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GROWTH AND DEVELOPMENT

With Special Reference to Domestic Animals

LI. Seasonal Metabolic and Endocrine Rhythms in the Domestic Fowl

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FOREWORD

The special investigation on growth and development is a cooperative enterprise in which the departments of Animal Husbandry, Dairy Husbandry, Agricultural Chemistry, and Poultry Husbandry have each contributed a substantial part. The parts for the investigation in the beginning were inaugurated by a committee including A. C. Ragsdale, E. A. Trowbridge, H. L. Kempster, A. G. Hogan, and F. B. Mumford. Samuel Brody served as Chairman of this committee and has been chiefly responsible for the execution of the plans, interpretation of results and the preparation of the publications resulting from this enterprise.

The investigation has been made possible through a grant by the Herman Frasch Foundation, represented by Dr. F. J. Sievers.

M. F. MILLER

Director Agricultural Experiment Station

ABSTRACT

The essential factual contributions of this bulletin are demonstrations of: 1) a parallelism between fasting energy metabolism and egg production in the domestic fowl during the first productive year; 2) a parallelism between heart rate (measured by an electric stethoscope while the bird rested in the closed, darkened respiration chamber), and metabolic rate, which parallelism, however, is not close enough to permit the calculation of metabolic rate from heart rate; and 3) a profound depression of egg production after thyroidectomy, with restoration of egg production following thyroxin injection. The thyroid, thus, is shown to play a vital part in the process of egg production. The effect of thyroxin administration on body weight and egg production is seen to be a function of the dosage. Egg production declines when excess thyroxin is administered, or when a deficiency of thyroxin is attained through thyroidectomy. It appeared that about 2 mg thyroxin per $(kg)^{.73}$ was the approximate dosage required to restore egg production in the thyroidectomized fowls used in the investigation to 60% of normal. This bulletin also describes a modified Regnault-Reiset metabolism apparatus employed for measuring the metabolism of fowls, an adaptation of an electric stethoscope to the measurement of heart rates of resting fowls in closed chambers, and a critical review of the literature of photoperiodicity and thermoperiodicity.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Professor S. Brody under whose direction these investigations were carried out; to Professors H. L. Kempster, A. G. Hogan, C. W. Turner, and A. C. Ragsdale by whom valuable advice and assistance were freely given; and to Mr. Hudson Kibler who rendered valuable assistance in the making of computations and figures.

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LI. Seasonal Metabolic and Endocrine Rhythms in the Domestic Fowl*

C. F. WINCHESTER

Seasonal rhythms of life processes are among the most striking of natural phenomena. The influence of changing day-length on reproductive processes of plants and animals is a particularly dramatic example. In temperate regions maize blooms only when length of day is decreasing while wheat blooms only when length of day is increasing. Deer, goats, and most sheep become sexually active when day-length is decreasing, while ferrets, field mice, hedgehogs, and nearly all birds come into sex activity when day-length is increasing. These rhythmic responses of animals to length of day tend to be disturbed by domestication.

The purpose of this bulletin is to present the results of a study of seasonal rhythmicity in the domestic fowl, with particular reference to the interrelations between egg production, energy metabolism, temperature, length of day, and hormone activity. A summary of part of the voluminous, but scattered, literature on seasonal rhythms is given in the review which follows.

I. REVIEW OF LITERATURE

Influence of Light on Sex Activity

The term photoperiodicity was first used in the pioneering (1920) paper by Garner and Allard who reported that "sexual activity can be attained by the plant only when it is exposed to a specifically favorable length of day. . . . The term photoperiod is suggested to designate the favorable length of day for each organism, and photoperiodism is suggested to designate the response of organisms to the relative length of day and night." The terms *photoperiod* and *photoperiodism* are now generally used to describe phenomena not only in plants, but in all living organisms.

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The seasonal regularity of bird migrations has always attracted the attention of naturalists. As long ago as 1907 Schäfer noted that bird migrations from year to year correspond closely to changes in day-length, but are nearly independent of environmental temperature. He reasoned that birds leave regions of shortening days and seek regions in which length of day is sufficient to allow them to satisfy their appetites.

Since the publication of Schäfer's paper, experimental methods have been used in attempts to determine the causative mechanisms of sexual periodicity.

Marcovitch (1924) observed that aphididae migrate, apparently, in response to a change in light-period, but showed that the migration is actually a direct response to chemical changes in the sap of the host which are caused by changes in day-length.

It remained for Rowan, in 1925, to demonstrate experimentally that testis size of birds is increased by lengthening the light-period to which the birds are exposed. Juncos were exposed to ordinary electric light each evening with the result that testis size increased enormously even though the environmental temperature was sometimes as low as 52° below 0°F. Later, Rowan (1928) reported a similar effect caused by forcing the birds to remain active for periods which at first were of a few minutes duration and which were gradually increased to 4 hours as the experiment progressed. Rowan (1931) stated that northward or southward migrations occur as a consequence of increasing or decreasing gonad activity and related endocrine changes. This idea was supported by the report (Rowan 1932) that crows did not migrate after castration.

Bissonnette (1931a), working with starlings, confirmed Rowan's reports on the influence of light on spermatogenesis, but not on the influence on spermatogenesis of forced activity of the birds at night. Wave-length of light was found by Bissonnette and Wadlund (1931) and Bissonnette (1932a) to be an important factor; red light was found to give greater modification of spermatogenic activity than white or green light when the intensity of light reaching the birds was equal in each case. Spermatogenesis was accelerated at an increasing rate with increase of light intensity from 10 to 25 and possibly to 40 (but not to 60) watt bulbs kept just outside the cages (Bissonnette 1931b). When, in addition to the red light, a sunlamp was used, the time required for the birds to reach maximum testis size was reduced from 60 to 32 days (Bissonnette and Wadlund 1932). Some of his earlier experiments led Bissonnette (1931a) to

suggest that in the starling "it is relative light ration as compared with the previous rations and not absolute light ration at any time that determines the reaction of the germinal elements of the testis to treatment." Shortly after the above statement was published further work led Bissonnette and Wadlund (1931), to state that "the slow increases of daily light in nature in spring are not necessary for consistent or uniform results, so far as germ cell activity is concerned, at least with this (1.7 foot candles) intensity." Bissonnette (1936) later reported that female ferrets, like starlings, respond to a large initial increase of the light period with full sexual activity, while male ferrets "require increasing lengths of day to be completely and quickly activated."

Baker and Ranson (1930) reported that a decrease of light-exposure period from 15 to 9 hours almost always prevents reproduction in field mice, the female being chiefly affected. Breeding habits of field mice in three different localities in England were studied by Baker and Ranson (1933). They reported that the breeding season lasts from February or March to September or October. A finding which the authors were not able to explain is that "breeding starts latest in the most southerly of the three areas, and continues latest in the most northerly."

Marshall (1936) has cited examples of sheep and deer which after having been transported from the northern to the southern hemisphere bred, not in the usual calendar months, but in autumn. This demonstrates that autumn breeding in these animals is an inherent response to environmental conditions.

Bissonnette, (1932b and 1938) exposed ferrets to artificial light at night with the result that young females were brought into estrus as early as 193 days before the normal time, and animals of both sexes became sexually active much earlier than the normal mating period. However, when these animals were bred pregnancy did not follow. It is the opinion of Bissonnette that only the males were infertile and that they require some "conditioning factor (other than light) for complete fertility."

Light influences the reproductive cycle of ferrets largely before, rather than after, the onset of estrus. This was shown by Marshall and Bowden (1934), who reported that female ferrets which had not entered the first stages of heat, when placed in light of very low intensity (incomplete darkness), did not come in heat; but those which had already reached the first stages of heat, under these same conditions, entered into full estrus in spite of the lack of normal

light. When artificial illumination was given for a period of 8 hours after night-fall the onset of estrus was accelerated by as much as 148 days. Hill and Parks (1934) demonstrated that ferrets in darkness, reached full sexual activity within the normal range of time.

There is abundant evidence that the eyes are essential for the response of animals to light. Double cataract was followed by complete anestrus for two full seasons even with added artificial light (Marshall and Bowden 1936) ; while cataract of one eye did not affect the cycle (Marshall and Bowden 1934). When light reaching the eyes of ferrets and ducks was reduced by hoods the sexual response to light was arrested (Bissonnette 1933 and 1935). The sex cycles of ferrets were freed from photic control when the optic nerve was severed, and instead there appeared "an inherent rhythm of the hypophysis—which leads to an inherent sexual cycle by alternate rise and fall in secretion or liberation of gonadotropic hormones" (Bissonnette 1938). These results led Bissonnette to conclude that the "optic nerves are necessary for the transmission of the stimulus to the brain and hypophysis and that such transmission is nervous through the optic nerves rather than humoral through the blood stream."

In contrast to material given in the preceding paragraph, there is evidence that organs other than the eyes may, in some cases, act as photoreceptors. Benoit (1934) reported that drakes did not respond normally to light when the eyes were hooded, but later reported (1935 a, b, and c) that severing of the optic nerves or even removal of the eye balls did not result in loss of the sexual response to light.* Ivanova (1935) reported that when the eyes of sparrows were covered and part of the skin (from which the feathers had been plucked) was exposed to light, testicular growth increased.

There is evidence that poikilotherms exhibit responses to light. Florentin and Stutinski (1936) found that in frogs kept in darkness the anterior pituitaries lose the chromatophobe cells and most of the basophilic cells. According to Koller and Rodewald (1933) the influence of light, through the hypophysis, causes changes in the color of the epidermis of frogs.

An interesting experiment was reported by Hoover (1937), who demonstrated that by first exposing brook trout to artificial light at night, and later discontinuing the artificial light treatments and in

*Rowan (1938), who believes that "the stimulation of the pituitary is affected by changes of metabolism and physiology by lengthening the period of diurnal activity", has pointed out that Benoit's "blinded birds . . . . . were kept together with normal birds, (and thereby) disturbance and enforced wakefulness were imposed on the blinded individuals".

addition excluding daylight during part of each day, it is possible to cause the fish to become sexually active 3 (females) to 3½ (males) months earlier than the normal spawning season.

Game birds and domesticated birds as well as juncos, starlings, and crows have been found to respond to light with modifications of the laying seasons. Cole (1933) obtained eggs from mourning doves months earlier than the usual laying season. Clark, Leonard, and Bump (1937) brought a group of grouse into laying about a month earlier than the control birds by the use of artificial lights. Scott and Payne (1937) exposed turkeys to artificial lights before daylight with the result that they laid about 2 months earlier than the controls.

It has been a common practice for many years to expose hens to artificial light in the early morning or evening hours to increase egg production, particularly during seasons when egg production is normally low. Kable, Fox, and Lunn (1928) reported that artificial lighting increased the production of pullets up to 6.6%, but that this was followed by less than normal production during the second laying season. Parkhurst (1930) reported similar results. Bausman (1936) studied egg production of several flocks with and without artificial light treatment, and concluded that while artificial lighting does not increase yearly egg production, it can shift the peak of production so that a higher percentage of eggs are laid when production would normally be low. Warren and Scott (1936) shifted the time of laying from day to night by reversal of the hours of light and darkness. They reported that fowls responded, after 60 hours, by changes in time of laying.

Influence of Temperature on Sex Activity

Present knowledge of the influence of environmental temperature on sex activity is even more limited than knowledge of the influence of light on reproductive functions. In studies of the influence of environmental temperature on sexual activity, the hibernating, spring breeding, thirteen-lined ground squirrel is of particular interest. Some environmental factor *other than light* seems to be instrumental in determining the breeding season of this animal according to Moore and associates (1934), and Wells (1935a) who reported that *temperature* may be a factor since genital function is greatly diminished during hot summer weather. Wells (1935b) later reported that male ground squirrels in a breeding state placed in an environment of 4°C, remained in that state until autopsy, which occurred between 2 and 5 months after control animals had undergone sexual involution. Male ground squirrels placed in a 4°C environment during low sexual

activity reached the peak of reproductive capacity about 3 weeks later than animals which had been kept at higher temperatures.

According to Baker and Ranson (1932), with 15 hours of light a day, but at temperatures of around 5°C, the field mouse breeds less than at summer temperatures, although fecundity is unaffected by these conditions.

An interesting finding is that of Burrows and Byerly (1938), who reported that broodiness in hens was induced by an environmental temperature of 90°F (32.2°C), darkness, and the presence of chicks.

Influence of Temperature on Thyroid Activity and Metabolic Rate

Evidence that the thyroid profoundly influences the organs of reproduction will be given later. If, as the reports reviewed here indicate, temperature affects thyroid function, it is possible that environmental temperature may exert an influence on the gonads through the thyroid. Riddle (1927a) reported that thyroids of pigeons increase in size in autumn and winter and decrease in spring and summer, while the gonads exhibit a seasonal size rhythm which is directly opposite to that of the thyroids. In addition the serum calcium level is highest in summer, and the spleen and liver exhibit seasonal changes parallel to those of the gonads (Riddle 1929). According to Zalesky (1935), the thirteen-lined ground squirrel exhibits two seasonal peaks in thyroid size; the winter peak corresponds to high colloid storage and low thyroid activity, while the May peak corresponds to an increase in parenchyma and represents the season of greatest functional activity. Zalesky believes that *epithelial volume* rather than absolute volume of the thyroid is representative of its period of *greatest activity*. Cruickshank (1930) reported that thyroid weight in fowls shows a marked seasonal variation, the weight being greatest from January to March (0.13 gm.) and least from mid-March to mid-July (0.085 gm.). The thyroid weight was maximum prior to the period of greatest egg production. Iodine content of the thyroids varied with the weight of the organs.

Lee and Lee (1937) found that geese after thyroidectomy were able to make metabolic adjustments for adaptation to a colder environment which were as great as those made by normal geese. However, Ring (1939) reported that the increased metabolism of rats at lowered environmental temperatures is largely controlled by the thyroid.

The question of a relationship between environmental temperature and heat production has interested several groups of workers. Riddle, Smith, and Benedict (1932) reported a seasonal rhythm in heat pro-

duction of tippler pigeons with lowest values in spring and highest values in autumn. The autumn values were 15% greater in males and 8% greater in females than the corresponding spring values when determined at 20°C. Collip and Billingsley (1936) reported that as the environmental temperature decreased from 31°C to 16°C the basal metabolic rate of guinea pigs increased by 240%, and when the temperature further decreased to 0° the basal rate increased by 275% over the 31° basal rate. This effect was found to be independent of the thyroid, but diminished by adrenalectomy or hypophysectomy.

Kleiber and Dougherty (1934) reported that heat production of 6 to 15-day old chicks raised at controlled temperatures increased from 17.4 Cal. per day at 40° to 35.4 Cal. per chick per day at 21°C. Winchester and Kleiber (1938), who raised chicks at temperatures as low as 16°C, reported that, for chicks 5 to 12 days of age, heat production per chick was 12.4 Cal. per chick per day at 38°C and that heat production increased to 34.6 Cal. per chick per day at 18°C. At 16°C heat production was only 28.8 Cal. per chick per day.

Interrelations Between Season, Nutrition, and Reproductive Activity

The seasonal rhythms in reproduction of animals may be associated with seasonal rhythms in the nutritive value of their diets as indicated by the following citations. Friedman and Friedman (1939) reported rapidly growing green plants to be rich in gonadotropic hormones. Kohler, Elvehjem and Hart (1938) have reported a dietary factor in grass which is essential for rats and guinea pigs. The grass juice factor is believed not to be any one of the previously known essential dietary factors. These 2 findings may help to explain the well-known stimulating effects of spring pasture on animals. In general, there is a seasonal rhythm in the availability of energy, protein, minerals and vitamins of feeds.

Vitamin deficiencies result in impairment of reproductive functions. Vitamin E, according to Evans and Burr (1927), is essential both for spermatogenesis and for embryonic development. Barrie (1937) reported that female rats not sufficiently depleted of vitamin E to cause resorbtion of the fetuses, and others which had received inadequate vitamin E dosage after having been vitamin E sterile, bore young which exhibited delayed ossification and failed to grow at the normal rate. In many ways these young rats resembled hypophysectomized rats, and on histological examination their pituitaries were found to

be abnormal. Wiesner and Bacharach (1937) reported that rats on a vitamin E-free diet were sexually inactive even when wheat germ oil was administered with the ration. Administration of gonadotropic hormone from pregnant mare's blood was followed within 3 to 5 days by mating in 60% of the sexually inactive males. Because wheat germ oil did not restore sex activity the authors believe they may have been dealing with some deficiency other than that of vitamin E.

Fetal death and prolonged gestation and parturition are caused by vitamin A deficiency, and even when vitamin E and vitamin A deficiencies are both present the resorptive process typical of one deficiency can be distinguished from the other (Mason 1935). Evans (1932) reported that male rats can be placed on diets inadequate in vitamin A at weaning, and rendered sterile by the third month of life, but that sterility does not appear until later in rats entirely without vitamin E but with adequate vitamin A.

Vitamin A deficiency results in localized edema of the testes (Wolback and Howe 1925) and in addition, according to Sampson and Korenchevsky (1932a), the weight of the penis and the prostate is increased. Sampson and Korenchevsky (1932b) found degenerative changes of the seminiferous epithelium of vitamin A deficient rats similar to those found in vitamin E deficient animals and in cryptorchids.

The first symptom of vitamin A deficiency in female rats is cornification of the vagina (Aberle 1933a). A placental extract standardized for its production of mucoid vaginal cells had no effect on these cornified cells (Aberle 1933b).

Lucas, Hume, and Smith (after Marshall 1936) reported that marmosets which refused to take cod liver oil developed rickets, and in addition failed to breed. After irradiation with ultra-violet light the symptoms of rickets disappeared and the animals resumed sexual activity.

Reproductive processes of animals may be influenced by inadequate protein in the ration. Guilbert and Goss (1932) reported that rats receiving diets containing inadequate amounts of protein ($3\frac{1}{2}$ to 4%) exhibited either long and irregular sex cycles or ceased ovulation altogether. Pearson, Hart, and Bohstedt (1937) found that the estrous cycle disappeared in rats which had received 5% of casein as the chief source of protein, but reappeared when 5% of gliadin was added to the ration.

Perry (1938) reported early maturity in English sparrows fed wheat which had been exposed to ultra-violet light. Perry concluded that: ". . . a chemical substance in the wheat is changed by the

ultra-violet radiations. Most probably this substance directly affects the anterior hypophysis, which in turn stimulates gonad activity."

Interrelations Between Endocrine and Reproductive Activity in Birds

It is generally recognized that sexual functions are largely controlled by the pituitary,* and that sex activity is abolished by hypophysectomy. Hill and Parks (1933 a and b) have shown that after hypophysectomy, ferrets do not show a sexual response to light but remain permanently infantile so far as sexual activity is concerned unless they receive replacement therapy.

Less is known about the relationship between the various hormones and reproduction in birds than about comparable relationships in mammals.

Walker (1925) reported that ovulation in the fowl is inhibited by the administration of fresh hypophyseal substance, apparently due to an injurious effect of excess of the hypophyseal substance on the developing ova. Gutowska (1936) orally administered a pituitary extract to laying hens, and reported increased activity of the ovaries and oviducts. Asmundson (1931) reported that estrogen in doses as large as 67.5 rat units was without apparent effect on egg production of pullets.

Broodiness was reported to have been induced in actively laying hens of races "genetically capable of broodiness" by means of prolactin injections (Riddle and associates 1935, Riddle and Bates 1936, and Riddle 1937a).

Herrick and Torstveit (1938) reported that adrenalectomized male fowls which were maintained for more than 80 days with injections of cortical hormone, plus sodium chloride treatment exhibited reduction in comb size 2 days after adrenalectomy. Later the testes showed marked degeneration, and the lumina of the tubules were obscured by the breaking down of the tubule walls.

Benoit and Aron (1934) reported that thyroidectomy of cocks resulted in losses as great as $^{9}/_{10}$ of the testis volume in 20 days. Similar results were obtained with drakes.

Crew (1925) reported rejuvenation of 5 cocks and 7 hens 5 to 8 years of age with heavy doses of dessicated thyroid. Soon after treatment was begun all the birds passed through a moult, and when new plumage appeared it was characteristic of younger fowls. The very low egg production of 6.67 eggs per hen in 6 months was increased during the thyroid treatment to 34 eggs for the same period,

*Reviews of the physiology of the pituitary body are given by Van Dyke (1936 and 1939) & Allen (1932 and 1939).

and during the 6-month period following the treatment, production was 24 eggs per hen. However, Asmundson (1931) reported reduced egg size, particularly size of yolk, as a result of thyroid feeding. Two of the 4 birds nearly ceased laying after the treatment was begun, but one of them resumed laying eggs of normal size when human placenta was fed with the thyroid material.

Taylor and Burmester (1940) reported that thyroidectomy reduced egg production by approximately $\frac{2}{3}$ to $\frac{3}{4}$, while production of incompletely throidectomized birds was reduced by $\frac{1}{3}$. Egg weight of thyroidectomized hens did not increase as rapidly as did that of normal fowls. The operated birds accumulated large amounts of fat. Thyroid administration did not influence egg production, but resulted in decreased body weight of thyroidectomized hens.

Interrelations Between Seasonal Metabolic Rhythms and Reproductive Rhythms

Seasonal energy rhythms in humans have been described by various workers. It is somewhat surprising that civilized man, living in a highly modified environment so far as temperature, light, and nutrition are concerned, should be subject to these rhythms. Fitt (1924) believes that man is subject to a seasonal energy rhythm inherited from remote ancestors who were forced to hibernate each winter in order to survive. He has collected a considerable amount of evidence to show that humans are, in general, most successful in the spring and least successful near the end of winter. Llewellyn (1932), Whitaker (1938), and Ashley-Montague (1939) have reported seasonal rhythms in human reproductive activity.

Brody (1938) has compared the seasonal rhythm in resting metabolism of goats prior to the first lactation with gain in weight of these animals, and with conceptions and births in mature goats. Heat production was maximum in early spring, as was weight gained and the number of births. The maximum number of conceptions occurred in autumn. Seasonal metabolic rhythm of young goats was shown to be very similar to that obtained for adult humans. Brody's tentative conclusion is that a high breeding level, in goats, is inversely related to high metabolic rate.

Seasonal Rhythms in Fowls

Whetham (1933), as a result of a study of egg production records of flocks in various parts of the world, reported that the highest rate of egg production tends to be correlated with increasing day-length. Brody, Funk and Kempster (1938) have shown that (in Missouri) the peaks of egg production curves usually occur in April and May

and the troughs in December. In addition to the production rhythm and the seasonal rhythms in thyroid size, function, and iodine content, which have already been discussed, there is evidence of the possible existence of other metabolic rhythms, as will be shown in the two following paragraphs.

The levels of fat and sugar in laying hens' blood are known to be very different from those of non-layers. Lorenz, Entenman, and Chaikoff (1938) found that the blood of laying hens contains much more fat than that of non-laying fowls. Total blood lipid was reported to be as high as 4719 mg. per cent in laying hens as compared with less than 500 mg. per cent in males and in immature females. Lorenz, Chaikoff, and Entenman (1938) reported a great increase in neutral fat in the livers of pullets with the onset of maturity. Riddle (1927b) and Riddle and Burns (1927) reported a 35% increase of blood fat in doves and pigeons and a 20% increase in blood sugar in pigeons (Riddle 1927b) following ovulation.

There is some evidence that heat production of hens is higher when they are laying than when they are not. Gerhartz (1914) reported that heat production of 2 hens was higher when the hens were laying than at the end of the laying season. Dukes (1937a) reported that fasting metabolic rates of 9 hens in the laying state were higher than the fasting metabolic rates when these birds were laying very few, or no eggs at all, but the differences in metabolic rate were large enough to be significant in only 2 cases.

This review may be summarized as follows: Seasonal rhythms of sex activity are influenced by nutrition, temperature, and light. A voluminous literature has come into being as a result of attempts to discover the mechanisms through which these various factors influence sex rhythms. This is particularly true of light as a factor, and while various hypotheses exist, the mechanism remains obscure. The explanation most widely accepted is that light receptors (either the eyes or certain skin areas) stimulate the hypophysis through the nervous system. Rowan (1938) believes that "the stimulation of the pituitary is affected by changes of metabolism and physiology induced by lengthening the period of diurnal activity." While this is perhaps the oldest hypothesis, it has received but little attention.

II. MATERIALS AND METHODS

The studies of metabolic phenomena were based on data on New Hampshire hens which were found to be particularly adaptable to laboratory work because of their relatively phlegmatic temperament. White Leghorn hens, chosen for high production, were used in the thyroid studies.

The 15 New Hampshire pullets on which metabolic measurements were first made were hatched from February to March 1937. These pullets were selected from a larger flock to represent a "good" flock of hens (by commercial standards). Mean production of these fowls during the pullet year was 158 eggs of 63.1-gram size. Of the 15 pullets in the flock, 3 laid over 200 eggs, 3 laid less than 100 eggs and the balance laid between 100 and 200 eggs during the first production year. This group of hens will be referred to as "Flock I". A second group of 12 pullets hatched in March and April 1938 will be referred to as "Flock II". The second group was less productive, but otherwise very similar to the pullets selected the preceding year.

The fowls were kept in chicken batteries which were housed in a frame hen house. During warm weather, air was circulated by means of a powerful electric fan. An automatically controlled, oil-burning heater was used to keep room temperature above freezing during the coldest part of the winter. Room temperature was recorded daily between 4 and 5 p. m.

Feed was measured into individual feeders for each fowl. Each egg was weighed and recorded. The weight of the broken eggs was estimated.

When normal heat production of fowls in Flock I was to be determined, the fowls were carried from the battery house to the laboratory at 5 p. m. and placed in the respiration chambers between 5:30 and 6:45 p. m. Usually the fowls in Flock II were kept at room temperature for 24 hours before normal heat production was determined. When fasting heat production was to be determined the fowls were kept (with water but without feed) for the one day between the determinations of normal and fasting heat production in batteries at temperatures ranging from 27 to 29 and occasionally to 32°C.

Body-temperature was determined with an ordinary thermometer (in the vagina). Body weight was determined with a large laboratory scale, accurate to 1 gram.

A Western Electric 3A Stethoscope (see Carter 1938) was used for counting the heart-beats while the hens were still in the closed chambers after completion of the respiration trials.

Feed, used in either pellet or mash form, was of the following composition:

Ingredient	Pounds	TDN according to Morrison's Conversion tables
Ground Corn	35	29.30
Ground Wheat	20	15.68
Ground Oats	15	10.73
Wheat bran	10	7.02
Alfalfa Meal	5	2.86
Meat Scraps	8	5.46
Dried Buttermilk	5	4.28
Cod Liver Oil	1	1.14
Sodium Chloride	0.5	
Limestone (finely ground)	2.0	
Total	101.5	76.47

A Volumetric Respiration Apparatus for Fowls

Of the numerous types of apparatus used for respiratory gas exchange measurements,* the open-circuit gravimetric respiration apparatus described by Haldane (1892), has been most frequently employed (Mitchell & Haines, 1927; Carpenter 1928; Phillips, Ashworth, & Brody 1932, and Dukes 1937a). This apparatus has given reliable results, but the method is somewhat laborious, and a serious error may occur in the oxygen determination since this depends on the increase in weight of the entire system (a difficult thing to measure when the chamber is large).

The writer decided to employ a volumetric method which would eliminate weighing of the respiration chamber as part of the technique of measuring the gas exchange.

In the apparatus designed for these experiments (Fig. 1), CO_2 is absorbed in a rocking battery containing a base, and oxygen is supplied from an oxygen container. In principle this is the method described by Regnault and Reiset (1849). The apparatus is similar in many respects to a respiration apparatus for rats designed by Kleiber (1936).

The inside of the wooden cabinet in which the apparatus is enclosed is 194 cm. long, 43 cm. wide, and 122 cm. high. Four doors with double panes of glass 5 mm. apart (for insulation) give access to the interior of the cabinet. Air, agitated by two "10-inch" electric fans, usually is maintained within 0.1°C of the desired temperatures by means of a mercury-ether thermoregulator controlling a relay switch which supplies current to four 100-watt electric light bulbs used as heating units.

*The history of metabolism apparatus used for fowls has been admirably traced by Benedict, Landauer, & Fox (1932).

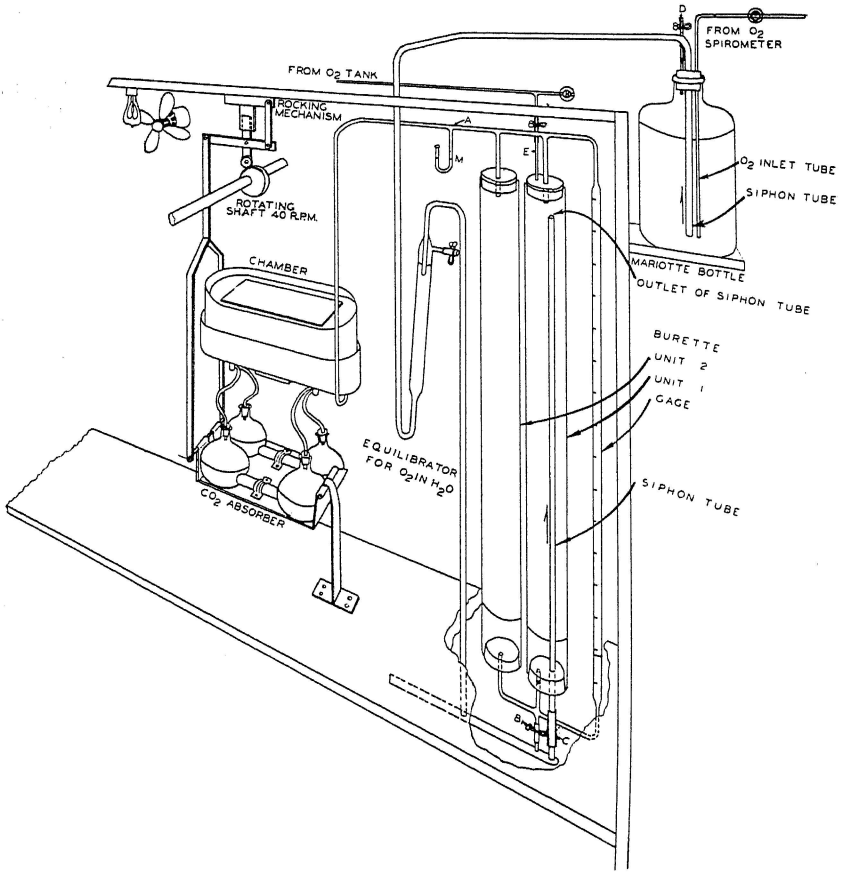

Fig. 1.—*A four-chamber respiration apparatus.* One unit, consisting of chamber, absorber system, and burette is shown. Tops of the large burette cylinders are sealed with mercury.

The cabinet contains 4 independent systems each consisting of a 6-liter respiration chamber, an 8-liter oxygen burette, and an absorbing battery (Fig. 1). The chambers are constructed of 22 gauge galvanized sheet iron, with the lower part of the chamber permanently mounted in the cabinet and the upper part removable. Double walls of the lower part of the chamber provide a water seal in which the single wall of the upper chamber is immersed to a depth of 6 cm. A window in the top of the chamber is covered with water to prevent leaks after the chamber is closed, and a cardboard cover over the chamber is used to prevent light from entering.

Each burette system consists of three glass tubes 110 cm. in length. The inside diameter of each of two of the tubes is 7 cm, and that of the third is 1 cm. The smaller tube is graduated for use as a gauge.

These tubes are so connected that either one or both of the large ones may be used at one time with the gauge depending upon the amount of oxygen estimated to be necessary in the trial. The volumes of the burettes were determined by water calibration.

Each unit of the absorbing battery consists of two 500 cc. flat-bottomed flasks joined near the bottoms with 2 cm. glass tubing. Two of these units are used with each chamber. A definite volume of 2N KOH and of molal $BaCl_2$ is measured into each battery; ordinarily 100 to 150 cc. of each solution is used. The battery is rocked at the rate of 40 cycles per minute. The solution in the battery, flowing from one flask to the other, acts as a piston which alternately draws chamber air into contact with the absorbing fluid and forces it back into the chamber at the rate of 12 liters per minute. Hydrochloric acid solution of known concentration is used to standardize the alkaline solution and to titrate the solution at the end of the trial.

The parts of the apparatus are connected with glass and rubber tubing as shown in Fig. 1. The equilibrator (Fig. 1) allows water saturated with 0_2 at room temperature in the Mariotte bottle to reach equilibrium in regard to oxygen concentration at chamber temperature before it passes into the burette to replace oxygen used by the animal. In the absence of this device, oxygen would be given off by the water if (as is usually the case) the Mariotte bottle were below chamber temperature; this would lead to a small error in oxygen determination.

During the first half hour of the trial no oxygen records are made, but during this period the following adjustments ordinarily take place: 1) the animal has an opportunity to become accustomed to the chamber, and it usually becomes quiet before the end of the first half hour*; 2) cabinet air and the enclosed apparatus are brought to the desired temperature (30°C); and 3) equilibrium apparently is established between the absorbing rate of the battery and the CO_2 production rate of the animal. Samples of chamber air at the end of the first half-hour and at the end of 4 hours contained approximately 1% of CO_2 and correspondingly less oxygen than room air. Since no oxygen records are taken until the end of the preliminary half-hour period it may be seen that there should be but little error in the oxygen determination due to changes in oxygen concentration within the chamber during the trial. Oxygen consumption multiplied

*Most of the trials were conducted between 6 and 11 p. m. Activity on the part of the fowls usually could be detected by the sound of the toenails against the sheet-metal floor, and by small movements of the chambers. Very little activity was noted after the preliminary half-hour period. If, during any half-hour period, persistent activity was noticed, the oxygen consumption during this period was not included in the final calculation. No correction was made for small, infrequent movements comparable to those of a human at bed-rest.

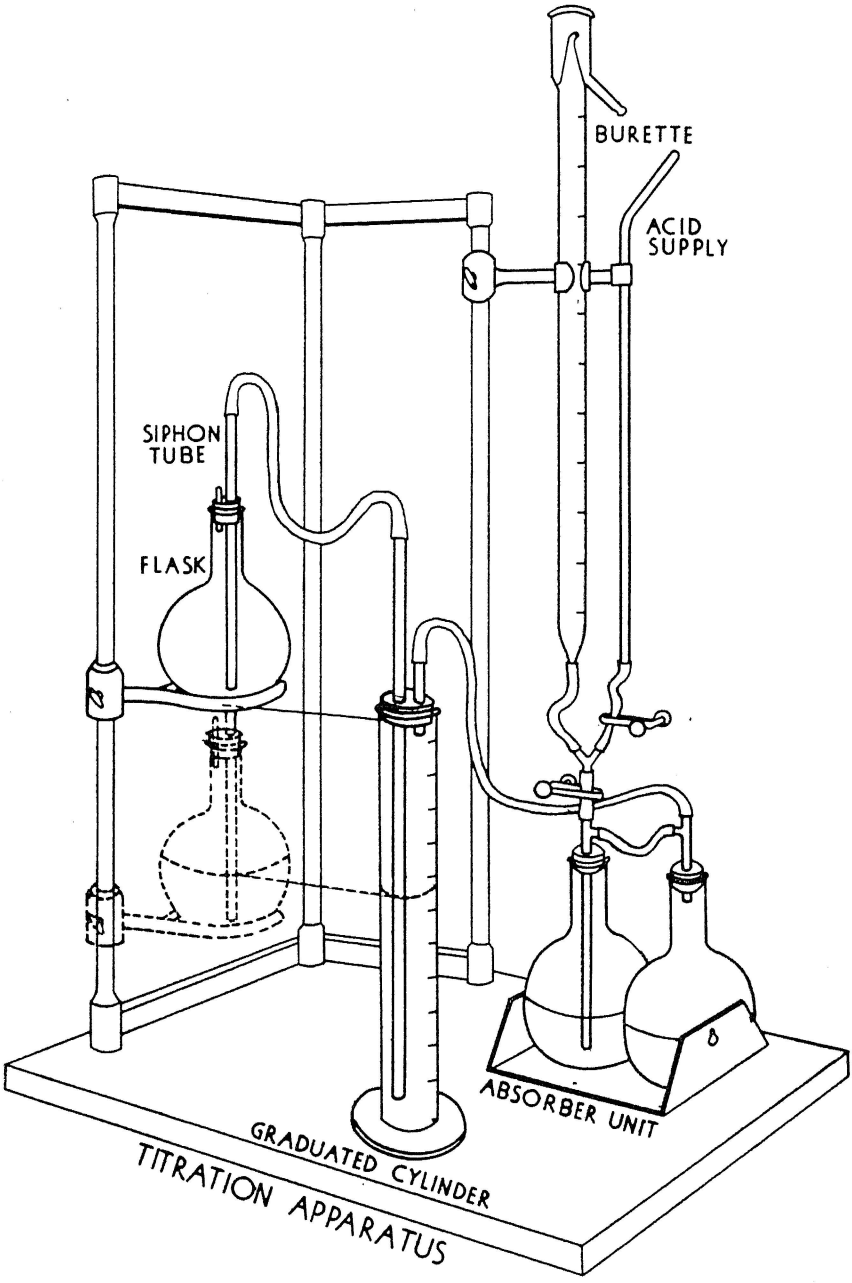

Fig. 2.—*Titration apparatus.* A device to prevent loss of CO_2 during titration. If CO_2 should be driven off by excess acid during the titration its volume can be measured in the graduated cylinder.

by a factor based upon the R. Q., yields the figure for heat production. An error is introduced into the CO_2 determination due to the change in CO_2 concentration in the chamber during the preliminary half-hour period (less CO_2 absorbed than produced). That this error does not greatly affect the R. Q. for the entire trial is shown by the fact that alcohol burned in the chambers yielded an R. Q. of 0.67 with less than 2% error.

The apparatus shown in Fig. 2 prevents loss of CO_2 during titration. Acid is liberated below the surface of the base during titration, and unless it is added very slowly some CO_2 may escape from the liquid. The device tends to retain the CO_2 in the battery where it usually is reabsorbed by the base. (The density of CO_2 is greater than that of air and hence the heavier gas tends to force the air upward and to remain in contact with the solution.) Toward the end of the titration, when the solution is nearly neutral, acid must be added very slowly to prevent loss of CO_2. As may be seen in Fig. 2, the volume of any CO_2 lost from the solution can be calculated from the volume of gas in the graduated cylinder. Ordinarily this calculation is not necessary. A mixed indicator of thymol blue and cresol red is used (Kolthoff and Menzel 1929).

Heat production of rats and other small animals was determined for some time by using, instead of the 6-liter chambers, a set of 1-liter chambers of similar shape. Later, an 8-chamber apparatus similar in design to the one described here was constructed for rats.

During respiration trials, readings of the water levels in the burette are made periodically, usually every half-hour. An example of the necessary calculations is given below.

A fowl was placed in one of the chambers at 7 p. m. and the first oxygen reading was made half an hour later. In the example given below only the initial and end readings are shown.

Volume of chamber air + tubes and absorbers ----------7.00 liters

Volume of bird (Specific gravity is assumed to be unity, and the figure for weight in kilograms is taken to represent volume of the animal.) ----------1.73 "

Chamber + tubes and absorbers—volume of the animal 5.27 "

Oxygen Consumption

1	2	3	4	5	6	7	8	9
Time	Burette Reading	Volume of gas in burette	Burette Vol. + air in the chamber, absorber, etc.	Temperature	Barometric Pressure	Factor for Volume	Volume (Standard)	Volume (Difference)
7:30	93.1	7.26	12.53	30.0	74.7	.847	10.61	
10:00	52.0	4.31	9.58	30.0	74.7	.847	8.11	2.50
Oxygen consumed		2.95	2.95					
Oxygen consumed (corrected) = 2.50 liters (2.95 × .847)								2.50
Oxygen consumed in 2.5 hours .....2.50 liters								
Oxygen consumed in 1.0 hour .....1.00 liter								

CO_2 Production

Volume of KOH solution in battery	200	cc.
Volume of $BaCl_2$ solution in battery	200	cc.
This was found to be equivalent to	166.5	cc. of 2.11 N HCl
Hcl used in titration	68.7	cc. of 2.11 N HCl
CO_2 absorbed equivalent to	97.8	cc. of 2.11 N HCl
1 cc. of 2.11 N HCl is equivalent to	23.63	cc. of CO_2
In 3 hours CO_2 production was	2311.0	cc. of CO_2
In 1 hour CO_2 production was	770.3	cc. of CO_2 or .77 liters

Respiratory Quotient

$$(R.\ Q.) = \frac{CO_2 \text{ produced per hour}}{O_2 \text{ consumed per hour}} = \frac{0.77}{1.00} = 0.77$$

Heat Production

If the R. Q. is 0.77 each liter of O_2 will represent approximately 4.8 calories* = $(1 \times 4.8) = 4.8$ Calories/hour.

Body weight of the fowl = 1.729 kg.

Body size (Kleiber 1932, and Brody and associates 1932 and 1934) of the fowl = 1.4 $Kg^{0.73}$

$$\text{Heat production } \frac{4.80}{1.48} = 3.24 \text{ Calories per hour/kg}^{0.73}$$

*According to the calculation of Mitchell and Haines (1927, p. 930), based on the data of Zuntz and Schumberg, when heat production is "computed on the assumption that the total respiratory quotient is non-protein . . . . the maximum error in the total heat production figures resulting from this simplification is 6.58 per cent and . . . . the usual error would be less than 2 per cent."

Ordinarily the changes in pressure and temperature are negligible during a trial, and the air in the chamber, absorber, and connecting tubes can be assumed not to have changed volume during the trial. This makes possible some saving of time in calculation, as the steps shown in columns 4, 8, and 9 of the oxygen calculation as well as the estimation of the volume of air remaining in the chamber, tubes, and absorber after the animal has been placed in the chamber, can be eliminated.

Comparison of Our Volumetric Respiration Apparatus with the Haldane Gravimetric Apparatus

To compare heat production data obtained with the volumetric apparatus and that obtained with the Haldane gravimetric apparatus, heat production of a group of rats was determined repeatedly with both systems. On a given day the heat production of 4 rats was determined first with the volumetric apparatus and later with the Haldane apparatus, and on a subsequent day the order was reversed. Heat production of the rat varies from hour to hour as has been shown by Herring and Brody (1938), and therefore some difference in the results obtained with the 2 systems is to be expected.

The 2 sets of data plotted one against the other are shown in Fig. 3. The correlation coefficient for data on the same rats obtained with

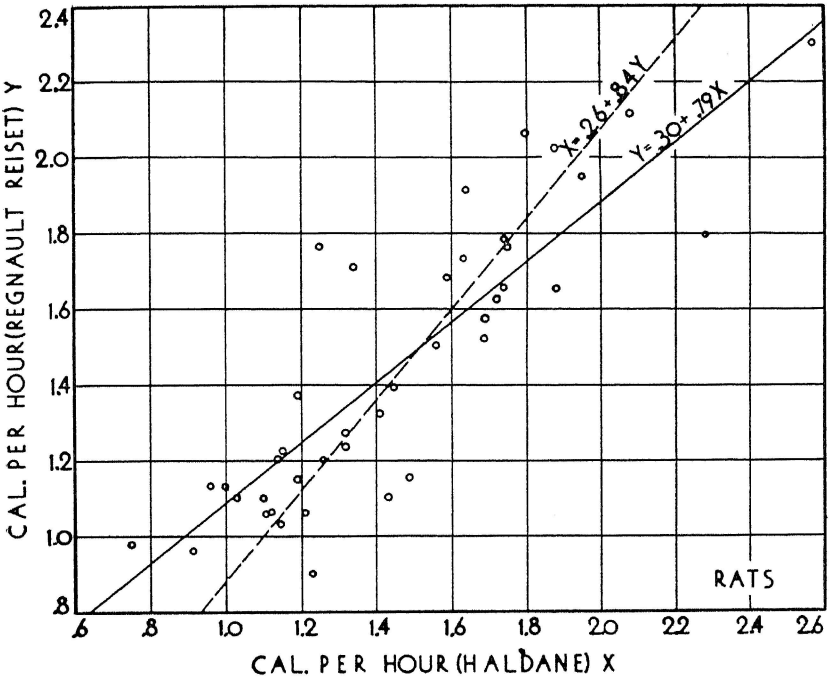

Fig. 3.—*Comparison of results obtained with two types of apparatus.* Oxygen consumption data on rats obtained with the Four-chamber Volumetric Apparatus plotted against similar data on the same rats obtained the same day with Haldane Gravimetric Apparatus.

the 2 different systems is 0.86. The arithmetic mean of all results obtained with the Haldane apparatus was 1.51 ± .06 and with the volumetric apparatus 1.49 ± .06 Cal/rat/hr. (The figures following the ± sign represent standard errors of means.) The difference between these two averages is obviously insignificant.

This comparison serves as an index of the reliability of the volumetric apparatus as compared with that of the well-known Haldane gravimetric apparatus. Actual accuracy of the apparatus can best be determined by means of alcohol tests. Such tests showed that the error involved in determination of the R. Q. with the volumetric apparatus is less than 2 per cent and the error in oxygen determination probably even less than 2 per cent.

III. RESULTS

Lability of Metabolic Processes in Laying Hens

Benedict and Ritzman (1935) and Ritzman and Benedict (1938) have called attention to the comparatively large variability in basal metabolism observed in cows. They used the term "lability" to de-

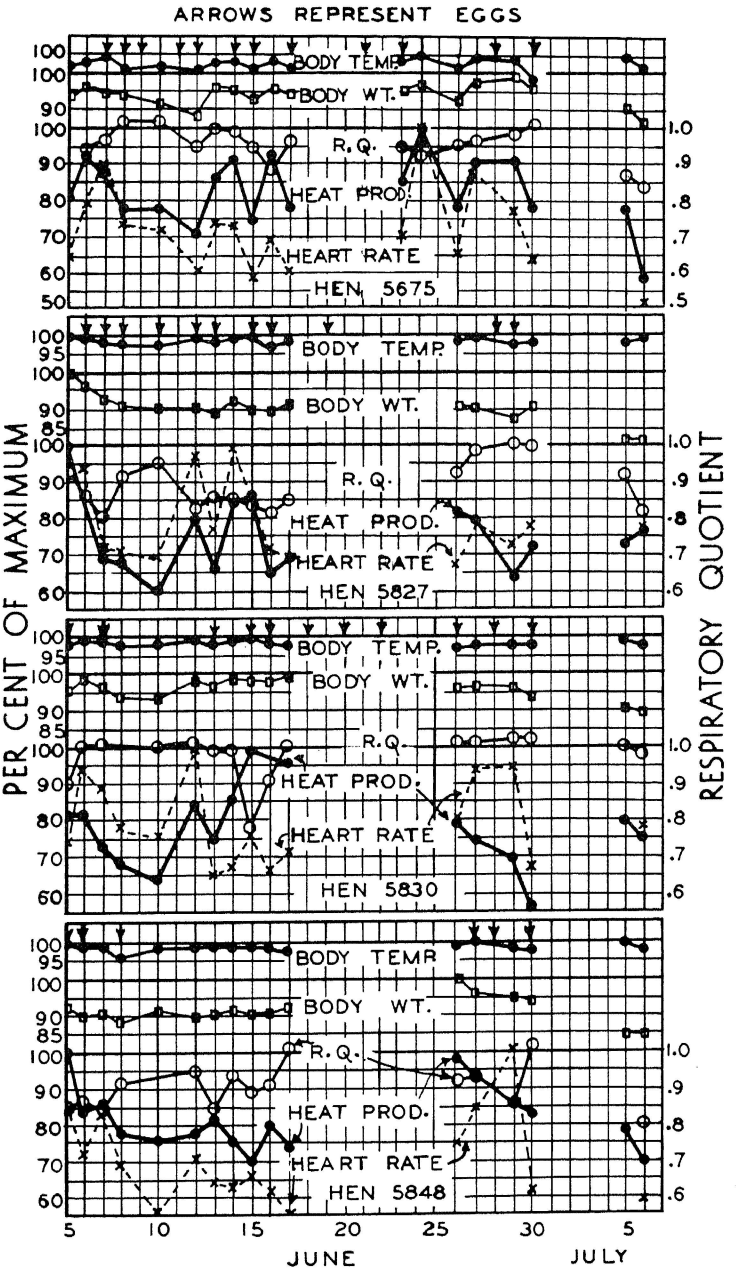

Fig. 4.—*Lability of Metabolic processes in laying hens.* Data on heart rate, heat production, and body temperature, at full feed obtained as frequently as possible are expressed as percentages of highest levels. Respiratory quotients are shown. Heart rate and normal heat production show great lability and tend to vary together.

scribe these variations, and concluded that ". . . . the more alert behavior or higher tension of the dairy type is the expression of a higher metabolic stimulus with consequently greater lability in metabolism . . . ." when comparison was made with beef type animals. Dukes (1937a) has called attention to lability in heat production of hens.

As a measure of lability, metabolic data were obtained on 4 laying hens either daily or as frequently as possible over a period of a month. The results expressed as percentages are given in Fig. 4. Day to day variations in normal heat production and heart rate were very large. In one case (Hen 5830) the extreme variation in heat production was over 40% of the maximum figure, and variations in heart rate were as large in the case of Hen 5848. In general, the curves of heat production and heart rate tend to rise and fall together. Body temperature exhibited variations within a limited range and tended to decline and rise with heat production. The fact that body weight was low, in some cases, when heat production was low suggests that the latter sometimes may have resulted from low food intake; however, the R. Q.'s in these cases do not indicate that the fowls were in a post-absorptive state.

The changes in metabolic rate may have been due to changes in the rate of egg production, but this relationship has not been demonstrated. These results emphasize one of the difficulties of seasonal studies of laying hens. The alternatives are either very frequent measurements of a small number of hens, or less frequent measurements of a fairly large group of fowls to minimize the influence of individual lability on group averages.

Alcohol tests made during the month indicated that the apparatus was functioning normally.

Fasting Heat Production

The hen does not reach her minimal level of heat production until after a prolonged period of fasting. Mitchell and Haines (1927) stated that "although the basal level of fowls was often reached after a fasting period of 24 hours, a fast of 48 hours was required before the basal level was reached in all cases". Brody, Funk, and Kempster (1932) found that heat production of fowls declined throughout a three-day period of fast. Dukes (1937a) reported a progressive fall in metabolic rate "until about the seventy-fifth hour", after which "the rate appears to be practically constant" but stated that after 24 to 30 hours of fast metabolic rate determinations could be repeated with accuracy. Dukes' respiratory quotients after 24 to 29 hours of fast range from 0.68 to 0.80 with a mean value of 0.74.

In this investigation heat production was determined between the

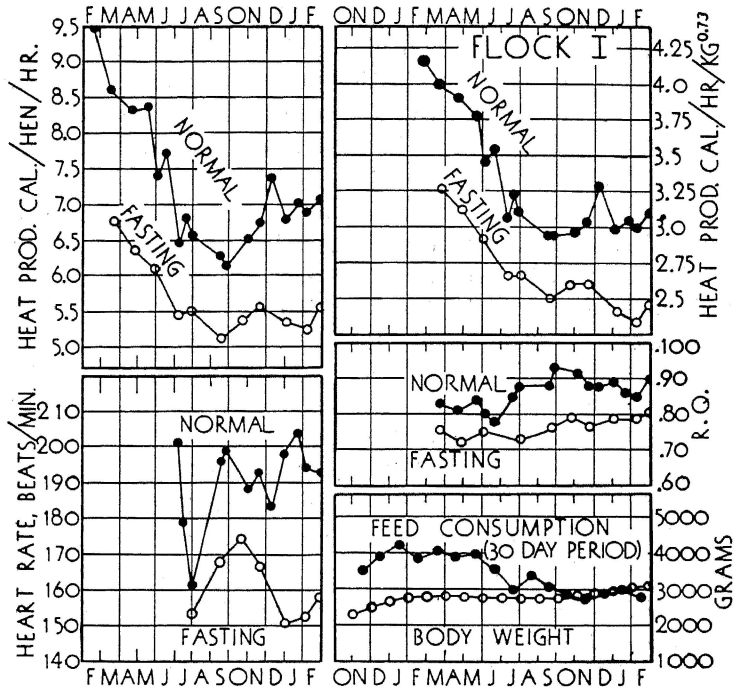

Fig. 5a, Flock I

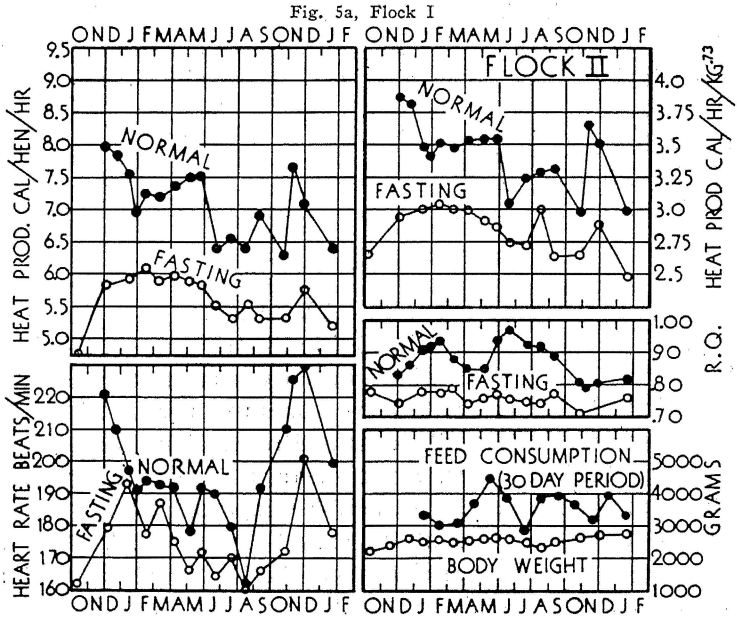

Fig. 5b, Flock II

Fig. 5.—*Metabolism data.* Normal and fasting heat production are represented both as Cal./hen/hr. and as Cal/hr./kg.$^{0.73}$. Heart rates and respiratory quotients during fast and after feeding, and feed consumption and body weight are plotted as time curves.

twenty-fifth and thirtieth hours of fast. It was particularly important that any procedure, such as prolonged fasting, which might have interfered with normal egg production, be avoided. Fig. 5 shows that while the mean R. Q. was sometimes as low as 0.71 or 0.72, it was more often than not between 0.75 and 0.79. This may be considered as evidence that the fowls were not ordinarily in basal condition. Very little activity was noted after the preliminary half hour period. When activity judged to be greater than that of a human at bed-rest occurred, the half-hour period during which it took place was not used in the final calculation. It is probable that the fowls were sleeping most of the time while in the chambers.

Heat production, determined between the twenty-fifth and thirtieth hours of fast and under the conditions just described, will be referred to as "fasting heat production", and the term "basal metabolism" will be used only in reference to work of those authors who have used the latter term.

Seasonal Rhythms of Heat Production

During the early laying season egg production increased very rapidly, and by November and December production had approached its maximum level. The accelerating phase* of the egg production curve was paralleled by a similar curve of fasting heat production (Fig. 6b). While egg production of Flock II did not reach its maximum until April, heat production reached maximum in February. Fasting heat production of Flock I was not determined until March. There is a striking similarity in the relationship between the curves of heat production and egg production of the two flocks which seems unlikely to have been entirely due to chance.

The pullets approached maximum egg production during the months of minimum production of mature fowls (November and December) and maintained relatively high production until (and in the case of Flock I after) the time at which production of older hens begins to decline (Fig. 6). It appears that egg production of pullets may be governed by endocrine factors limited to a greater degree by age-changes than by environmental conditions which appear to be limiting factors for production of 2 year old and older hens. The declining phase of production of pullets may be a response to environmental conditions. In the case of Flock I the limiting factor may have been temperature, since the decline appeared with the onset of hot weather. However, the decline in production of Flock II appeared while temperature was low and length of day was increasing, and in this instance it does not appear that these were the limiting factors.

*The egg production curve may be thought of as consisting of an accelerating, a linear, and a declining phase.

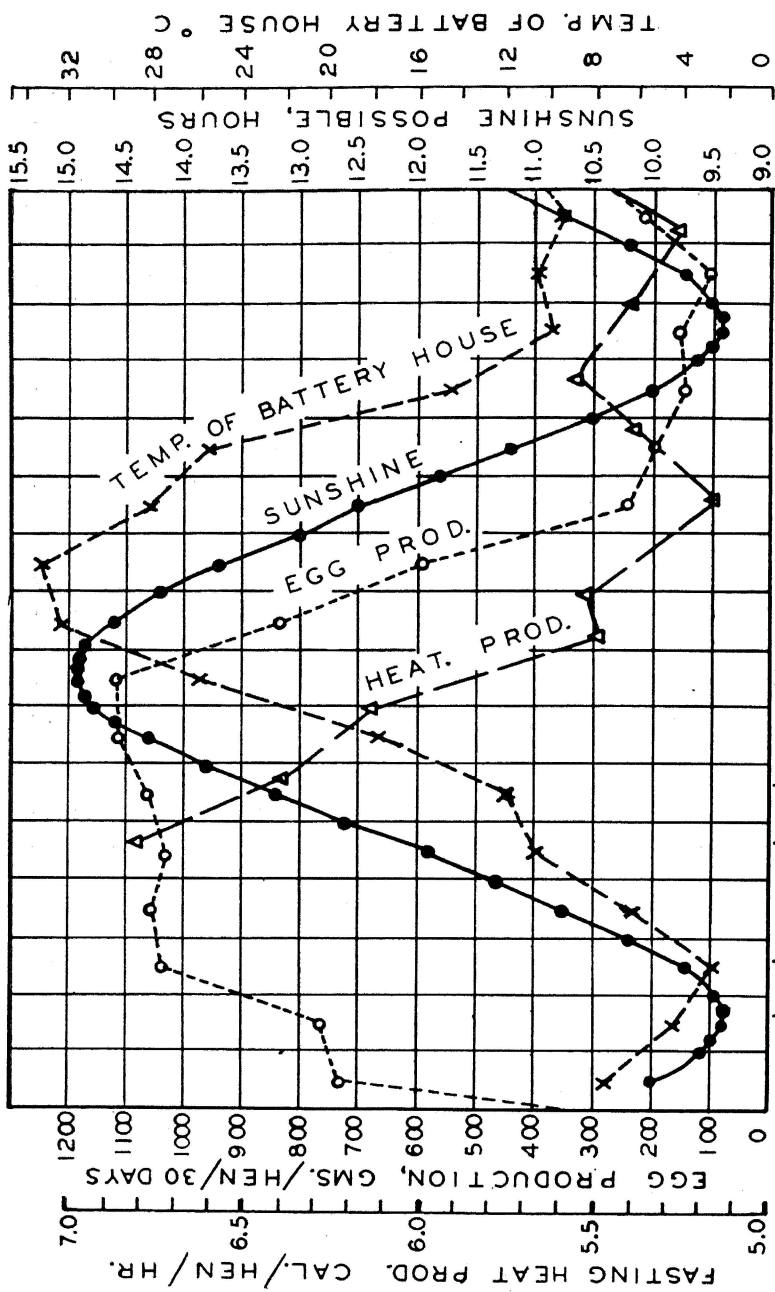

Fig. 6a.—Egg production and fasting heat production of Flock I compared with time curves of day length and environmental temperature.

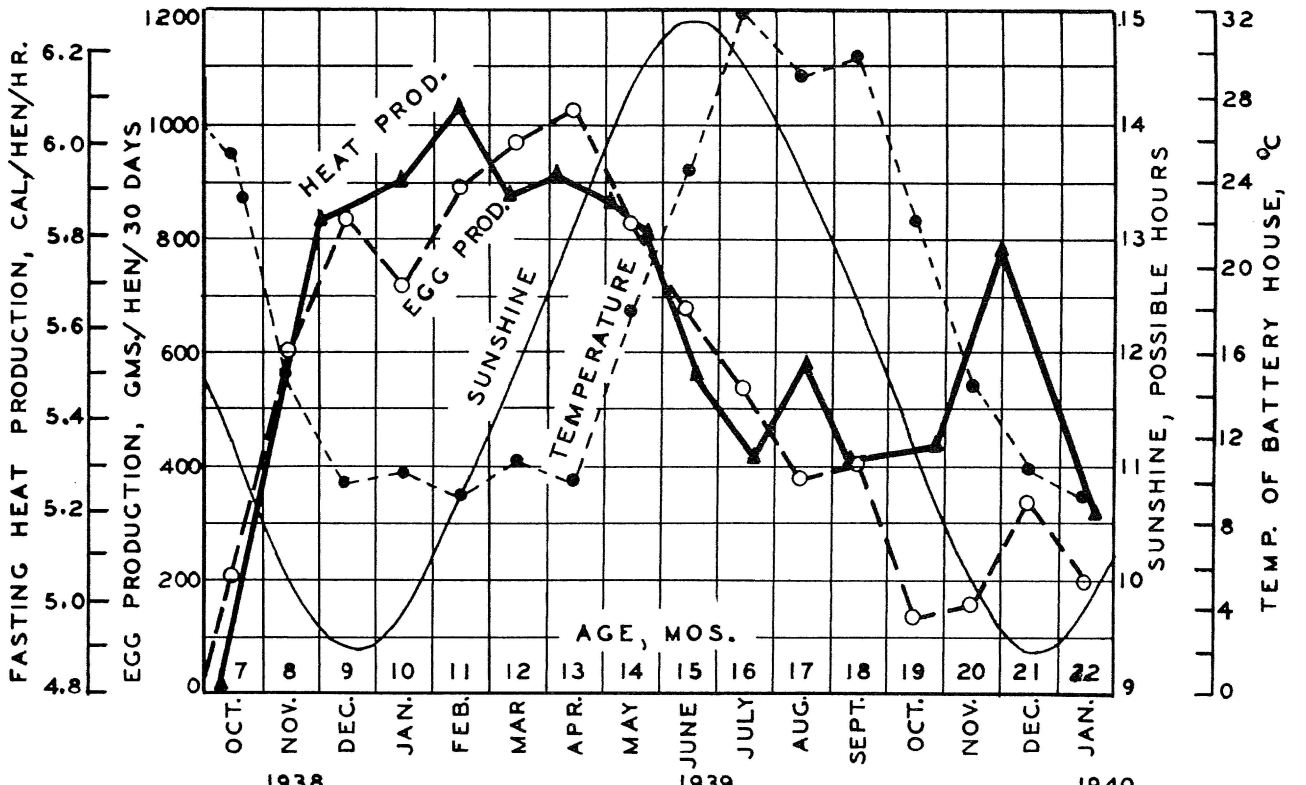

Fig. 6b.—Egg production and fasting heat production of Flock II compared with time curves of day-length and environmental temperature.

TABLE 1.—METABOLISM DATA FOR FASTING HENS (Arithmetic Means)

Flock I

		1938								1939		
		Mar. 21	Apr. 25	June 1	July 7	July 30	Sept. 17	Oct. 23	Nov. 20	Jan. 2	Feb. 6	Mar. 2
No. of hens		11	11	15	15	15	15	13	13	12	12	12
Heat production, Cal./hr./hen		6.80	6.39	6.13	5.49	5.53	5.15	5.38	5.55	5.39	5.26	5.60
	σ_M	0.32	0.29	0.20	0.15	0.16	0.15	0.15	0.16	0.24	0.12	0.14
Heat production, Cal./hr./kg.$^{0.73}$		3.26	3.12	2.92	2.66	2.66	2.50	2.59	2.59	2.42	2.34	2.49
	σ_M	0.13	0.10	0.07	0.05	0.08	0.08	0.10	0.10	0.10	0.10	0.07
Body temperatures after 28 to 30 hours fast		41.2	41.0	40.9	40.7	40.7	40.6	40.9	40.9	40.7	40.9	41.1
	σ_M	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1
Respiratory Quotients		0.76	0.72	0.75	...	0.73	0.76	0.79	0.76	0.78	0.78	0.81
	σ_M	.011	.006	.006	...	.003	.006	.004	.005	.004	.003	.005
Heart Rates after 28-30 hrs. fast, beats/min.		...	...	...	...	153	167	174	166	150	152	158
	σ_M	...	...	...	...	9.0	10.2	7.7	8.0	6.0	6.0	5.3

TABLE 2.—METABOLISM DATA FOR FULL-FED HENS

Flock I

		1938													1939			
		Feb. 23	Mar. 19	Apr. 20	May 19	June 3	June 17	July 6	July 18	July 29	Sept. 14	Sept. 24	Oct. 30	Nov. 19	Dec. 7	Jan. 1	Jan. 22	Feb. 5	Mar. 1
No. of hens		11	11	11	14	15	15	15	15	15	15	15	13	13	13	12	12	12	12
Heat production Cal./hr./hen		9.48	8.62	8.34	8.41	7.43	7.74	6.49	6.85	6.60	6.30	6.14	6.54	6.76	7.41	6.78	7.02	6.90	7.10
	σ_M	0.55	0.32	0.24	0.27	0.27	0.22	0.24	0.18	0.15	0.18	0.19	0.18	0.20	0.30	0.19	0.19	0.31	0.12
Heat production, Cal./hr./kg.$^{0.73}$		4.16	4.00	3.92	3.78	3.44	3.59	3.06	3.22	3.11	2.94	2.94	2.96	3.06	3.33	2.98	3.04	2.99	3.12
	σ_M	0.26	0.13	0.09	0.15	0.13	0.12	0.10	0.08	0.08	0.07	0.06	0.10	0.13	0.15	0.08	0.10	0.14	0.14
Body temperature		...	41.6	41.5	41.5	41.4	41.3	41.0	41.0	40.9	41.1	41.2	41.1	41.2	41.6	41.2	41.4	41.5	41.8
	σ_M	...	0.25	0.08	0.12	0.11	0.13	0.09	0.05	0.07	0.13	0.06	0.04	0.11	0.11	0.15	0.14	0.16	0.15
Respiratory Quotients		...	0.83	0.81	0.84	0.80	0.77	...	0.85	0.88	0.88	0.93	0.91	0.88	0.87	0.89	0.86	0.85	0.90
	σ_M	...	.018	.015	.015	.019	.010	...	.016	.012	0.012	0.020	.015	.014	.015	.016	.012	.018	.021
Heart rate (beats/min.)		...	...	...	...	...	...	201	179	161	196	199	188	193	183	198	204	194	193
	σ_M	...	...	...	...	...	...	8.7	6.6	4.2	8.8	8.8	8.6	10.8	6.2	11.8	6.6	9.5	10.9

TABLE 4.—METABOLISM DATA FOR FASTING HENS (Arithmetic Means) *Flock II*

		1938 Oct. 9	1938 Dec. 2	1939 Jan. 15	1939 Feb. 15	1939 Mar. 10	1939 Apr. 6	1939 May 5	1939 May 26	1939 June 20	1939 July 19	1939 Aug. 17	1939 Sept. 10	1939 Oct. 26	1939 Dec. 1	1940 Jan. 20
No. of hens		12	12	12	12	12	12	12	12	11	12	11	12	11	10	9
Heat production, Cal./hen/hr.		4.84	5.84	5.92	6.09	5.89	5.94	5.88	5.82	5.51	5.32	5.54	5.31	5.35	5.78	5.20
	$\sigma_M\pm$	.15	.26	.17	.23	.23	.32	.28	.20	.33	.36	.30	.22	.29	.38	.12
Heat production, Cal./hr./kg.$^{0.73}$		2.71	2.95	3.01	3.04	3.00	2.99	2.92	2.87	2.74	2.72	3.00	2.64	2.66	2.88	2.48
	$\sigma_M\pm$	.04	.08	.09	.07	.08	.08	.07	.09	.12	.14	.12	.11	.10	.09	.06
Body temperature, °C.		41.1	41.1	41.1	41.2	41.4	41.3	41.2	41.5	41.0	40.8	41.3	41.0	41.4	41.5	40.8
	$\sigma_M\pm$	.06	.16	.17	.11	.10	.17	.16	.10	.25	.10	.17	.15	.17	.29	.09
Respiratory Quotient		0.78	0.75	0.78	0.78	0.79	0.74	0.76	0.77	0.76	0.75	0.74	0.78	0.71	*..	0.78
	$\sigma_M\pm$	.004	.007	.009	.010	.011	.006	.005	.008	.012	.013	.016	.019	.009	...	.009
Heart-rate (beats/min.)		162	180	193	177	187	175	166	172	164	170	160	166	172	201	178
	$\sigma_M\pm$	5.5	8.1	9.6	5.8	9.6	7.7	6.4	5.7	10.1	11.8	5.4	8.3	9.3	5.7	11.9

*Absorber solution not standardized.

TABLE 5.—METABOLISM DATA FOR FULL-FED HENS *Flock II*

		1938 Dec. 1	1938 Dec. 22	1939 Jan. 14	1939 Jan. 29	1939 Feb. 14	1939 Mar. 9	1939 Apr. 5	1939 May 4	1939 May 25	1939 June 19	1939 July 18	1939 Aug. 16	1939 Sept. 9	1939 Oct. 25	1939 Nov. 8	1939 Nov. 30	1940 Jan. 20
No. of hens		12	12	12	12	12	12	12	12	12	11	12	11	12	11	11	10	9
Heat production, Cal./hr./hen		7.99	7.85	7.54	6.94	7.24	7.19	7.32	7.47	7.51	6.40	6.56	6.42	6.92	6.29	7.68	7.10	6.40
	$\sigma_M\pm$	.42	.30	.25	.22	.23	.22	.28	.36	.27	.48	.42	.53	.51	.46	.32	.27	.18
Heat production, Cal./hr./kg.$^{0.73}$		3.87	3.83	3.49	3.39	3.52	3.48	3.53	3.56	3.55	3.04	3.24	3.29	3.32	2.98	3.66	3.50	2.98
	$\sigma_M\pm$	.13	.10	.15	.07	.10	.10	.07	.10	.10	.17	.15	.19	.19	.14	.07	.10	.13
Body temperature, °C.		42.1	41.9	42.0	41.7	41.7	41.5	41.8	41.9	42.0	41.5	41.4	41.3	41.6	41.2	42.1	41.9	41.4
	$\sigma_M\pm$	.22	.14	.10	.10	.11	.09	.22	.16	.15	.19	.18	.26	.18	.21	...	.27	.13
Respiratory Quotient		0.83	0.86	0.91	0.92	0.94	0.88	0.86	0.85	0.94	0.97	0.92	0.92	0.89	0.81	0.79	0.81	0.82
	$\sigma_M\pm$	.02	.01	.02	.02	.02	.02	.01	.02	.02	.01	.02	.03	.02	.01	.02	.04	.02
Heart-rate (beat/min.)		221	210	197	191	194	193	192	178	192	190	180	162	192	210	226	230	199
	$\sigma_M\pm$	9.7	9.5	7.7	7.3	7.9	8.2	9.6	8.0	7.9	16.4	10.7	7.3	15.3	12.4	7.4	9.6	9.3

TABLE 3.—FEED CONSUMPTION, EGG PRODUCTION, AND BODY WEIGHT (Arithmetic Mean Values)

Flock I

	1937 Nov.	Dec.	1938 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	1939 Jan.	Feb.
More productive Group																
No. of hens	7	7	7	7	7	7	7	7	7	7	6	6	6	6	6	
Feed consumption,** hen/30 days, gms.	3682	4009	4277	4317	3951	3987	4006	3412	2807	3249	2934	2705	2900	2844	3036	3097
Egg production, hen/30 days, gms.	924	1025	1239	1248	1180	1195	1377	1413	1051	743	320	345	285	269	185	240
Body weight after 30 hrs. fast, kg.	2.16	2.43	2.53	2.60	2.67	2.70	2.67	2.66	2.60	2.59	2.60	2.47	2.52	2.68	2.77	2.82
Less Productive Group																
No. of hens	8	8	8	8	8	8	8	8	8	8	8	7	7	7	6	6
Feed consumption,** hen/30 days, gms.	3272	3768	3975	3874	3842	3685	3670	3593	2827	3251	3067	2830	2466	2764	2713	2807
Egg production, hen/30 days, gms.	563	533	865	884	898	949	880	862	659	458	181	64	18	55	18	175
Body weight after 30 hrs. fast, kg.	2.28	2.48	2.67	2.85	2.86	2.89	2.86	2.85	2.85	2.84	2.85	2.96	3.01	3.00	3.14	3.19
Entire Flock																
No. of hens	15	15	15	15	15	15	15	15	15	15	13	13	13	12	12	
Feed consumption**/hen/month, gms.	3463	4010	4253	3808	4023	3826	3954	3509	2911	3358	3005	2864	2666	2894	2970	2755
Feed consumption**/hen/30 days, gms.	3463	3881	4116	4080	3893	3826	3827	3509	2817	3250	3005	2772	2666	2801	2874	2952
Egg production/hen/months, gms.	732	788	1075	983	1064	1064	1149	1119	870	611	246	200	141	159	105	194
Egg production/hen/30 days, gms.	732	763	1039	1053	1030	1064	1112	1119	842	591	246	194	141	154	102	208
Body weight after 30 hrs. fast, kgs.	2.27*	2.45*	2.60*	2.71*	2.76*	2.79	2.76	2.75	2.71	2.70	2.71	2.73	2.78	2.88	2.96	3.01

*Empty body weight calculated from full body weight (1st day of month), kgs.
**These figures include wastage which could neither be avoided nor accurately estimated.

TABLE 6.—FEED CONSUMPTION, EGG PRODUCTION, AND BODY WEIGHT

Flock II

	1938 Oct.	Nov.	Dec.	1939 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	1940 Jan.
More Productive Group																
No. of hens	6	6	6	6	6	6	6	6	6	6	5	5	5	5	4	4
Feed consumption,[1] hen/30 days, gms.	....	....	....	3381	2915	3005	3661	4469	3750	2830	3995	4050	3451	3072	3890	3351
Egg production, hen/30 days, gms.	287	729	1000	842	976	1140	1285	929	850	708	472	385	88	132	344	174
Body weight after 30 hrs. fast, kg.	2.16	**	2.56	2.44	2.52	2.46	2.44	2.53	2.51	2.41	2.24	2.52	2.31	**	2.44	2.73
Less Productive Group																
No. of hens	6	6	6	6	6	6	6	6	5	5	5	4	4	4	4	3
Feed consumption,[1] hen/30 days, gms.	....	....	....	3270	3233	3215	3747	4405	4040	2859	3670	3802	3678	2895	3628	3322
Egg production, hen/30 days, gms.	119	478	673	588	797	806	776	566	475	267	229	266	115	123	211	227
Body weight after 30 hrs. fast, kg.	2.27	**	2.63	2.60	2.67	2.56	2.66	2.73	2.75	2.70	2.41	2.60	2.90	**	2.98	2.82
Entire Flock																
No. of hens*	12	12	12	12	12	12	12	12	11	12*	11	12*	11	11	10	9
Feed consumption,[1] hen/month, gms.	....	....	....	3435	2871	3213	3704	4584	3882	2935	3980	3962	3813	3200	4081	3494
Feed consumption[1]/hen/30 days, gms.	....	....	....	3325	3075	3110	3704	4437	3882	2841	3850	3962	3691	3200	3950	3382
Egg production/hen/month, gms.	210	603	864	739	828	1006	1030	857	680	558	394	404	137	159	352	203
Egg production/hen/30 days, gms.	203	603	838	715	887	974	1030	830	680	540	381	404	133	159	341	196
Body weight after 30 hrs. fast, kgs.	2.22	**	2.59	2.52	2.60	2.53	2.55	2.63	2.62	2.50	2.34	2.55	2.62	**	2.74	2.77

*Hens added to flock in July and September are not included in either the more productive or less productive group.
**Hens were not fasted during month.
[1]These figures include wastage which could neither be avoided nor accurately estimated.

Gross Efficiency of Egg Production

Gross efficiency may be defined as the ratio between energy in the product, and the energy required to produce the product, as given by the equation

$$\text{Gross efficiency} = \frac{\text{Energy in eggs during unit time}}{\text{Energy in feed during unit time}}$$

Using the values determined by Brody, Funk, and Kempster (1938, p. 8) for energy value of whole eggs and of feed having the same formula as that used in the present experiment the equation becomes

$$\text{Gross efficiency} = \frac{1.6 \times \text{gms. eggs}}{3 \times \text{gms. feed}}$$

Gross Efficiency from January to June Inclusive

Flock I

	Good Layers (7 hens)	Poorer Layers (8 hens)	Flock I (15 hens)
Egg material produced, gms/hen	7695	5367	6453
Egg material produced, Cals/hen (gms × 1.6 = Cals)*	12312	8587	10325
Feed consumed, gms/hen**	24067	22762	23374
Feed consumed, Cals/hen (gms × 3.0 = Cals)	72201	68286	67122
Gross efficiency, per cent	17.0	12.6	14.7
Relative efficiency, % (or $\frac{\text{gross efficiency of poor layers}}{\text{gross efficiency of good layers}} \times 100$)			In unit time 74.1

Flock II

	Good Layers (7 hens)	Poor Layers (6 hens)	Flock II (12 hens)
Egg material produced, gms/hen	6224	3943	5084
Egg material produced, Cals/hen	9958	6309	8134
Feed consumed, gms/hen**	21347	21381	21364
Feed consumed, Cals/hen	64041	64143	64092
Gross efficiency, per cent	15.5	9.8	12.7
Relative efficiency, per cent			63.2

Flock I
Gross Efficiency for the Year November 1937 to October 1938

	Good Layers (6 to 7 hens)	Poorer Layers (7 to 8 hens)	Flock I (13-15 hens)
Egg material produced, gms/hen	12209	7882	9900
Egg material produced, Cals/hen	19534	12611	15840
Feed consumed, gms/hen*	43882	42199	42984
Feed consumed, Cals/hen	131646	126597	128952
Gross efficiency, per cent	14.8	10.0	12.3
Relative efficiency, per cent			67.6

*Brody, Funk & Kempster (1938, p. 8).
**These figures include a certain amount of unavoidable wastage of feed.

Gross efficiency calculated for the entire year results (obviously) in a lower figure than that obtained during the season of high production. One hen was lost from each group in October, but the effect of this on the calculation of gross efficiency is slight.

These data emphasize the necessity for careful culling of flocks.

Relative Levels of Heat Production of Good and Poor Layers

It has been shown that the groups of poorer layers were only about 70% as efficient (gross efficiency) as the better groups. Tables 3 and 6 show that the quantities of feed consumed by the poorer groups were about the same as those consumed by the better groups. What are the comparative rates of heat production of the two groups?

When heat production is expressed as Cals/hr/$kg^{0.73}$ full fed and fasting metabolism of the better group of Flock I is consistently greater than that of the remainder of the flock, but the difference is not significant. When heat production is expressed as Cals/hen/hr, heat production of neither group is consistently higher than that of the other. Similar relationships are exhibited by Flock II. These data confirm the conclusion reached by Brody, Funk, and Kempster (1932) that "there seems to be no marked difference between the heat production of good . . . and poor . . . laying pullets."

If metabolic rate and feed consumption of the better and poorer producers are similar how can the feed energy which corresponds to the difference in energy converted into egg material as between the better and poorer layers be accounted for? Some of this energy was undoubtedly stored as fat by the groups of poorer layers as is indicated by the fact that the poorer groups (Tables 3 and 6) were heavier than the groups of better producers. In order to answer this question satisfactorily, complete energy balance experiments would be necessary.

Interrelations Between Body Weight and Metabolic Rate

It has been shown independently by Kleiber (1932) and by Brody and associates (1932 & 1934) that for animals of different species, from the size of the mouse to that of the elephant, heat production varies very nearly as the 0.73 power of body weight. It has not been proven that heat production of animals within a given species varies with a power of body weight, although this is sometimes assumed to be true. The results of an attempt to find a mathematical relationship between metabolic rate and body weight of the fowls in Flock I are given in Fig. 7. Heat production varied as the 0.75 power of body weight in April, as the 0.53 power in June, as the 0.55 power in early July, and as the 0.43 power in late July, while

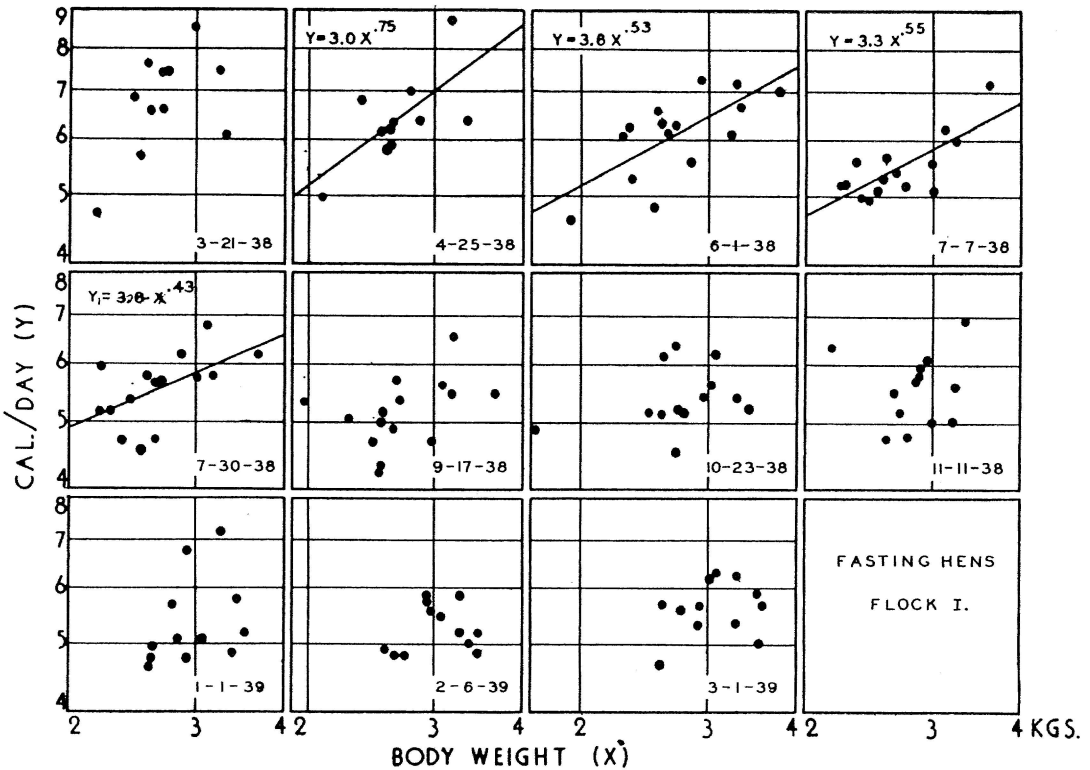

Fig. 7.—*Heat production as power of body weight.* A significant correlation was found only in the 4 periods for which curves are given. The range of body weight is too small to permit generalizations based on these data.

during the balance of the year no significant relationship appeared. Range of body weight was, of course, very limited.

These data are included because they show that the use of a power function of body weight to express relative body size within the species studied here may be subject to limitations. In this research heat production is expressed both as Calories per 0.73 power of body weight and as Calories per hen. The latter expression is, perhaps, preferable to the former in research, such as that reported here, in which the same individuals are studied over extended periods.

Calorigenic Effect of Feed

The difference between normal (full fed) and fasting heat production represents the calorigenic effect of feed. This may be expressed as percentage of digestible nutrients in the feed consumed (Table 7). The calorigenic effect is shown to vary within a range of 5.5 to 13.5%.

In the case of Flock I there appears to be an inverse relationship between environmental temperature of the battery house and the calorigenic effect of feed due, possibly, to the fact that these fowls usually were brought to the laboratory about 2 hours before the beginning of each determination of full fed heat production (see "Materials and Methods"), and they remained in a room of the laboratory building 25 to 26 hours preceding the fasting heat determinations. This resulted in a period in which adjustment to room temperature was possible (in cold weather) preceding fasting heat production determination, but little or no opportunity for adjustment preceded those at full feed. In the case of Flock II the hens were kept in the laboratory for about 24 hours before the determination of heat production at full feed. The calorigenic effect of feed does not show a relationship to environmental temperature in the case of Flock II.

TABLE 7a.—CALORIGENIC EFFECT OF FEED *Flock I*

	Mar.	Apr.	June	July	1938 Aug.	Sept.	Oct.	Nov.	1939 Jan.	Feb.
Feed/hen/day, gms.*	129.8	127.5	117.0	93.9	108.3	100.2	92.4	88.9	95.8	98.4
Energy in feed/hen/day, Cals.	389.4	382.5	351.0	281.7	324.9	300.6	277.2	266.7	287.4	295.2
Daily heat production at full feed, Cals.	206.9	200.2	178.3	155.8	158.4	151.2	157.0	162.2	162.7	165.6
Daily heat production of fasting hens. Cals.	163.2	153.4	147.1	131.8	132.7	123.6	129.1	133.2	129.4	126.2
Calorigenic effect of feed. Cals./day	43.7	46.8	31.2	24.0	25.7	27.6	27.9	29.0	33.3	39.4
Calorigenic effect of feed, per cent	11.2	12.2	8.9	8.5	7.9	9.2	10.1	10.9	11.6	13.3

*These figures include wastage which could neither be avoided nor accurately estimated.

TABLE 7b.—CALORIGENIC EFFECT OF FEED *Flock II*

	Jan.	Feb.	Mar.	Apr.	May	1939 June	July	Aug.	Sept.	Oct.	Dec.	1940 Jan.
Feed/hen/day, gms.*	110.8	102.5	103.7	123.5	147.9	129.4	94.7	128.3	132.1	123.0	131.7	112.7
Energy in feed/hen/day, Cals.	332.4	307.5	311.1	370.5	443.7	388.2	284.0	384.9	396.3	369.3	395.1	338.1
Daily heat production at full feed, Cals.	181.0	173.8	172.6	175.7	179.7	153.6	157.4	154.1	166.1	151.0	170.4	158.9
Daily heat production of fasting hens. Cals.	142.1	146.2	141.4	142.6	140.4	132.2	127.7	133.0	127.4	128.4	138.7	124.8
Calorigenic effect of feed. Cals./day	38.9	27.6	31.2	33.1	39.3	21.4	29.7	21.1	38.7	22.6	31.7	34.1
Calorigenic effect of feed, per cent	11.7	9.0	10.0	8.9	8.8	5.5	10.5	5.5	9.8	6.1	8.0	10.1

*These figures include wastage which could neither be avoided nor accurately estimated.

Body Temperature

The small variations found in body temperature do not appear to be related to egg production. This confirms the report of Heywang (1938) that the body temperatures of 6 hens were approximately equal during laying and non-laying periods.

Heart Rate of Fowls

Because the heart rate as a criterion of other physiologic phenomena was not touched on in the general review of literature, a few cogent references are given here.

Fishburne and Cunningham (1938) reported that thyroidectomy led to a decrease of one-sixth to one-third in heart rate of rats.

Means and Aub (1919) reported a close correlation between heart rate and metabolic rate in some of their patients suffering from exopthalmic goiter. Murlin and Greer (1914) reported that pulse rate multiplied by pulse pressure gave a somewhat better index of oxygen consumption in humans than the heart rate alone. This finding was confirmed by Read (1924), who devised a formula using pulse rate and pulse pressure for predicting the basal metabolic rate in humans. Read reported that the pulse rate and pulse pressure vary directly with the basal metabolic rate, and that the coefficient of correlation between the basal rate and pulse rate is 0.74, and between the basal rate and a pulse pressure, 0.62. However, Benedict and Finn (1928) reported that pulse rate of a normal woman was usually lowest when the oxygen consumption was low, but that the higher pulse rates did not occur simultaneously with high oxygen consumption. Sutliff and Holt (1925) reported that from 5 to 20 years of age the pulse rate and basal metabolic rate decline together; but that while the basal metabolic rate declines with increasing age, the pulse rate remains nearly constant throughout adult life.

This study was made because nothing was known concerning the relationship between heart rate and heat production of fowls.

The first attempts to obtain heart rate were made with a diaphragm stethoscope while the hen was held in the hand. The heart rates obtained in this way were extremely variable. The behavior of the hens indicated that they were frightened by the necessary restraint, and even after weeks of training the hens were invariably uneasy during hart rate determinations. Later, it was found that when the microphone of an electrically amplified stethoscope* was held against the sheet metal floor of the closed chamber just below the fowl's breast the heart beat usually could be heard quite clearly. Ordinarily

*Western Electric 3A Stethoscope (See Carter 1938).

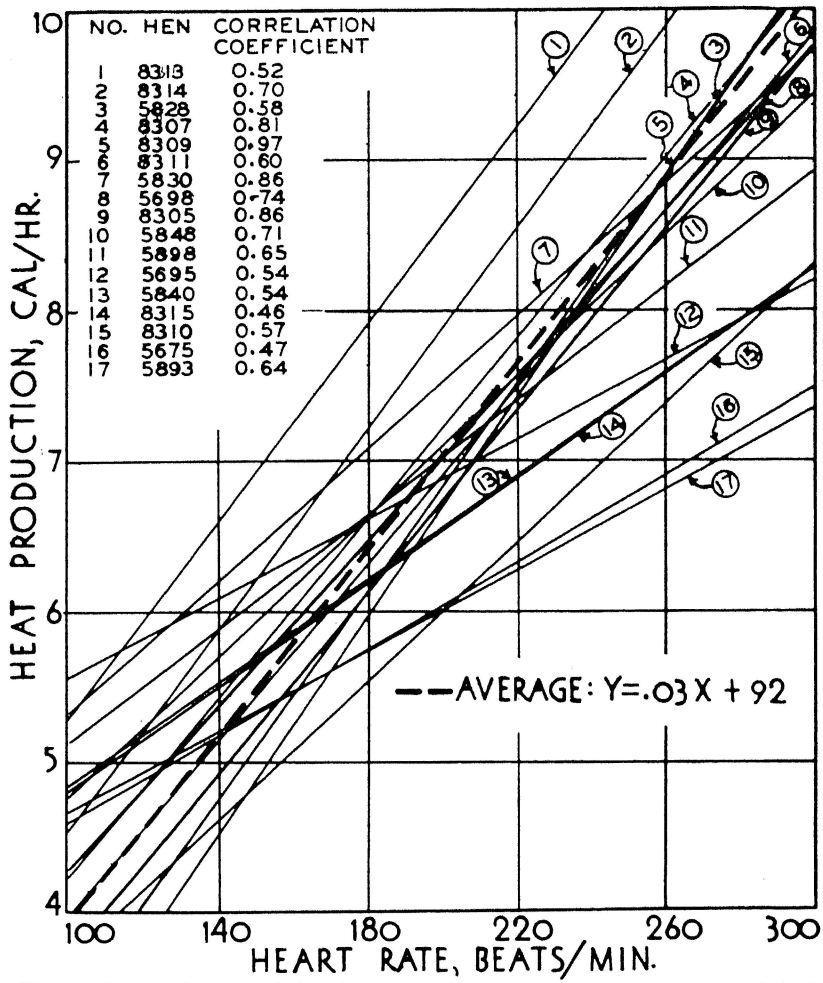

Fig. 8.—*Curves showing relationship between heat production and heart rate of fowls.* Curves for individuals in which the correlation proved to be significant are shown. The heavy, broken line represents an average of the curves for individuals.

the birds rest with their breasts against the floor of the chambers. Heart rates of individuals obtained in this way were as low as 84 beats per minute, and they were almost invariably found to be lower than they had been when the diaphragm stethoscope had been used with the fowl held in the hand. Mean heart rates of groups of hens measured while they were in the closed chambers are given in Tables 1, 2, 4, and 5. Heart rates which had been obtained earlier with the diaphragm stethoscope were within the range of 280 to 400 beats per minute reported by Dukes (1937b, p. 109, after Kaupp).

It may be assumed that the fowls were sleeping in the closed chambers and that heart rates of resting fowls in a wakeful state might be higher than those measured. The conditions under which heart rate was obtained were virtually the same as those existing during heat production measurements, and the possibilities for comparing measurements of the two different phenomena were therefore excellent. It should be noted that heart rates could not have been obtained in this way unless the fowls were very quiet.

Fig. 5 shows curves of heart rate and of heat production under conditions of fast and full feed. It will be seen that fasting heart rate was always lower at a given period than that of the same group of fowls at full feed.

As shown by Fig. 4 there are remarkable similarities between heart rate and heat production curves of individual hens. The heat production record (Cals/hen/hr) of each individual hen in Flock I was plotted against corresponding heart rate. In 17 out of 25 cases the correlation proved to be significant with coefficients of correlation ranging from 0.46 to 0.97. These 17 cases are shown in Fig. 8 with a curve representing an average of those of the individuals. The equation for this average relationship shows that for each increase in heart rate of one beat per minute, heat production increased by 0.03 Cal. per hour in these 17 hens. Body weight of the hens was disregarded in this calculation.

Summarizing, there is a tendency for heat production to vary with heart rate in fowls, but the correlation is not close enough to make possible the prediction of one from the other.

Thyrotropic Hormone Levels

The metabolic rate is regarded as a satisfactory measure of thyroid activity, particularly when other factors which might influence heat production are controlled. The seasonal rhythm of heat production in fowls may therefore be regarded as an index of thyroid activity. In this connection seasonal measurements of pituitary thyrotropic hormone levels are of particular interest.

A study of the seasonal rhythm of pituitary thyrotropic hormone in fowls was attempted. The assay method first used was similar to those described by Smelser (1937 and 1938) and by Bergman and Turner (1939). Four daily intramuscular injections of hens' pituitary† suspended in water were given to newly hatched chicks; thyroid weight was determined on the fifth day. After the approxi-

†Mean weight of 230 anterior lobes of hens' pituitaries was 9.7 mg., which is not far from an average of the weight of rat pituitaries, and less than half of the mean weight of pituitaries of rabbits within the fowls' range of body weight.

TABLE 8a.—INFLUENCE OF INJECTED PITUITARY ON THYROID WEIGHT OF CHICKS (In February)

Chicks No.	Chicks Mean Body Wt.	Dosage	Mean Thyroid size	Increase over controls
	gms.	mg.	gms.	%
28	42	0.5 (muscle)	1.9 ± 0.18	0
39	43	0.25 (pituitary)	2.4 ± 0.17	26.3
59	43	0.50 (pituitary)	3.0 ± 0.16	57.9

Additional Data on Pituitaries Used:

No. of White Leghorn hens (Source of Pit.)	Mean Body Weight kg.	Mean Ant. Pit. Wt. mg.	Chick units per gland	Chick units per gm. of pituitary	Chick units per kg. of hen's body weight
7	1.51	11.5	23	2000	15.2

TABLE 8b.—INFLUENCE OF INJECTED PITUITARY ON NORMAL METABOLISM OF CHICKS

Date	No. Chicks	Body Wt. of group gms.	Dosage of Pituitary mgs.	Heat Prod. Cal./hr.	Heat Prod. Cal./hr./gm.	Diff. in Heat Prod. Exp.—Controls	Increase in Metabolism %
Feb. 9	11	402.9	3.0	2.30	.0057	.0017	42.5
Feb. 9	11	407.9	1.50	2.16	.0053	.0013	32.5
Feb. 9	11	404.6	0.98	2.35	.0058	.0018	45.0
Feb. 10	10	371.9	Muscle	1.50	.0040	....	...
Feb. 10	10	379.4	0.72	2.16	.0057	.0013	29.5
Feb. 10	10	389.6	0.36	2.35	.0060	.0016	36.4
Feb. 10	10	369.6	0.18	1.88	.0051	.0007	15.9
Feb. 10	10	320.0	Muscle	1.41	.0044	....	...
Feb. 9	8	323.4	0.09	1.86	.0058	.0005	9.4
Feb. 9	7	274.6	Muscle	1.46	.0053	....	...

*Pituitaries were from hens of medium heavy breeds killed at slaughter houses.

mate minimal dosage had been determined by preliminary trials, the "chick unit", or minimal dosage of hens' anterior pituitary substance required to give an increase of approximately 50% in thyroid size of chicks of both sexes was determined (Table 8).

A technique making use of an increase in metabolic rate was also used. This technique is less time consuming than the thyroid weight method. The two methods, if used together, should add greatly to the accuracy and dependability of the determinations. Groups of 7 to 11 or more chicks were injected with pituitary suspension 36, 24, and 12 hours* before they were placed in a 6 liter respiration chamber. Heat production was measured during a period of 2 or more hours. Metabolic rate of control groups which had been injected with muscle suspension was measured simultaneously. Differences in metabolic rate of 2 control groups measured simultaneously were as large as 12%, and this served as an index of the possible error of the method. Some results obtained with this method during the month of February are given in Table 8. Up to a dose which gave a 36%

*O'Donovon and Collip (1938) and Billingsley, O'Donovan, and Collips (1939) have shown that the "Specific Metabolic Principle" of the pituitary causes an increase in metabolic rate which is maximum at about 3 to 4 hours after injection, after which there is a slow return to normal. Any effect of this hormone on the metabolic rate of the assay chicks should have disappeared within 12 hours after injection.

increase in metabolic rate, the percentage increase in heat production and in thyroid size were remarkably similar for given dosages.

During March and April hens' pituitary tissue was injected in increasingly large doses, but the metabolic rates of the injected chicks could not be regarded as significantly higher than those of the controls.

In May, pituitaries of adolescent fowls were injected because these were the only kind available in large numbers. Doses up to 263 mg. gave no significant results. A dose of 263 mg. was followed by an increase of only 26% in heat production. Doses of 310 mg. of pituitary tissue proved to be lethal, although muscle tissue administered in the same quantity and manner apparently was harmless and did not significantly increase the metabolic rate of injected chicks.

These data, although fragmentary, suggest that the pituitary thyrotropic hormone level is high in February (the month in which the highest heat production was determined) and that the level declines rapidly following February.

Influence of Thyroidectomy and of Replacement Therapy on Egg Production

Reports by various authors indicate that reproductive processes are influenced by the thyroid. The reports of Crew (1925), Benoit and Aron (1934), and Taylor and Burmester (1940) which are particularly pertinent have already been reviewed (see pages 13 and 14). A few additional references are given here.

The effects of thyroidectomy on male and female mammals are similar to those on fowls. Zalesky and Wells (1937) reported that thyroidectomy depressed spermatogenesis in male thirteen-lined ground squirrels. According to Fishburn and Cunningham (1938) sex cycles of female rats were lengthened following thyroidectomy.

In addition to its influence on the reproductive organs, the thyroid influences milk production. Graham (1934) reported that thyroidectomy depressed milk production of cows; thyroid feeding temporarily restored milk production of thyroidectomized cows, and temporarily increased production of normal cows. Herman, Graham, and Turner (1938) reported that thyroid feeding increased milk production for a limited time following treatment.

The influence of thyroid on feather growth has been investigated by Cole and Reid (1924), Cole and Hutt (1928), Martin (1929), and Schwarz (1930 and 1933).

The purpose of this part of our research is the determination of possible relationships between the thyroid and egg production by means of thyroidectomy and replacement therapy.

Thyroidectomy in the fowl is a comparatively difficult operation. The thyroid is located not in the neck, but within the body on the ventral side of the common carotid artery at a point where it is closely associated with the jugular vein. Since the thyroid is so close to these important vessels, and since it is in addition a highly vascular organ, hemorrhage tends to be great. A technique was evolved by which the thyroid was separated from the vessels by blunt dissection before the organ was fully removed, with reduced hemorrhage as a result. Twenty-two hens were thyroidectomized under light ether anesthesia.

The fowl ordinarily seems to possess accessory parathyroid bodies; but as a precautionary measure against low blood calcium, 5% of calcium lactate was given in the water.

After thyroidectomy the fowls, which had been laying at the time of the operation, rapidly assumed the appearance of non-laying hens. Average egg production of the flock was very low during the two months following the operation (see Table 9a). Of the 17 hens surviving at the end of this period the 7 most affected by the operation were selected for replacement therapy, while those less affected were kept as a control group, and 4 hens showing no effects of the operation were equally divided between the two groups. However, data on these 4 hens are not included in the tables since it happened that deletion of these data did not influence the ratio of egg production of the thyroxin-injected and non-injected thyroidectomized groups, while inclusion of records of these 4 apparently normal birds would have resulted in larger figures for egg production during replacement therapy, and therefore might have led to a false conclusion regarding the effectiveness of thyroxin in restoring the production of the operated birds.

Thyroxin, suspended in water, was injected twice a week intramuscularly at an initial weekly level of 0.93 mg per $kg.^{0.73}$ of body weight. After 9 days at the original level, the thyroxin dosage was doubled and was kept at this higher level in order to maintain heat production of the fowls near the preoperative level.

As shown by Table 9a, thyroidectomized hens produced between 3.8 and 4.5 times as many eggs (per hen per week) while receiving the thyroxin treatment as they had produced during the period of approximately 2 months between the time of operation and the first thyroxin injection.

Data on fasting heat production are given in Table 9b. Since fasts occurring at intervals of less than a month might have adversely affected egg production, fasting heat production was determined only once each month. As shown by Table 9b fasting heat production declined after thyroidectomy and was at least partially restored to the preoperative level by injected thyroxin, but the preoperative level was probably not exceeded. Mean body weights of the 2 groups of operated hens as determined at intervals during the investigation are given in Table 9b.

The mechanism through which the thyroid influences reproductive processes is not yet known. The fact that synthetic thyroxin partially restored egg production of thyroidectomized hens suggests that it is *thyroxin,* and not some other possible thyroid hormone, that influences reproduction.

TABLE 9a.—INFLUENCE OF THYROIDECTOMY ON EGG PRODUCTION, AND OF REPLACEMENT THERAPY ON PRODUCTION OF THYROIDECTOMIZED HENS

	Group I Thyroidectomized fowls injected with thyroxin			Group II Thyroidectomized fowls not injected with thyroxin		Normal fowls
Number of birds used	7			6		17
	Production eggs/hen/ week	% of production of normal fowls	Weekly dosage	Production eggs/hen/ week	% of production of normal fowls	Production eggs/hen/ week
Production during months prior to operation. March 23 to April 22 inc.	3.77	...	0	3.50	...	4.31
σ_M	0.39	...	...	0.44	...	0.17
Production during period between operation and 1st thyroxin injection April 23 to June 21 inc.	0.42	10.2	0	0.81	19.7	4.12
σ_M	0.18	...	...	0.32	...	0.21
Production during period June 22 to 30 inc. Thyroxin 1st given on June 21	1.88	43.5	0.93 mg. thyroxin per kg.$^{0.73}$	0.13	...	4.33
σ_M	0.41	...		...	...	0.20
Production during July	1.77	50.6	1.86 mg. thyroxin per kg.$^{0.73}$	0.75	21.3	3.52
σ_M	0.41	...		0.21	...	0.23
Production during August ..	1.58	60.1	1.86 mg. thyroxin per kg.$^{0.73}$	0.75	28.5	2.63
σ_M	0.36	...		0.21	...	0.32

TABLE 9b.—FASTING HEAT PRODUCTION AND BODY WEIGHT. INFLUENCE OF THYROIDECTOMY AND OF REPLACEMENT THERAPY ON THEROIDECTOMIZED HENS.

During fast of 24-28 hours	During month prior to operation. Mar. 23 to April 22 inc.	Period between time of operation and time of first injection Apr. 25 to June 21 inc.	Period of thyroxin injection at first level. June 22 to 30 inc.		Period of thyroxin injection at second level			
					July		August	
			Weekly dosage Thyroxin /kg.$^{0.73}$		Weekly dosage Thyroxin /kg.$^{0.73}$		Weekly dosage Thyroxin /kg.$^{0.73}$	
Mean rate of fasting heat production	Calories /kg.$^{0.73}$	Calories /kg.$^{0.73}$	Calories /kg.$^{0.73}$	mg.	Calories /kg.$^{0.73}$	mg.	Calories /kg.$^{0.73}$	mg.
Group 1								
Injected fowls	3.27	2.81	3.02	0.93	2.89	1.86	3.09	1.86
σ_M	0.11	0.09	0.10	...	0.08	...	0.18	...
Group 2								
Non-injected fowls	3.03	2.85	...	0	2.98	0	2.80	0
σ_M	0.05	0.18	...	...	0.17	...	0.09	...
Mean body weight	kg.	kg.	kg.	mg.	kg.	mg.	kg.	mg.
Group 1								
Injected fowls	1.56	1.42	1.48	0.93	1.47	1.86	1.59	1.86
σ_M	0.06	0.05	0.10	...	0.08	...	0.10	...
Group 2								
Non-injected fowls	1.51	1.49	...	0	1.43	0	1.62	0
σ_M	0.10	0.13	...	...	0.05	...	0.06	...

IV. SUMMARY AND CONCLUSIONS

The results of a two-year investigation of the seasonal metabolic rhythm of domestic fowls during the pullet year may be summarized as follows:

1. There is a rough parallelism between fasting heat production, heart rate, and egg production. The correlation between heart rate and metabolism is not great enough to permit the prediction of one from the other.

2. There was considerable *daily* fluctuation in metabolism and heart rate, which was as great as 40% of the largest figures obtained during a period of one month. This unexpectedly great lability in metabolism suggests the need of relatively large numbers of individuals if statistically significant results are to be obtained.

3. Thyrotropic hormone content of hens' pituitaries was determined both by increase in thyroid size and by increase in metabolic rate of injected chicks. Assays made in February showed a high thyrotropic potency of hens' pituitaries. Those made later indicated that the thyrotropic potency of hens' pituitaries was much less following February than it had been during that month.

4. The influence of thyroid on egg production was studied. Seven hens which had been laying 3.77 $\pm$ 0.39 eggs per hen per week before thyroidectomy, laid only 0.42 $\pm$ 0.18 eggs per hen per week after the operation. Weekly injections of about one mg. of thyroxin per $kg^{0.73}$ were followed by an increase of production to 40% of that of a group of normal hens, and injections of about 2 mg. per $kg^{0.73}$ were followed by production of 60% of that of the normal group. Six thyroidectomized non-injected hens remained at a low production level throughout the experiment. The preoperative fasting metabolism level was restored, but probably was not exceeded during the course of injections. Mean body weight of the group was not significantly influenced by the injections.

5. This investigation involved the design and construction of a 4-chamber Regnault-Reiset metabolism apparatus, and the adaptation of an electric stethoscope to the measurement of heart rates of fowls while they were resting quietly in closed chambers; both of these are described in the text. This bulletin also includes a critical discussion of photoperiodicity, and thermoperiodicity with particular reference to their bearing on the egg production problem.

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