and Physiology—L. W. Taylor, Chairman, College of Agr., Berkeley, California.
Walter Landauer, Connecticut Agr. Exp. Sta., Storrs, Connecticut.

Nutrition—H. J. Sloan, Chairman, Univ. of Minnesota, St. Paul, Minn.

Pathology—E. P. Johnson, Chairman, Virginia Polytechnic Inst., Blacksburg, Va.
W. R. Hinshaw, Univ. of California, Davis, Calif.
H. Van Roekel, Massachusetts State College, Amherst, Mass.

Economics and Poultry Farm Management—Alfred VanWagenen, Chairman, Cornell Univ., Ithaca, New York.
R. R. Slocum, Bur. of Agr. Economics, U.S.D.A., Washington, D.C.

Plan of Sessions:

By action of the Directors of Poultry Science Association, sessions of the Association this year will be from 9 to 12 A.M., and 1:30 to 4:30 P.M. The business meeting of the Association will occupy the time Friday morning, August 13. Tuesday afternoon, August 10; Wednesday morning, August 11; Thursday morning, August 12, and Friday afternoon, August 13, will be devoted to a general program, wherein there will be presented papers of greatest general interest selected from the various sections. Wednesday afternoon, August 11 and Thursday afternoon, August 12, will be devoted to meetings of the sections at which time papers of special interest to those sections will be presented and discussed.

Joint Committee on Poultry Housing:

Poultry Science Association—D. H. Reid, Chairman, Texas A. & M. College, College Station, Tex.
C. W. Carrick, Purdue Univ., Lafayette, Ind.
Carl Frisch-Knecht, Utah State Agr. College, Logan, Utah.
C. L. Morgan, S. Car. Agr. College, Clemson College, South Carolina.

American Society of Agricultural Engineers—F. L. Fairbanks, Chairman, Cornell Univ., Ithaca, N.Y.
M. A. R. Kelly, Bur. of Agr. Eng., U.S.D.A., Washington, D.C.
C. P. Tobin, The Celotex Corp., 919 So. Michigan Ave., Chicago, Illinois.
C. H. Van Vlack, Iowa State College, Ames, Iowa.

Poultry Research Competition Committee—
D. C. Warren, Chairman, Kansas Agr. College, Manhattan, Kansas.
L. C. Norris, Cornell Univ., Ithaca, N.Y.
A. J. G. Maw, Macdonald College, Can.
W. R. Hinshaw, Univ. of California, Davis, Calif.
G. D. Buckner, Kentucky Agr. Exp. Sta., Lexington, Ky.

Last Call for Papers:

If you wish to present a paper at the annual meeting and have not gotten in touch with a member of the appropriate committee, you must do so at once.

INSTRUCTIONS TO CONTRIBUTORS TO POULTRY SCIENCE

POULTRY SCIENCE is devoted to the publication of the results of research concerning the various aspects of the science of poultry husbandry. The plan does not provide for reviews or abstracts, but critical papers may be accepted if of sufficient interest. For the purpose of this journal poultry science is interpreted as including the scientific aspects of all fields of poultry husbandry, some of which are embryology, genetics, nutrition, physiology, pathology, and economics.

Manuscripts. Manuscripts intended for POULTRY SCIENCE should be sent to the Managing Editor. Approval by at least two members of the Editorial Board is necessary for acceptance.

While no arbitrary limit is placed on the length of papers which will be accepted, it is urged that papers intended for the journal be as brief as is consistent with adequate presentation of the subject matter. Preliminary notices will not be accepted.

Manuscripts should be typed, double space with wide margins. The left-hand margin should be at least 1½ inches. The title pages should include title of paper, name of author, institution, and number of figures or plates. A condensed title not to exceed 65 letters (counting spaces as letters) should be given. Figures and tables should not be in the body of the manuscript, but on separate sheets.

Cuts. Figures, groups of figures, or plates must conform to the dimensions of the page [text figures either 2½ or 5¼ inches in width (one or two columns); plates 5 x 7½, double plates 11¼ x 7½ inches] after the desired reduction. The amount of reduction desired should be indicated in each case. For numbering or lettering figures or graphs, printed numbers or letters of sufficient size to allow for the desired reduction should be used.

Drawings intended for photographic reproduction should be made in black ink on white or blue-white paper or bristol board, not on cream-white or yellow-white. For graphs in which ruling of paper is not to appear, blue-lined-ruled paper should be used. If ruling is to appear, red-line-ruled paper should be used. Photographs intended for reproduction should be printed on glossy paper with good contrast and mounted with colorless paste, not glue. Legends for figures and graphs are desirable and should explain lettering or other symbols as well as indicate as briefly as possible the significance of the figure or graph. Legends should be typed on sheets separate from the text and the figures or graphs.

Authors will be allowed a single column cut or its equivalent for each two pages, with a maximum allowance on any paper of $15.00. Authors will be asked to pay the extra cost for an excessive amount of especially expensive tabular material. Contributors who are not members of the Poultry Science Association will pay for all cuts.

Proofs. Galley proofs and engravers' proofs of figures will be sent to authors. Page proofs will not be sent the author unless specifically requested on the galley. Alterations from copy exceeding 10 per cent of the cost of composition will be charged to authors. It is required that manuscripts be returned with corrected galley proofs. Original photographs may be retained by the author. Places for insertion of figures, graphs, and tables should be indicated on the corrected galley proof when returned. Proofs should be returned to the Managing Editor, POULTRY SCIENCE, Cornell University, Ithaca, New York.

The corrected galley proof should be accompanied by an author's abstract for publication in *Biological Abstracts*. Instructions for preparation of the abstract will accompany the galley proof.

Reprints. Fifty reprints without covers will be furnished free and additional copies will be furnished at cost. Requisitions for additional reprints and reprint covers should be drawn on the Banta Publishing Company, Menasha, Wisconsin.

References. (1) All literature cited should be listed under the title "References" at the close of each article. (2) The references should be listed by author in alphabetical order, the year of publication immediately following the author or authors. (3) The initials of the senior author should follow the surname, whereas those of the other authors should precede. (4) All references to volume number should be in arabic numerals, without the use of the abbreviation "Vol." and the issue number omitted. (5) In listing references to articles appearing in periodicals and bulletins the first and last pages should be given. (6) In giving titles of papers only the proper nouns should be capitalized. (7) In the body of the article the reference to the publication should be made by listing the year of publication in parentheses, thus: St. John and Johnson (1931).

The arrangement of references in the current issue of POULTRY SCIENCE will serve as a guide to those who in the future may prepare manuscripts for this journal.

Authors should not quote references unless they have consulted the original source material. If the original publication to be quoted is not available to the writer, he should indicate the source from which his quotation is taken thus: "according to St. John and Johnson as quoted by Jull (1931)," and the publication of Jull should be listed in the references at the conclusion of the article. In case of more than one paper by the same author or authors, the order should be chronological.

POULTRY SCIENCE

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Contents

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