THE EFFECT OF VITAMIN A DEFICIENCY UPON THE URIC ACID EXCRETION IN THE FOWL

by

EMERY JACK COULSON

B. S., Kansas State Agricultural College, 1927

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1930

TABLE OF CONTENTS

INTRODUCTION

The quantity of uric acid excreted in the urine of the mammalia, amphibia, and fishes is small and is but a small proportion of the total nitrogen outgo. However, in reptiles and in birds, which were evolved from reptiles, this substance takes the place of urea and most of the nitrogen excretion is in this form. This substitution of uric acid for urea as the form of nitrogen waste in reptiles and birds seems to be an adaptation for the conservation of water, thus fitting them for a dry climate. Uric acid has very little affinity for water, therefore it is excreted with a minimum amount. Urea, on the other hand, is very soluble and has a great affinity for water and so removes considerable amounts from the body when it is excreted.

Although there has been a great deal of experimental work done on the nitrogenous constituents of the urine of other animals, there exists today a lack of knowledge about the constituents of bird urine. Perhaps one of the greatest reasons for this lack of data is that, due to their anatomical structure, the urine of the fowl is quite difficult to obtain without being contaminated with feces. When chemical, mechanical, or operative methods of

separation of the excreta are resorted to, assumptions are often made which are not readily proved.

Uric acid in birds is derived principally from the protein material of the tissues and the food, and is formed through a process of synthesis analogous to the formation of urea in man. This formation of uric acid occurs chiefly in the liver of the fowl; a comparatively small fraction of the total uric acid excretion of birds may result directly from nuclein material. O. Minkowski (1) has proved conclusively that uric acid is formed chiefly in the liver of fowls. He has extricated the liver in geese and ligating the colon just ahead of the cloaca, by means of which he obtained pure urine, observed that in birds in which the liver had been removed the uric acid constituted only 3 to 6 per cent of the total urinary nitrogen, while 50 to 60 per cent of the total nitrogen was excreted as ammonia. In normal birds he found that uric acid constituted 60 to 70 per cent of the total nitrogen.

It has been observed that in the blood of hens in advanced stages of avitaminosis A that the concentration of uric acid rises to a very high degree. The other outstanding nitrogenous constituents of blood namely, urea and creatine tend to increase, but the increase is in no

way parallel to the increased concentration of the uric acid.

The retention of uric acid in avitaminosis A hens suggested the problem as to what condition was causing this decided increased concentration of uric acid. At first there seemed to be but two possible causes; first, is uric acid produced at an abnormal rate in these hens, or second, is uric acid eliminated at a decreased rate? Other possible causes were presented as the problem progressed.

As an attempt to solve this problem, analyses were made on the urine of normal hens and a comparison made with corresponding analyses on the urine of hens suffering from the lack of vitamin A in their diet. Since the hens were always fasting when the advanced stages of the disease occurred, analyses of the urine of fasting hens were also made for comparison.

METHODS

White leghorn hens were used in this experiment and put upon a diet consisting of white corn and tankage. The analyses of the urine of the normal hens were made a short time after they were placed on the diet, and before the effects of any vitamin deficiency could be registered.

In about five or six months the hens that had been left on this diet began to show abnormal symptoms, the familiar symptoms of avitaminosis A hens. They first began watering slightly at the eyes and refused to eat. They do not appear to be other than normal hens in their actions at this time except that they are perhaps a little more quiet. However, they soon become weak and their eyes swell very much depending seemingly upon the amount of water they take. A hen in this condition when left to herself takes no water or food and soon dies, but in several cases water was administered to the hens forcibly to see if the uric acid could in this manner be washed out of the system. Although these hens lived longer than those that took water voluntarily, they succumbed in exactly the same manner. The water administered did not prevent the retention of uric acid although the unusually high values were noticeably absent. The eyes of the hens given water daily swelled to an extreme degree.

In hens which received no water but what they take voluntarily, the uric acid concentration of the blood increased from one to sixteen times the normal concentration usually, with a few cases running higher. In one case a concentration of 160 mg. of uric acid per 100 cc. of blood was encountered.

These high values of uric acid seem almost impossible when one considers the solubility of uric acid. However, Gibbs (2) has achieved the higher figure of 170 mg. of uric acid per 100 cc. of blood by the simple procedure of tying the fowl's ureters and allowing the bird to live a long time before bleeding. Gibbs gives evidence which shows that the uric acid occurring in fowls' blood is in a special soluble form.

On post mortem examination crystals of uric acid were usually found distributed throughout the tissues. These were particularly noticed in the kidneys which take on a grayish appearance due to the solid material in the tubules. The ureters which are normally not easily visible are distended to many times their normal size.

URINE COLLECTION

To overcome the difficulty of separating the urine from the feces, a special technique, first used by Davis (3) was employed, which is applicable to unanesthetized, unoperated birds. The procedure used is as follows:

The hen is first given 100 cc. of water by means of a stomach tube and immediately fastened on her back on a specially constructed board. This apparatus consists of a

flat board about 16 inches long by 12 inches wide. To this was fastened, about four inches from the end, two upright pieces slanting toward the top of the board and to the inside so that the legs of the hen could be fastened in their natural position. Through two holes in the board was a strap which encircled the hen, around her breast and wings. The whole apparatus was slanted at an angle of about 45 degrees. This arrangement secured the hen in such a manner that she could not move during the time of collection. (Fig. 1)

The feces were first removed from the cloaca and the cloaca carefully washed out. A catheter consisting of a glass tube flared at one end was inserted into the urodeum near the openings of the ureters. The urine from the ureters was usually quite copious and in the form of a clear or translucent mucoid-like liquid in which are usually found white crystals of uric acid. The urine was collected in a solution of lithium carbonate, in order to dissolve the crystals of uric acid and prevent its further precipitation.

The period of collection usually extended over about one and one-half hours. During this time 30 to 90 cc. of urine was collected from the normal hens. Considerably less urine was collected from the sick hens as will be noted later.

Plate I

Method for Collecting Urine

The objection to the above procedure lies in the fact that the total daily urinary excretion cannot be obtained as it is obviously impossible to leave the hens trussed up on the board for twenty-four hours at a time. Other investigators have obtained the total urinary excretion by operating upon the birds and forming an artificial anus in the anterior abdominal wall, which permits the separation of the feces and urine by means of suitable collection devices. This plan has been followed by Minkowski (1), Milroy (4), Paton (5), and Katayama (6). The chief objection to this procedure lies in the fact that an abnormal condition is created which, without doubt, has some effect upon the bird's behavior.

The total daily urinary excretion would be indispensable if one were conducting digestibility experiments and, indeed, it would be of great assistance in solving this problem, but rather than run the risk of affecting the uric acid secretion by the operation, and because of the large number of hens to be examined, the non-operative method was employed. We must then content ourselves by determining the uric acid excretion in relation to the total urinary nitrogen.

The results of Davis (3) and Ambard and Wolf (7) have

shown that the water diuresis, caused by the water administered before collection of thr urine, has no effect upon the relative amounts of nitrogen compounds in the urine.

CHEMICAL METHODS

The total nitrogen was estimated by the Kjeldahl method, urea by the urease method of Folin and Youngberg (8). Ammonia was determined by the Folin and Bell (9) permutit method. This method was checked in three cases by the aeration method and was found to agree quite closely. Creatine and creatinine were determined together by the Folin-Benedict (10) method. Creatinine occurs in very minute amounts, if at all, in the urine of birds. Uric acid was determined by the Folin and Wu (11) silver lactate isolation method.

These several determinations failed to account for all the nitrogenous material present by an average of about 12 per cent of the total nitrogen. A decision was therefore made to discover, if possible, what other nitrogenous products were present and thus make a more complete analysis possible.

If purine bodies other than uric acid are present, these should be found also precipitated as silver salts

along with the uric acid in the silver lactate precipitate, which is the first step in the determination of uric acid by the Folin and Wu method. Consequently, the silver lactate precipitate was carefully washed and the total nitrogen determined by the Kjeldahl method. In every case there was more nitrogen present than was accounted for by the uric acid determined on the silver lactate precipitate. This procedure was then followed throughout the rest of the determinations of purine bases. In more detailed form this method is as follows:

Two 10 cc. samples of urine are measured into 15 cc. centrifuge tubes and enough silver lactate solution is added to precipitate all the purine bases. These are allowed to stand in the dark for 5 minutes and then centrifuged. The clear supernatant liquid is poured off and the precipitate carefully stirred up with distilled water containing 2 or 3 drops of the silver lactate solution. After washing the precipitate three times in the above manner one of the samples is transferred quantitatively to a Kjeldahl flask and nitrogen determined in the usual manner. This gives total purine nitrogen and the uric acid nitrogen determined on the other sample, subtracted, gives purine nitrogen other than uric acid.

The remainder of the unaccounted nitrogen is thought to be largely allantoin. At any rate, quantitative determinations of allantoin by the acid hydrolysis method of Plimmer and Skelton as modified by Harding and Young (12) gave results which when added to the above determinations checked closely to 100 per cent of the total nitrogen. There are, however, objections to this method. Benedict (13) found that the Folin (14) procedure for the determination of urea, which is of the same principle, (where it is subjected to the action of hydrochloric acid and magnesium chloride), hydrolyzes all of the allantoin nitrogen, 2 per cent of uric acid nitrogen and 1 per cent of creatinine nitrogen into ammonia. More recently, however, Morgan and Osburn (15) have checked the above allantoin procedure on rat urine and report a recovery of from 92.3 per cent to 97.1 per cent of added allantoin, which would tend to indicate no hydrolysis of uric acid. However, the uric acid in rat urine amounts to only .5 to 1.7 per cent of the total urinary nitrogen.

Other methods for the determination of allantoin were so long and tedious that it was not thought worth while, where so many determinations were to be made, that these methods should be followed; also since the major problem with which we were concerned was a study of the uric acid.

The formal titration of Henriques and Sorensen (16) failed to indicate the presence of amino acids.

In Table I are shown the results of the determinations of the nitrogenous constituents in the urine of normal hens.

DISCUSSION

The uric acid nitrogen was found to constitute an average of 65.80 per cent of the total urinary nitrogen, varying between the limits of 54.10 per cent and 76.80 per cent. This wide variation of results, due probably to the inherent individuality of the fowl, makes a comparison between normal and abnormal hens a hazardous undertaking. However, it is thought that a large number of determinations when averaged will give an indication, at least, of the tendency of the increase or decrease in the excretion of uric acid.

Minkowski (1), using the operative method for obtaining urine, found the uric acid nitrogen to account for from 60 to 70 per cent of the total urinary nitrogen in normal hens. Milroy (4), also using the operative method, reports that the uric acid accounts for from 60 to 65 per cent of the total nitrogen excreted in the urine. Davis (3) using the same procedure as described here found the uric acid

nitrogen to vary between 59.4 to 69.5 per cent of the total urinary nitrogen, with an average of 62.9 per cent. Sharp (17), who obtained pure urine from birds by inserting cannulas into the ureters, found that uric acid nitrogen accounted for about 30 per cent of the total nitrogen. He, however, used the ammonium sulphate method for the precipitation of uric acid which is not thought to be as accurate as the later methods.

Collection of urine from the sick hens was not as easily accomplished as in the case of the normal ones. As has been mentioned, the hens when left alone would quit eating and drinking as soon as they entered the last stages of the disease. In every case it was found that the ureters were clogged with crystals of uric acid. These were tightly packed and very dry so that even when a large amount of water was given to the hen just before collection, no urine could be collected. That this was not a mechanical stoppage, however, was shown by the fact that if the hen were given water by a stomach tube once or twice the day before collection took place, the body became hydrated and the ureters became moist and then urine could be collected. However, only small amounts could be collected during the 90-minute period. Nevertheless, this urine was very

concentrated and it was necessary to dilute it before making the analysis. The results, however, are reported as undiluted urine.

In Table II are shown the analyses of the urine of a group of hens all in the last stages of avitaminosis A. As will be seen from an examination of Table II the uric acid nitrogen constitutes an average of 63.62 per cent of the total urinary nitrogen, as compared to 65.80 per cent in the urine of normal hens. This is probably not significant, but if this amount of retention did go on for 24 hours a day, a large amount of uric acid could be accounted for. If it were possible to obtain the total daily excretion, a closer comparison might be made.

Since all of these hens were well advanced into the last stages, and since their former level of uric acid excretion was not determined, it was thought that a better conclusion could be drawn if their former level of uric acid excretion were determined, and the progress of the disease were followed from the time the hen showed the first symptoms of the disease, and before any appreciable amount of increased concentration of uric acid in the blood had taken place.

This was done with the last three hens. As soon as these hens showed the first sign of approaching the last stage, they were placed in separate cages and the analysis of their urine began. In every case the blood of these hens analyzed as normal blood, but as time went on the uric acid concentration increased in the blood and seemingly decreased in the urine. The other outstanding constituents of the blood, i.e. urea and creatine, increased 2 or 3 times their normal amount, while the uric acid increased 6 to 8 times its normal amount. The unusually high values of uric acid were not encountered here because of the frequent watering it was necessary to give the hens, so that collection of the urine would be possible. The results of these analyses are shown in Table III.

An examination of Table III reveals that the excretion of uric acid decreases in proportion to the total urinary nitrogen up until almost the last, when there seems to be a belated attempt by the body to rid itself of this product.

That there is a renal deficiency at this stage is also clearly evidenced by a few preliminary kidney efficiency tests which were run on a few of the abnormal hens while in this last stage. One cc. of a solution containing 1.5 milligrams of phenolsulphonephthalein was injected intramuscularly into the right thigh of the hen

and the urine collected in half-hour periods. In the first half hour 11 to 17 per cent of the drug was recovered in the urine of the sick hens as compared to about 40 per cent from normal hens. In the second half hour period 12 to 16 per cent was recovered as compared to about 24 per cent from normal hens.

An interesting fact was noted in the case of these abnormal hens. It seems that not until the fowl showed outward appearance of a pathological condition, and frequently quite sometime later, does the uric acid concentration increase to a very high degree, and not until sometime later does the urea and creatine nitrogen increase (18) This suggests the possibility first mentioned by Gibbs (19) that the fowl's kidney actively secretes uric acid and that this mechanism is distinct from that involving water excretion. The following is quoted from Gibbs (19):

"Certainly the uric acid does not simply filter through the renal cells as through a colloidin membrane, since the process requires the intervention of energy as a concentration of over 3,000 times, and which is also shown by the fact that asphyxia promptly stops secretion, and this may occasionally be shown in a very marked manner.

"We have, however, a beautifully analogous case in the secretion of bile. This substance is taken up from the blood stream in a specific manner by the liver cells, and then gently pushed out the other side. The amount dealt with in this way depends upon the amount in the blood stream; back pressure promptly interferes with this mechanism, the bile piling up in the blood stream in the same way as uric acid does in the fowl, when the renal mechanism is affected. Also like the fowl's kidney, no storage takes place. It is suggested therefore that the process of uric acid secretion in the bird is by a process involving a similar type of mechanism to that of biliary secretion, which picks up the bile from the blood, though this is modified to suit the different substance. Such a mechanism would satisfy all the data obtained in a long series of experiments on this subject."

We would have to suppose, however, that the renal deficiency affected only the uric acid secretion mechanism of the kidney, while the water excretion mechanism remained normal.

In gout the human kidney is said to lose the power of properly eliminating uric acid and it collects in the blood in abnormally high concentration.

Since, as stated previously, these hens fast completely in this last stage of the disease, it occurred to us that perhaps the fast also might have something to do with that retention of uric acid. Consequently, a group of hens was placed upon a complete fast and an analysis of their urine made from time to time. (See Table IV). The blood uric acid was also determined from time to time. Aside from the fact that these hens did not become sick with the sore eyes, etc., as noticed in the avitaminosis A hens, they reacted in quite a similar manner. The uric acid content began to increase in the blood with a corresponding decrease in the urine. However, these hens drank more water and passed more urine than did the avitaminosis A hens. Consequently, the uric acid concentration did not increase to such a high degree in the blood.

Lennox (20) reports an increase of uric acid concentration in the blood of fasting human patients as the result of decreased uric acid elimination. He further found that the non-protein nitrogen of the blood did not parallel the concentration of uric acid in the blood. That this disturbance of renal function is only temporary is clearly evidenced by the fact that as soon as the patient began eating again the elimination of uric acid became normal.

Paton (5) gives evidence to show that there is a retention of muscle nitrogen within the body of the fasting fowl.

The variation of the creatine nitrogen is also interesting and significant. The creatine nitrogen excretion increases in the fasting and avitaminosis A hens relatively to the total urinary nitrogen. This fact was also noted in the fasting fowl by Paton (5), who says "----- the increased creatine excretion indicates an increased muscle catabolism and since creatine is an end product, from the amount excreted, conclusions may be drawn as to the amount of muscle tissue catabolised." This catabolism of body protein may also account for an increased production of uric acid.

The urea and ammonia nitrogen seems to remain quite constant in the normal and abnormal hens. The allantoin nitrogen excretion is greater in the fasting and avitaminosis A hens. This looks like an attempt by the body to change the uric acid nitrogen into the more water soluble product as an attempt to eliminate this waste product from the system.

Morgan and Osburn (15) found that the uric acid excretion for rats suffering from vitamin A deficiency is the

highest with the greatest loss of weight. They write, "Apparently then the lack of vitamin A does not decrease the excretion of uric acid, but rather increases it as the proportion of total nitrogen derived from breakdown of body tissues (and therefore purine compounds) rises." They do not report any renal deficiency in the case of the rat and evidently did not do any analyses on the blood of the animals.

Whether the renal deficiency in the avitaminosis A hens is due entirely to the fact that they are fasting, or whether the lack of vitamin A affects the kidneys is not known. However, Wolback and Howe (21) report that the outstanding pathological change in the rat when deprived of the fat-soluble vitamin A, seems to be a keratinization of the epithelium throughout the body, including the epithelium of the bladder, ureters, and pelvis of the kidney. If this condition occurs in the kidney of the fowl when deprived of vitamin A, it seems logical to believe that the deprivation of vitamin A would cause a renal deficiency. This might be checked up by forced feeding of these hens to provide the necessary energy to maintain their body weight. This has been attempted (22) but so far it has been impossible to keep the hens from losing in weight. The concentration of the uric acid in

the blood of the avitaminosis A hens seems to increase more rapidly than it does in the fasting hens.

CONCLUSIONS

The results indicate that there is very little difference in the relative amounts of the nitrogenous constituents in the urine of the fasting hens and the avitaminosis A hens, and that the retention of uric acid may in a large part be due to the fasting in the last stage of the disease, rather than to the lack of vitamin A.

In both the fasting and avitaminosis A hens the results seem to indicate some decreased elimination of uric acid.

The increased excretion of creatine nitrogen and rapid loss of weight of the fowls indicates the breakdown of body proteins and accounts for an increased production of uric acid.

The extremely high concentrations of uric acid which are sometimes found in the blood of the avitaminosis A hens are due to the complete cessation of the renal function which is brought about by the precipitation of uric acid in the ureters, thus plugging them so that urine cannot flow.

Table I

Normal Hens

Date	Hen No	Time of Collection	Amount Collected	Total Nitrogen	Uric Acid Nitrogen		Purines other than Uric Acid Nitrogen		Urea Nitrogen		Ammonia Nitrogen		Creatine Nitrogen	Creatinine	Possible Allantoin Nitrogen		Total	
		Minutes	cc.	mg.	mg.	Per cent	mg.	Per cent	mg.	Per cent	mg.	Per Cent	mg.	Per cent	mg.	Per cent	mg.	Per cent
2-1-28	400	40	40	58.00	38.12	65.72	----	----	7.27	12.53	5.00	8.62	2.40	4.13	----	----	52.79	91.01
6-5-28	638	65	85	80.00	53.06	66.30	----	----	----	-----	4.36	5.45	----	----	----	----	57.42	71.77
6-11-28	638	?	?	76.45	53.33	69.90	----	----	5.06	6.73	5.54	7.25	----	----	----	----	63.93	83.62
6-11-28	655	80	37	68.68	42.83	62.40	----	----	3.80	5.53	4.41	6.42	----	----	----	----	51.04	74.31
6-21-28	56	60	70	64.60	36.25	56.10	----	----	2.88	4.45	----	----	2.24	3.46	----	----	41.37	64.04
6-21-28	76	60	58	71.20	38.50	54.10	----	-----	2.84	4.00	5.50	7.72	3.92	5.50	----	----	50.76	71.29
6-28-28	20	60	60	68.80	47.70	69.33	----	----	----	----	----	----	----	----	----	----	47.70	69.33
7-5-28	20	75	63	51.50	35.10	68.20	----	----	----	----	4.34	8.42	2.42	4.70	----	----	41.86	81.28
7-9-28	699	100	48	68.00	40.40	58.40	10.30	15.17	2.39	3.52	5.35	7.87	5.57	8.20	3.46	5.08	67.47	99.22
7-5-28	3	75	63	36.00	20.95	58.20	4.45	12.30	1.67	4.64	3.23	8.98	2.91	8.08	2.70	7.50	35.91	99.75
7-12-28	3	90	60	47.00	26.40	56.20	4.40	9.57	3.73	7.94	----	----	2.14	4.56	----	----	36.67	78.02
9-6-28	765	60	33	86.00	59.20	68.80	10.80	12.60	5.30	6.20	5.50	6.40	5.28	6.15	0.40	0.46	86.48	100.55
9-6-28	767	60	50	93.80	72.00	76.80	6.40	6.83	3.30	3.56	6.03	6.43	3.09	3.30	1.87	2.00	92.69	98.81
9-6-28	468	75	50	49.70	29.40	59.10	8.40	16.90	2.56	5.16	4.42	8.90	2.88	5.80	1.98	4.00	49.64	99.88
9-6-28	677	75	50	79.80	60.70	76.10	6.50	8.15	3.08	3.86	6.85	8.60	1.88	2.37	1.97	2.46	80.98	101.47
6-20-29	141	60	38	113.91	86.95	76.33	8.30	7.29	10.71	9.40	4.36	3.83	1.94	1.70	5.93	5.21	118.19	103.75
6-26-29	813	90	28	120.00	91.92	76.60	1.08	0.90	8.57	7.14	11.76	9.80	2.69	2.24	5.17	4.31	121.19	100.99
7-10-29	819	90	29	73.13	48.10	65.77	4.40	6.02	8.89	12.16	6.59	9.01	2.68	3.66	2.52	3.45	73.18	100.06
Average						65.80		9.57		6.45		7.58		4.56		3.83		97.79

Table II

Avitaminosis A Hens

Date 1928	Hen No.	Time of Collection Minutes	Amount Collected cc.	Total Nitrogen mg.	Uric acid Nitrogen mg.	Uric acid Nitrogen Per cent	Purines other than Uric Acid Nitrogen mg.	Purines other than Uric Acid Nitrogen Per cent	Urea Nitrogen mg.	Urea Nitrogen Per cent	Ammonia Nitrogen mg.	Ammonia Nitrogen Per cent	Creatine Creatinine Nitrogen mg.	Creatine Creatinine Nitrogen Per cent	Possible Allantoin Nitrogen mg.	Possible Allantoin Nitrogen Per cent	Total mg.	Total Per cent	Condition of Hens at Time of Collection
6-8	638	?	?	80.00	53.06	66.30	-----	-----	-----	-----	4.36	5.45	5.19	6.50	----	----	62.61	78.26	Beginning of last stage
6-10	638	85	65	76.45	53.33	69.70	-----	-----	5.06	6.63	6.72	8.80	6.40	8.38	----	----	71.51	93.53	Weak, eyes swollen
6-6	655	?	?	55.24	28.52	51.60	-----	-----	-----	-----	----	----	2.96	5.36	-----	----	31.48	56.99	Good
6-8	655	80	37	68.68	42.83	62.40	-----	-----	3.80	5.53	5.36	7.80	3.98	5.80	5.59	8.14	61.56	89.63	Poor
6-8	700	45	20	103.70	60.30	58.20	-----	-----	9.60	9.25	10.38	10.01	4.96	4.78	----	----	85.24	82.20	Very poor
6-8	766	45	15	185.90	94.39	50.77	-----	-----	12.35	6.66	10.69	5.76	11.50	6.19	----	----	128.93	69.35	Very poor
6-12	683	75	53	111.03	81.78	73.60	-----	-----	6.26	5.64	11.05	9.95	8.10	7.30	----	----	107.19	96.54	Poor
6-13	759	75	54	79.18	43.95	55.50	-----	-----	5.45	6.89	7.51	9.48	5.54	7.00	----	----	62.45	78.87	Poor
6-13	763	75	48	139.66	91.60	65.60	-----	-----	6.28	4.50	11.84	8.49	10.56	7.57	----	----	120.28	86.12	Very poor, eyes swollen
6-25	680	90	25	88.00	52.71	59.89	-----	-----	6.21	7.06	4.84	5.50	7.07	8.04	4.95	5.63	75.78	86.11	Beginning of last stage
6-25	761	90	61	86.10	50.24	58.35	-----	-----	4.56	5.29	9.00	10.45	5.22	6.06	4.24	4.93	73.26	85.08	Good
6-28	761	90	61	143.75	95.70	66.57	-----	-----	-----	-----	11.50	8.02	----	----	----	----	107.20	74.57	Has eaten no food
6-25	643	90	48	65.29	36.12	55.32	-----	-----	3.43	5.26	9.42	14.43	4.98	7.64	3.90	5.97	57.85	88.60	Good
6-28	643	90	19	162.60	116.70	71.77	-----	-----	-----	----	----	----	14.07	8.66	----	----	130.77	80.42	Has eaten no food
6-25	658	90	48	64.13	36.77	57.34	-----	-----	4.74	7.39	5.41	8.44	1.65	2.57	----	----	48.57	75.73	Good
6-28	658	90	44	92.35	67.38	72.95	-----	-----	3.71	4.02	3.86	7.42	6.24	6.76	----	----	81.19	87.91	Good
7-9	661	100	28	145.00	102.90	71.00	15.10	10.41	-----	----	----	----	10.71	7.40	----	-----	128.71	88.76	Fair, eyes commencing to swell
7-18	634	95	06	696.25	397.00	57.00	-----	-----	21.20	3.04	41.70	6.00	74.80	10.73	----	----	534.70	76.80	Poor
7-18	663	95	16	320.00	199.80	62.40	32.20	10.05	22.10	6.90	22.60	7.05	29.40	9.21	4.60	1.43	310.70	97.09	Very weak
Average*						63.62		6.20		6.81		8.28		6.10		5.78		96.80	

* This average includes Table III.

Table III

Avitaminosis A Hens

Date	Hen No.	Time of Collection	Amount Collected	Total Nitrogen	Uric Acid Nitrogen		Purines Other than Uric Acid Nitrogen		Urea Nitrogen		Ammonia Nitrogen		Creatine Creatinine Nitrogen		Possible Allantoin Nitrogen		Total		Condition of Hens at Time of Collection
1929		minutes	cc.	mg.	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	
6-17	822	90	25	102.00	64.64	63.37	9.11	8.93	11.10	10.88	6.31	6.18	4.56	4.47	----	----	95.72	93.83	Good
6-20		90	33	205.25	129.50	63.09	9.25	4.51	17.70	8.62	14.88	7.25	13.09	6.38	9.42	4.59	193.84	94.44	Eyes swollen shut
6-21		90	25	190.00	111.07	58.46	14.93	7.86	6.95	3.66	14.42	7.59	15.31	8.06	24.63	12.96	187.31	98.58	Very sick
6-24		90	27	255.20	161.60	63.32	16.40	6.43	13.79	5.40	20.00	7.84	12.93	5.07	22.21	8.70	246.93	96.76	Barely breathing
6-26	830	90	32	100.05	70.20	70.16	9.80	9.79	7.27	7.27	8.21	8.21	2.74	2.74	3.27	3.27	101.49	101.43	Beginning last stage
6-28		90	37	62.50	42.30	67.68	2.70	4.32	7.69	12.30	5.16	8.26	2.90	4.64	.15	.24	60.90	97.44	Eyes commencing to swell
7-1		90	19	159.38	94.08	59.03	9.04	5.67	----	----	12.50	7.84	10.11	6.34	----	----	125.73	78.88	Very weak
6-26	813	90	28	120.99	91.92	76.60	1.08	0.90	8.57	7.14	11.76	9.80	2.69	2.24	5.17	4.31	121.19	100.99	Good
7-1		90	33	68.75	46.35	67.42	----	----	6.45	9.38	6.02	8.76	2.18	3.17	3.53	5.13	61.00	93.86	Good
7-5		90	25	82.50	51.60	62.55	2.03	2.45	7.38	8.95	9.79	11.87	3.66	4.43	5.33	6.46	79.79	96.71	Poor
7-8		90	7	624.00	440.00	70.51	19.20	3.08	38.58	5.86	42.67	6.84	21.19	3.40	56.75	9.09	616.39	98.78	Very poor, died next day

Table IV

Fasting Hens

Date 1928	Hen No.	Length of fast	Time of Collection	Amount Collected	Total Nitrogen	Uric Acid Nitrogen		Purines other than Uric Acid Nitrogen		Urea Nitrogen		Ammonia Nitrogen		Creatine Creatinine Nitrogen		Possible Allantoin Nitrogen		Total		Condition of Hens at Time of Collection
		days	minutes	cc.	mg.	mg.	per cent	mg.	per cent	mg.	per cent	mg.	per cent	mg.	Per cent	mg.	per cent	mg.	per cent	
6-19	688A	10	90	45	79.20	45.10	56.90	----	----	5.49	6.93	7.28	9.20	5.12	6.46	4.03	5.08	67.02	84.62	Good
6-19	1206A	10	90	40	40.65	27.43	67.60	----	----	----	----	----	----	2.90	7.15	----	----	30.33	74.61	Good
7-5	1206A	26	90	30	84.50	57.50	68.00	----	----	----	-----	7.49	8.87	----	----	----	-----	64.99	76.91	Very weak
7-9	1206A	30	100	14	364.00	226.60	62.30	----	----	24.80	6.82	23.80	6.54	21.57	5.92	38.40	10.54	335.17	92.08	Very weak
7-12	1206A	34	120	10	650.40	393.00	60.40	67.60	10.40	50.10	7.20	61.40	9.44	62.20	9.56	45.50	7.00	679.80	104.52	Died five days later
6-19	1204A	10	90	40	61.05	36.92	60.00	----	----	4.94	8.08	7.18	11.76	3.63	5.94	-----	----	52.67	86.27	Good
7-5	1204A	26	150	20	226.80	138.30	61.00	----	----	----	----	15.85	6.72	8.37	3.69	[illegible]	----	162.52	71.65	Very weak
7-9	53	23	100	34	96.00	67.10	69.90	6.70	7.00	----	----	5.95	6.20	5.55	5.78	-----	----	85.30	88.85	Only slightly weak
7-12	53	27	90	35	167.80	97.00	57.80	----	----	6.30	3.75	18.00	10.72	----	----	-----	----	121.30	72.29	Weak
7-12	86	18	120	35	22.44	[illegible]	-----	----	----	6.35	2.83	12.95	5.78	13.50	6.02	25.20	11.24	58.00	25.84	Fair
7-18	56	24	100	55	100.25	55.60	55.30	9.86	9.81	----	----	----	----	8.29	8.25	----	----	73.75	73.56	Weak
7-18	97	24	100	60	68.40	44.60	65.20	6.90	10.10	2.85	4.17	7.03	10.30	4.04	5.92	3.42	5.04	68.84	100.64	Weak
	Average						62.22		9.33		5.68		8.55		6.47		7.78		100.03	

BIBLIOGRAPHY

(1) O. Minkowski
Arch. exp. Path. u. Pharmakol., 1886, XXI, 40.

(2) Owen S. Gibbs
Science, 1929, LXX, 241.

(3) R. E. Davis
Jour. Biol. Chem., 1927, LXXIV, 509.

(4) T. H. Milroy
Jour. Physiol., 1903, XXX, 47.

(5) D. N. Paton
Jour. Physiol., 1910, XXXIX, 485.

(6) T. Katayama
Bul. Imperial Agri. Exp. Sta., Japan, 1924, III, 1.

(7) L. Ambard, and M. Wolf
Compt. rend. Soc. biol., 1924, XC, 784.

(8) Folin and Youngberg
Jour. Biol. Chem., 1919, XXXVIII, III.
Ibid 1922, XLV, 319.

(9) Folin and Bell
Jour. Biol. Chem., 1917, XXIX, 329.

(10) S. R. Benedict
Jour. Biol. Chem., 1914, XVIII, 191.

(11) Folin and Wu
Jour. Biol. Chem., 1919, XXXVIII, 459.

(12) Harding and Young
Jour. Biol. Chem., 1919, XL, 231.

(13) S. R. Benedict
Jour. Biol. Chem., 1910, VIII, 405.

(14) O. Folin
Am. Jour. Physiol., 1905, XIII, 45.

(15) A. F. Morgan and D. O. Osburn
Jour. Biol. Chem., 1925, LXVI, 573.

(16) Henriques and Sorensen
Zeit. Physic. Chem., 1910, LXIV, 120.

(17) N. C. Sharp
Am. Jour. Physiol., 1912, XXXI, 75.

(18) Unpublished data.

(19) O. S. Gibbs
Am. Jour. Physiol., 1929, LXXXVIII, 87.

(20) W. G. Lennox
Jour. Biol. Chem., 1925, LXVI, 521.

(21) S. Burt Wolbach, and Percy R. Howe
Jour. Exp. Med., 1925, XLII, 753.

(22) J. S. Hughes
Unpublished data.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation and thanks to his major instructor, Doctor J. S. Hughes, Professor of Physiological Chemistry, for his many valuable suggestions and assistance in carrying out this investigation.