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A STUDY OF THE FLUCTUATION OF SOME BLOOD CHEMICAL CONSTITUENTS IN RELATION TO THE REPRODUCTIVE CYCLE OF THE MALLARD DUCK

by

Edward H. Decker

A Thesis submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
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INTRODUCTION

The purpose of this study was to determine normal levels of several blood chemical constituents (the minerals sodium, potassium and calcium and the organic substances serum cholesterol and serum albumin) for the female mallard duck (*Anas platyrhynchos*) and to examine their relationships as a possible index to reproductive development.

Much research has been done on the chemical composition of the avian egg and only those constituents which are of concern in this study will be discussed in detail. According to Romanoff (1949), studies of the chicken egg have shown that only a few mineral elements are present in relative abundance in the egg. In addition to the three mentioned above phosphorus, chlorine, magnesium, iron, and sulfur are also present in amounts of considerable quantity. The largest amounts of certain minerals, especially calcium, are found in the eggshell which is 95 per cent inorganic matter. In comparison, mineral elements constitute only 1 per cent of the egg contents (albumen and yolk) but the greatest variety of minerals is present in the egg contents. Calcium constitutes 98.2 per cent of the total mineral elements of the eggshell and six per cent of the total inorganic matter in the egg contents. Neither sodium nor potassium is present in the chicken eggshell but both are present in the egg contents. Sodium constitutes 14.5 per cent of the total mineral elements in the egg contents and potassium 16.8 per cent. Each constitutes .16 per cent of the albumen of the chicken egg but in the yolk potassium is present in slightly greater amounts (.11 per cent) than sodium (.07 per cent).

Romanoff (1949) has also done extensive research into the organic constituents of the chicken egg. He reported that of the 6.2 grams of lipids in the chicken egg, about 99 per cent are in the yolk, with sterols constituting 4.9 per cent of all yolk lipids. Cholesterol, one of the most important sterols, comprises about 30 per cent of yolk sterols or 1.6 per cent of the total yolk. Yolks of duck eggs contain a somewhat greater amount (1.8 per cent) of cholesterol (Gaujoux and Krijanowsky, 1932). The abundance of cholesterol in nervous tissue, and its chemical relationship to bile acids and sex hormones, indicate the importance of its presence in the egg since nervous tissue and perhaps hormones are produced early in embryonic life.

Although proteins are present in both yolk and albumen of the chicken egg, the simple protein albumin is found only in the albumen. Ovalbumin, or egg albumin, constitutes about 75 per cent of the total albumen with two other simple proteins (ovoconalbumin and ovoglobulin) and two glyco-proteins (ovomucoid and ovomucin) comprising the remaining portion.

In previous studies of avian blood chemistry only albumin and calcium have been studied intensively. Chorine (1938), Dyer and Roe (1934), Howe (1925), and Sturkie and Newman (1951), all working with chickens, found albumin amounts ranging from 1.48 - 2.53 grams per 100 ml. of blood but no studies have been directed towards fluctuations corresponding with laying. Calcium (present in two fractions in the blood, a filterable or diffusible fraction and a nonfilterable or nondiffusible fraction) has been found present in non-laying chickens at levels from 9.9 - 17.1 mg. per 100 ml. of blood (Sturkie, 1954) and in various other sexually inactive females from 8.1 - 15.4 mg. per 100 ml. of blood (Simkiss, 1961). Calcium has been found at levels from 21.5 - 24.0 mg. per 100 ml. of blood in laying chickens and from 19.9 - 29.3 mg. per 100 ml. in laying females of other species. An increase in blood calcium just prior to and coincident with ovulation and laying is mainly in the nondiffusible or bound fraction, there being little or no change in the diffusible or inorganic fraction (Sturkie, 1954 and Simkiss, 1961).

Since the minerals and organic constituents involved in this study are found in various amounts in different parts of the egg, their concentration in the blood of the female may be related with the sequence of events which results in egg production. Prior to ovulation, the yolk material is laid down by the ovary. Of the chemicals under concern in this study, two of the minerals, potassium and sodium, make up appreciable amounts of the yolk. Cholesterol is also involved here since it comprises about 1.6 per cent of the total yolk. The infundibulum of the oviduct engulfs the ovum when it is ovulated and from here the ovum passes into the magnum, which is the largest single portion of the duct. Here the albumen is laid down, again involving potassium and sodium and also the simple protein, albumin, which constitutes about 75 per cent of the egg white. From the magnum, the ovum passes into the isthmus where the inner and outer shell membranes are formed. From the isthmus the ovum moves into the shell gland where in a period of 18 - 30 hours the outer shell is formed. It is this sequence of events and the corresponding variations of the utilization of these chemicals which allows for the possibility of monitoring reproductive development through blood chemical analysis.

METHODS AND MATERIALS

Perfecting the Sampling Technique

Prior to the start of the actual project, several birds were kept in an environmentally controlled laboratory (12 degrees centrigrade and 11-12 hours daylength) in order to perfect the blood sampling technique. The metatarsal vein on the inside of each foot was decided upon as the most convenient vessel for the blood collecting because it lies just under the skin in an area free of feathers. Also, in most birds the metatarsal vein is a large vessel which makes the sampling procedure less time consuming and thus less strenuous on the animal. Initially, it was thought that blood could be drawn most efficiently using a syringe and hypodermic needle. Although this method proved very satisfactory in some cases it was time consuming and resulted in undesirable stress on the birds. Also, because avian blood clots rapidly, hemolysis usually resulted because of the difficulty involved in the transfer of clots from the syringe to a test tube prior to centrifugation. The technique was simplified by omitting use of the syringe. The vein was pierced with a hypodermic needle and the blood allowed to flow directly from the vein into a test tube. This method proved satisfactory and was used to obtain blood samples from the birds during the actual study.

Sampling of the Birds

The twenty-one birds (11 females, 10 males) used for the study were kept in a garden in the center of Wood Hall, Western Michigan University (Figure 1). Trapping (the trapping procedure is discussed later in this section) and blood collecting were performed on all females once every three days beginning approximately three weeks prior to laying and continuing through the breeding season. Some of the birds had shown ovulatory activity prior to coming into our possession but laying subsided during the early stages of the study, probably due to environmental change.

The blood collecting was done between the hours of 9:00 A. M. and 12:00 noon. Immediately after trapping the males were removed from the trapping box since only the females were to be sampled. Also, the presence of the males in the trapping box along with the females produced undesired activity which conceivably could alter blood chemical levels. Following removal of the males, the female ducks were taken out one at a time (Figure 2) and brought to a temporary

Figure 1: View of garden in the center of Wood Hall.
Pool is in center of picture.

Figure 2: Removing a female from the trapping box.

laboratory which was set up in the garden entrance hall. The bird was held over a pail of warm water so that the feet were submerged. This part of the procedure served to remove loose dirt and to dilate the blood vessels. The feet were dryed, the head of the bird was tucked under one of its wings, and the bird was placed head first into a muslin bag, the edges of the bag being pulled approximately half-way up the length of the animal (Figure 3). This made holding of the bird easier and also aided in keeping the animal inactive. The duck was then turned upside down and held firmly. The foot of the bird was held so that the main joint of the foot pointed down with the metatarsal vein running vertically. Alcohol was then used to clean and disinfect the skin surface and a hypodermic needle was made ready to pierce the vein. A wide mouth test tube was held in position at the joint and upon piercing of the vein, the blood flowed down toward the joint and into the tube (Figure 4). Occasionally it became necessary to clamp off the vein proximally so as to block the flow of blood and force it out the wound. At least 1.5 milliliters of blood was collected from each bird. A coagulant was spread over the wound to aid in clotting and a piece of adhesive tape was used as a bandage. The bird was

Figure 3: Putting bird head-first into muslin bag. Bird is upside-down in this view.

Figure 4: View showing the blood collecting. After the vein was pierced, the blood flowed down to the joint and directly into the tube.

then released into the garden and the next female was removed from the box for sampling.

After all eleven female birds were processed the blood samples were allowed to clot at room temperature and were then chilled thoroughly in a refrigerator. Centrifugation of the clotted samples left a supernatant serum which was transferred to glass test tubes by means of a rubber-bulb pipet. Analysis was performed either immediately after centrifuging or the samples were frozen until a later date when sufficient time was available. To insure that freezing did not alter the results, several samples were analyzed both before and after freezing.

Analyzing of Serum Samples

The concentrations of sodium, potassium and calcium were determined by means of flame photometry. The instrument used was a Hatachi Perkins-Elmer Spectrophotometer with flame attachment. The flame photometry procedure as outlined by Annino (1960) was followed step by step with the exception of the detergent strength, which for this study was .11 per cent as compared to .02 per cent in the original procedure. Prior to the start of the project, dilution curves for sodium, potassium, and calcium were made using a commercially prepared control "serum" (Lab-trol) as a standard.

The Liebermann-Burchard test was employed for serum cholesterol determination (Boltzelaer and Zondag, 1960) and the Biuret test was used for serum albumin (Natelson, 1961). A cholesterol stock solution (200 mg. per cent) was used for the cholesterol standard and Lab-trol was used as a standard for the Biuret test. A Bausch and Lomb Spectronic 20 colorimeter was used in both the serum albumin and serum cholesterol tests.

During the early stages of the study, when it became apparent that hemolysis could not be avoided in some cases, comparisons were made of flame photometry results of hemolyzed and non-hemolyzed samples. It was found that there was no consistent alteration in the hemolyzed samples when compared to the non-hemolyzed samples. Thus, any alterations caused by hemolysis, even though no doubt present, were not considered great enough to cause concern.

Processing of Raw Data

The raw data were put through the IBM 1620 Central Processing Unit located in the Computer Center at Western Michigan University. The five chemical constituents studied were established as the independent variables with the ovipository time as the dependent variable. No permanent nests were established during the study and therefore it was not possible to determine which bird laid which egg. When the data was processed, each bird, with its five independent variables, was analyzed against all the eggs laid. Since blood collecting was done every three days, it was decided to group the eggs according to this three-day period. Thus, the eggs collected on the sampling day and the two post-sample days were tabulated and three-digit combinations were prepared from the data. For example, if an egg was laid on the day of blood collecting and none were laid on either of the two post-sample days, the three digits 1 - 0 - 0 were used as the dependent variable. If eggs were laid on all three days, the digits 1 - 1 - 1 were used. If no eggs were found during the three day period, the digits 0 - 0 - 0 were used. Thus, each digit space refers to the corresponding day of the three-day sampling period. In the one case where two eggs were laid on one day (April 29) the digit used was still 1 because each bird could have laid only one egg on one day. The ovipository times, then, can be one of seven possible combinations (0 - 0 - 0, 1 - 0 - 0, 1 - 1 - 0, 1 - 0 - 1, 1 - 1 - 1, 0 - 1 - 1, and 0 - 0 - 1). The computer system checked the data for intercorrelation, Beta regression coefficients, regression constant, standard error of estimate, multiple correlation, F and *t* tests of B, and partial correlation coefficients. All of these results are listed for each of the 11 females and can be found in Appendix B.

Miscellaneous Methods and Materials

Eight of the 11 females used for the study were donated by Charles VanKirk of Goodrich, Michigan. These birds are a type of "call" duck but their classification, like all mallards, is *Anas platyrhynchos*. All ten males and the remainder of the females were obtained from R. D. VanDeusen of the Kellogg Bird Sanctuary, Hickory Corners, Michigan. To identify individuals, different combinations of colored leg bands were used. Four different colors (tan, green, blue, and red) were employed and subsequently in this study the birds will be referred to by the leg band color combinations; e.g., Single Tan, Blue and Tan, and Double Tan. The three females from VanDeusen were Single Tan, Blue and Green, and Red and Green. All of the remaining eight females, then were VanKirk birds. The latter group had shown egg-laying activity prior to coming into our possession.

Trapping of the birds was by means of a box five feet in length by two feet in width by a foot and a half in height (Figures 5 and 6). All of the birds were driven in the open end and the paddle was turned so that the open end was closed. The paddle was then moved toward the closed end, pushing the birds ahead of it. The bottom of the paddle was locked in place by means of wooden knobs. Hasps were screwed onto the top of the box along the length-wise slit and two of these were used to keep the paddle-handle in position.

Each afternoon the birds were fed generous amounts of corn and wheat; occasionally some lettuce or various other greens were put in the pool. Another daily activity involved checking for eggs at 9:00 - 10:00 A.M. and at 2:00 - 3:00 P.M. Although some nesting activity did take place and some of the eggs were laid in these nesting areas, the majority of the eggs were laid at various locations away from nests, usually in the open away from sufficient cover.

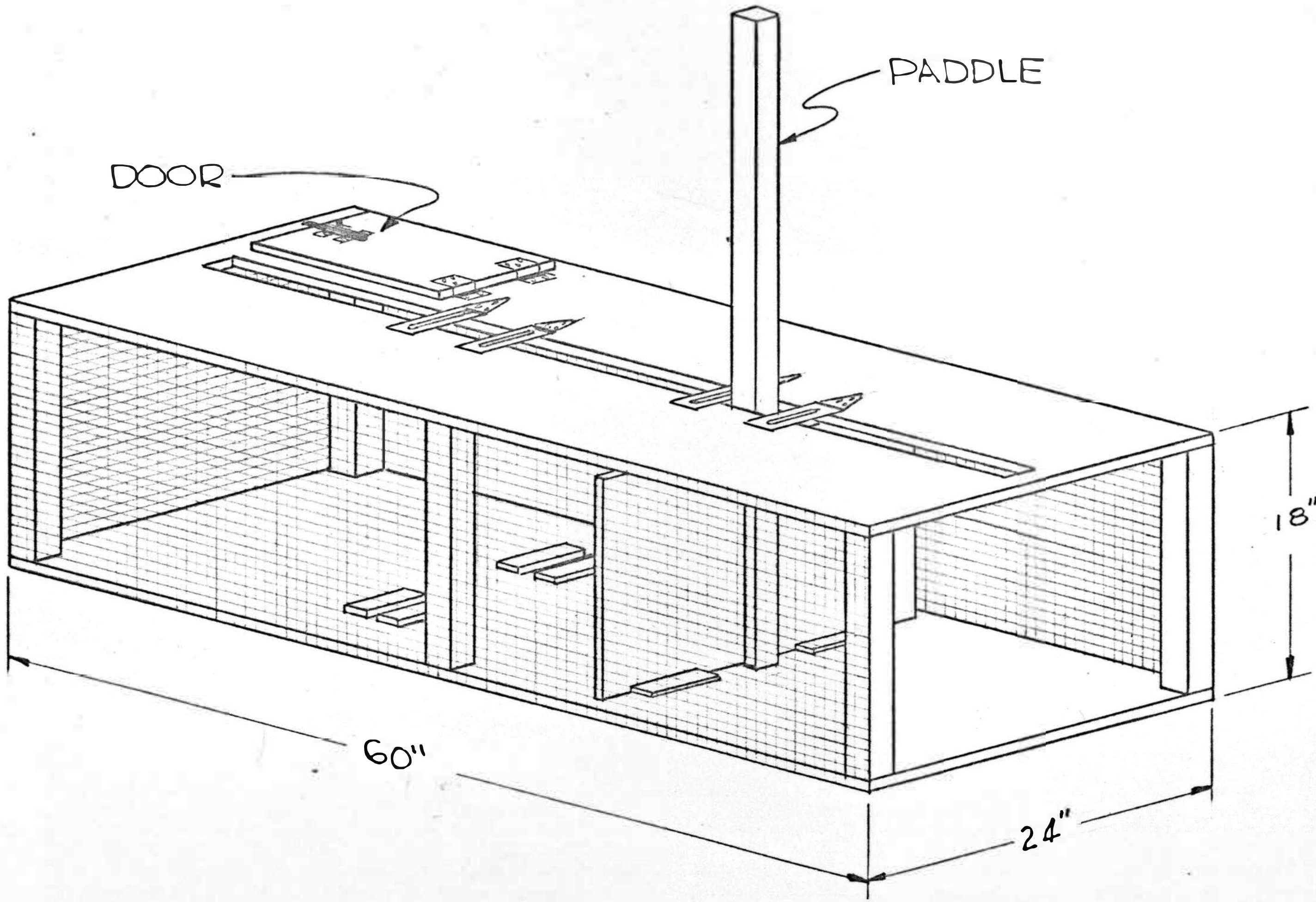

Figure 5: Orthographic drawing of trapping box.

Figure 6: Trapping box in position in garden. Structure to the right of the box aiding in funneling the birds into the open end.

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RESULTS AND DISCUSSION

Since none of the eggs laid could be positively identified with any specific females, the raw data for each bird were processed against a day by day tabulation of the total eggs laid. Results were then determined on the basis of a statistical correlation between fluctuations in blood chemical constituents and the incidence of egg-laying.

Of the 11 females used for the study, five showed statistical correlation beyond the 10 per cent level in two paramenters (potassium and albumin). The raw data for these five statistically significant birds are presented in Tables 1 through 5. Of these five, all except Blue and Tan were observed to have paired during the study. There was no evidence that Blue and Tan was mated, but as was pointed out by Wood (1965) in a study on Canada geese, some successful nesting pairs fail to show the aggressiveness normally associated with mating and nesting.

The raw data for the six birds which did not show correlation beyond the 90th percentile for potassium and albumin appear in Appendix A. This group includes two paired females, Double Tan and Red and Green.

Table 1: Variations in five blood chemical constituents for Single Tan.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	173.5	4.98	5.55	212	3.98
April 7	180.5	4.87	6.22	216	4.17
April 10	177.0	5.23	6.95	236	5.36
April 13	164.0	4.42	6.23	239	3.92
April 16	156.0	3.86	5.25	242	3.54
April 19	156.0	3.90	9.75	208	4.23
April 22	142.0	4.87	5.12	193	3.97
April 25	149.0	3.86	6.22	212	3.76
April 28	170.0	6.21	6.51	219	3.68
May 1	156.0	4.71	6.37	204	3.64
May 4	159.5	5.23	7.21	208	3.98
May 7	177.0	5.04	7.21	248	4.39
May 10	173.5	6.15	7.21	248	5.26
May 13	166.5	6.15	6.80	242	4.47
May 16	156.0	5.51	6.08	226	4.30
May 19	173.5	5.58	6.65	260	4.84
May 22	159.5	6.15	7.09	189	4.30

Table 2: Variations in five blood chemical constituents for Single Green.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	166.5	4.51	6.95	189	3.73
April 7	177.0	4.98	7.21	280	3.75
April 10	163.0	5.17	6.37	324	5.30
April 13	161.0	4.41	5.92	253	4.37
April 16	158.0	4.03	5.55	204	3.88
April 19	158.0	4.13	11.40	248	4.57
April 22	149.0	4.80	8.62	200	4.92
April 25	152.5	4.24	9.87	196	4.13
April 28	159.5	5.43	6.80	346	4.92
May 1	145.5	4.37	5.25	242	3.47
May 4	159.5	5.10	5.95	212	3.82
May 7	152.5	5.10	6.22	242	4.16
May 10	156.0	4.66	7.09	289	4.39
May 13	156.0	5.32	5.82	254	4.16
May 16	151.0	4.82	7.65	189	4.16
May 19	156.0	4.87	8.35	178	4.16
May 22	173.5	5.67	7.77	200	4.39

Table 3: Variations in five blood chemical constituents for Single Blue.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	177.0	4.75	10.02	236	5.59
April 7	191.0	5.17	15.00+	254	5.37
April 10	166.5	4.75	12.62	212	5.25
April 13	169.0	4.41	9.31	207	4.91
April 16	159.5	3.86	8.90	193	4.61
April 19	170.0	3.73	15.00+	147	4.02
April 22	145.5	4.75	9.17	133	4.97
April 25	149.0	4.24	10.23	186	5.00
April 28	170.0	4.98	12.62	208	4.26
May 1	149.0	7.00	8.35	176	4.92
May 4	159.5	5.10	9.03	183	3.88
May 7	151.0	4.82	9.60	267	4.84
May 10	187.5	5.67	12.77	236	4.35
May 13	151.0	5.04	8.90	189	4.02
May 16	149.0	4.98	9.32	208	5.04
May 19	145.5	4.37	8.20	229	4.20
May 22	163.0	4.87	6.95	452	5.04

Table 4: Variations in five blood chemical constituents for Blue and Tan.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	175.0	5.04	7.77	236	5.31
April 7	201.5	4.24	9.60	306	4.96
April 10	152.5	4.19	6.37	282	3.59
April 13	145.5	3.62	7.77	182	3.87
April 16	138.5	3.17	8.35	159	4.23
April 19	166.5	3.86	10.30	200	4.16
April 22	149.0	5.32	5.12	193	4.16
April 25	149.0	4.66	5.01	186	4.13
April 28	184.0	6.03	7.50	217	4.16
May 1	142.0	5.67	7.50	179	4.20
May 4	184.0	5.62	9.17	170	4.61
May 7	149.0	4.66	8.47	196	4.39
May 10	184.0	5.81	12.08	193	5.15
May 13	170.0	5.51	10.30	193	4.92
May 16	156.0	4.60	8.62	193	4.16
May 19	163.0	5.32	9.17	183	3.88
May 22	180.5	5.04	10.85	236	4.39

Table 5: Variations in five blood chemical constituents for Blue and Green.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4*					
April 7	173.5	4.41	6.22	282	4.91
April 10	152.5	4.66	5.00	268	4.91
April 13	149.0	4.37	8.75	247	4.73
April 16	121.0	3.95	13.25	200	4.29
April 19	173.5	3.45	15.00+	158	4.09
April 22	156.0	4.46	8.47	158	3.67
April 25	145.5	4.37	9.03	165	3.82
April 28	170.0	6.50	7.50	245	4.47
May 1	138.5	4.24	7.77	204	4.02
May 4	173.5	4.98	12.08	173	4.20
May 7	137.0	4.60	7.77	200	3.59
May 10	149.0	3.86	6.51	346	3.85
May 13	149.0	4.60	5.55	267	4.23
May 16	149.0	3.81	9.32	170	4.02
May 19	163.0	4.03	9.32	170	3.95
May 22	159.5	5.51	8.20	158	4.09

* No values for this date since bird was not obtained until April 7.

Table 6 presents a sequential post-sample tabulation of the eggs laid during the study. The three-digit combinations shown were used as the dependent variable during data processing and are discussed at length under the section "Processing of Raw Data."

Potassium and albumin showed the highest and most consistent correlations to egg laying of the five chemical constituents studied. Potassium _t_ values obtained by multiple correlation showed a positive correlation whereas albumin showed a negative correlation. All of the potassium _t_ values for the five females whose data are presented in this section vary from the .03 level to the .005 level (Table 7). Two of these females, Single Blue and Blue and Green also show _t_ values for albumin significant at the .01 level. The albumin _t_ values for the remaining three are at the .10 level. Calcium _t_ values for Single Tan and Single Blue were significant at the 95th percentile and those for Single Green and Blue and Tan at the .10 level.

The partial correlation coefficients (Table 8) for potassium for all the birds but Single Blue are in the area of 70 per cent. Single Blue shows a correlation coefficient of 58 per cent. The negative albumin correlation coefficients show values ranging

Table 6: Sequential post-sample tabulation of the eggs laid during the study. The first day of each three-day period was a sampling date. The three-digit combinations following the dates are identical to the three-digit combinations used in data processing.

Dates	Eggs Laid During the Three Days	Dates	Eggs Laid During the Three Days
April 4-6	0 - 0 - 0	May 1-3	1 - 1 - 0
April 7-9	0 - 0 - 0	May 4-6	0 - 0 - 1
April 10-12	0 - 0 - 0	May 7-9	1 - 1 - 1
April 13-15	0 - 0 - 0	May 10-12	1 - 0 - 1
April 16-18	0 - 0 - 0	May 13-15	0 - 1 - 0
April 19-21	0 - 0 - 1	May 16-18	0 - 0 - 1
April 22-24	1 - 0 - 0	May 19-21	1 - 1 - 0
April 25-27	0 - 1 - 0	May 22-24	1 - 1 - 1
April 28-30	1 - 2* - 0		

* Although two eggs were collected on April 29, the digit 1 was used in the data processing since any one bird could have laid only one egg on one day.

Table 7: *t* values for Single Tan, Single Green, Single Blue, Blue and Tan, and Blue and Green.

	Single Tan	Single Green	Single Blue
Sodium	-.705573	-2.426848	-2.625377
Potassium	3.052884	3.298747	2.423316
Calcium	1.883964	1.605933	1.921577
Cholesterol	.599568	.904195	3.984069
Albumin	-1.422544	-1.382609	-2.754342

	Blue and Tan	Blue and Green
Sodium	-.508221	-.686101
Potassium	3.071529	3.028718
Calcium	1.771411	-.036191
Cholesterol	.121989	.774401
Albumin	-1.609257	-3.985915

Table 8: Partial correlation coefficients for Single Tan, Single Green, Single Blue, Blue and Tan, and Blue and Green.

	Single Tan	Single Green	Single Blue
Sodium	-.20808	-.59051	-.62066
Potassium	.67724	.70519	.58995
Calcium	.49391	.43580	.50131
Cholesterol	.17789	.26302	.76854
Albumin	-.39418	-.63888	-.63888

	Blue and Tan	Blue and Green
Sodium	-.15146	.21203
Potassium	.67947	.69169
Calcium	.47111	-.01144
Cholesterol	.03675	.23785
Albumin	-.43653	-.78339

from 38 to 78 per cent.

Figure 7 presents a graphic description of potassium levels in two of the females, Single Green and Blue and Tan. These two birds were selected because their potassium _t_ values are the highest. A rather consistent pattern in fluctuation of potassium levels began about the time that the first eggs were laid. These patterns continued throughout the length of the study and seem to fluctuate regularly around the mean, indicating the possibility of potassium being a short-term index. The VanKirk birds, as was mentioned previously, had shown ovulatory activity before the start of our study and the early April pattern seems to correspond with this fact. The precise reason for the pronounced decrease during the middle of April is not known although it is possible that this pattern which led to a decline in significant correlation was due to inhibition resulting from movement.

Albumin levels also showed interesting patterns of fluctuation (Figure 8) but do not present the short-term fluctuation around the mean as does potassium. Instead, the pattern seems to be one of a general depression of serum albumin concentration as the egg-laying period began, indicating the possibility of albumin as more of a long-term index.

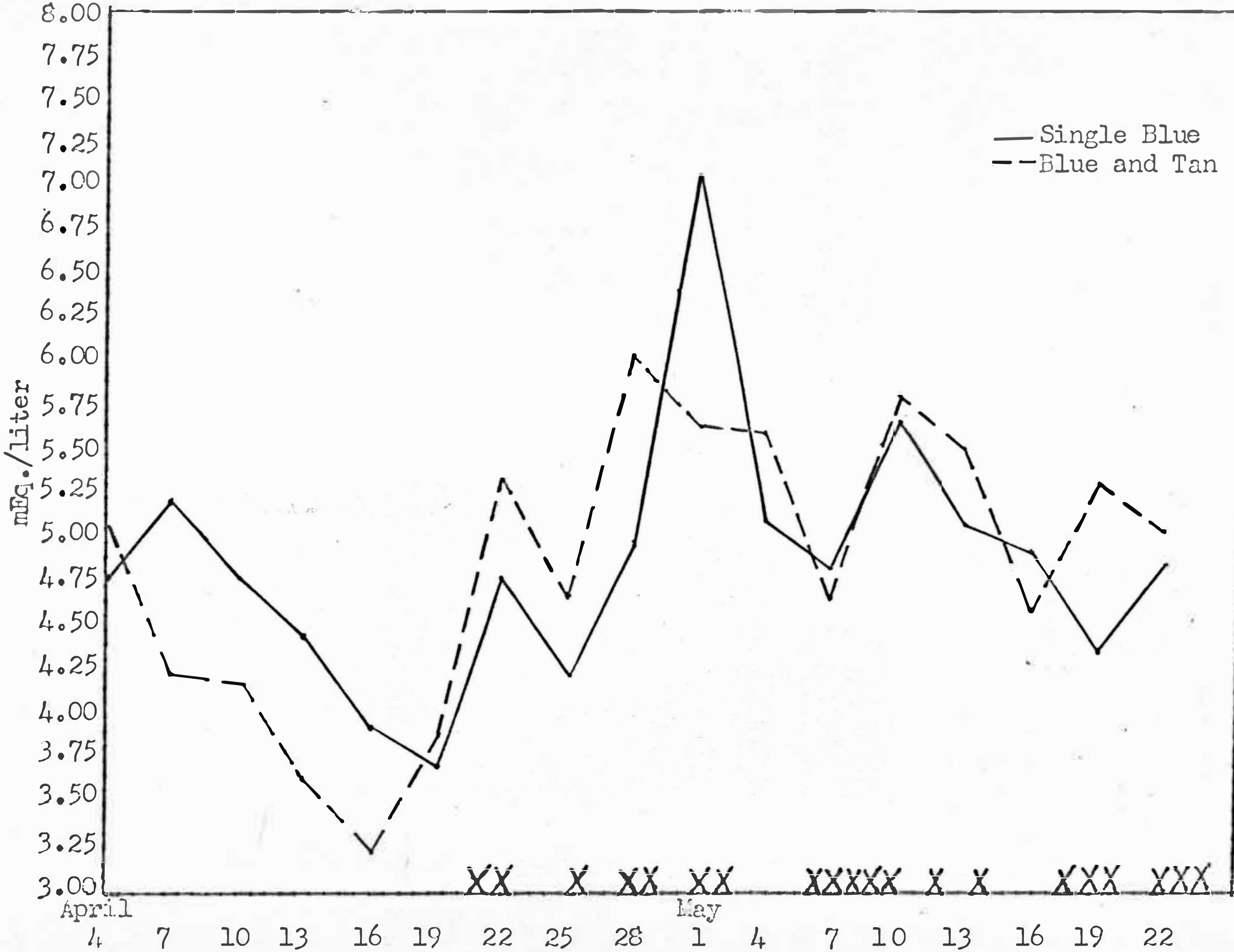

Figure 7: Potassium values for Single Blue and Blue and Tan. The "X" marks indicate dates that eggs were laid by the 11 females.

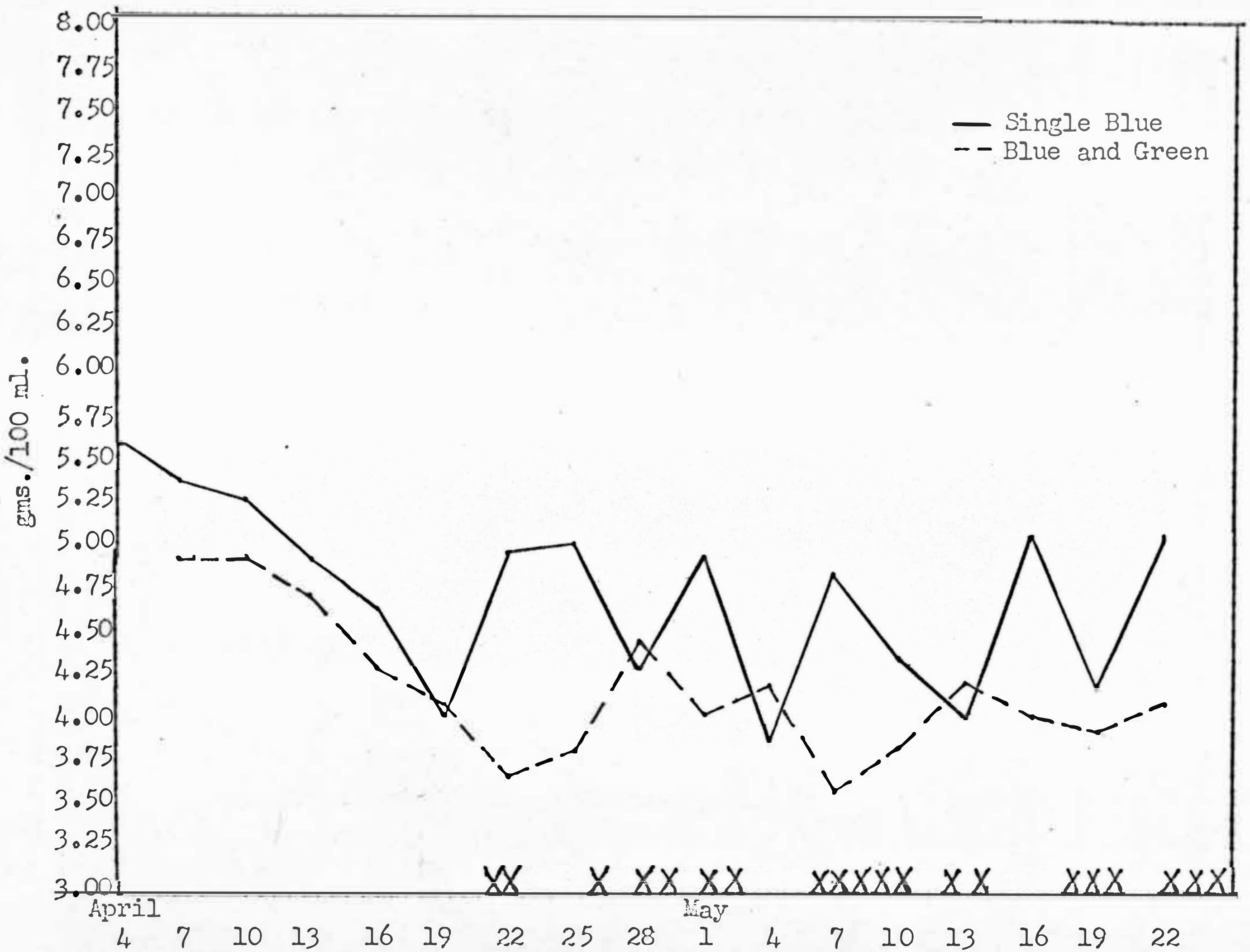

Figure 8: Albumin values for Single Blue and Blue and Green. The "X" marks indicate dates that eggs were laid by the 11 females.

Simkiss (1961) reported that in the pigeon, ring dove, domestic fowl, English sparrow, and quail the the calcium level of the blood of a laying bird is double that in the reproductively inactive female. Her values for the mallard duck, however, were given only for the male and sexually inactive female. A survey of the data in Tables 1 through 5 and in Appendix A shows that in several instances the level of calcium nearly doubles from one sampling date to another. An excellent example is Single Blue (Table 3), where the level of calcium rose from 8.90 mEq/L. on April 16 to more than 15.00 mEq/L. on April 19, just 72 hours later. Riddle and Reinhart (1926) found that the rise in blood calcium started approximately 108 hours before ovulation so in the instance mentioned above, the rise may have begun before the April 16 sampling time or it may have continued beyond April 19. The calcium values for this study seem to be higher than those for the various other species reported by Simkiss (1961), and it is possible that a lack of significance in our data was due to the experimental model. Since, however, calcium values for sexually active female mallards were not given, a specific comparison is not possible.

An interesting observation in regard to the parameters studied is the lack of consistent correlation between related constituents (Intercorrelation data - Appendix B). A pronounced decrease in one cation level was not consistently compensated for by a pronounced increase in another although one would expect that such compensating changes might well occur. It may be that such a phenomenon does exist. It is quite evident from these results that the parameters used for this study are incomplete by themselves and perhaps a much more intensive survey including other parameters is needed.

Additional factors were observed which could have caused modification of the data presented in this section and Appendix A. Emaciation was very apparent in Single Red, Double Red, and Double Blue; and no doubt the strange environment had pronounced influence on their feeding habits which in turn could cause fluctuations of blood chemicals. Low temperatures during the latter part of April may have also caused modifications because of the reduced activity and feeding.

Three of the females (Single Tan, Blue and Green, and Red and Green) showed signs of frequent copulation, another factor which could have caused blood chemical fluctuations through a response to stress. Although several other females were observed copulating, the three mentioned above were completely void of feathers on the head and neck indicating frequent copulation.

It should be re-emphasized that none of the eggs which were laid could be positively identified with any one female. Also, at no time during the study was there any true nesting (eggs were not consistently placed). Consequently, it is possible that laying followed a non-concentric pattern that is, there was no regular pattern of laying. If an acentric pattern of laying is assumed and if the coincidental fluctuation of blood potassium is truly related to egg formation, then the results of this study indicate that potassium is an index of short duration (24 hours or less).

This short-term index possibly becomes evident when the potassium fluctuation levels are compared to the pattern of the eggs which were laid. For example, in Figure 7 the potassium levels of Single Blue show four pronounced increases from previous sample levels. In each case an egg was laid on the day of the increase. The same is true of Blue and Tan, also represented by Figure 7. Of the other three females showing significant fluctuations in potassium, this observation holds true except for Single Tan on May 4 and Single Green and Blue and Green on May 4 and May 13.

This high correlation between potassium increases and egg laying holds true only for a 24-hour period. This point can be illustrated by again comparing potassium levels with egg dates. For example, the first pronounced increase in potassium for Single Blue is followed by an egg in 24 hours but no additional eggs were found 48 hours or 72 hours after the increase. The second increase is followed by an egg within 24 hours and an additional egg within 48 hours but no egg was found before the end of 72 hours. The information in Table 9 presents this data for three of the females showing significant fluctuations in potassium. In all of the three birds presented, in every instance but one an egg was present in the first 24 hours after the increase but only six were found within the first additional 24 hours and only three within the next additional 24 hours. This then accounts for the nearly 100 per cent statistical correlation observed. Single Green and Blue and Green (see Tables 2 and 5) also showed this high correlation of potassium increases and eggs found within 24 hours after the increase.

Verification of the precise correlation between levels of blood constituents and reproductive development needs further study. Future studies should in-

	Single Blue				Blue and Tan				Single Tan				
	First increase	Second increase	Third increase	Fourth increase	First increase	Second increase	Third increase	Fourth increase	First increase	Second increase	Third increase	Fourth increase	Fifth increase
Twenty-four hours after the increase	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Forty-eight hours after the increase	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	No	Yes
Seventy-two hours after the increase	No	No	No	Yes	No	No	No	No	No	No	No	Yes	Yes

Table 9: A correlation between eggs found and the three twenty-four hour periods which followed the potassium increase. Data for three of the females are given. If at least one egg was found during the appropriate time period the word "Yes" was inserted.

clude a critical examination of the individual and a more thorough and intensive study into various parameters involved in reproductive development.

SUMMARY

Eleven female mallard ducks were trapped every three days for a 51-day period and a blood sample was collected. The sample was centrifuged and the supernatant serum was drawn off. Flame photometry was used to analyze the serum for sodium, potassium, and calcium. Serum cholesterol and serum albumin were tested by means of the Liebermann-Burchard and Biuret procedures respectively. The raw data obtained by serum analysis was then processed by means of an IBM 1620 Central Processing Unit, with the levels of the chemical constituents serving as the independent variables and the egg-laying time as the dependent variable. Since none of the eggs laid could be positively identified with any specific female, the raw data for each bird were processed against all the eggs laid.

Of the 11 females used for the study, five showed statistical correlation beyond the 10 per cent level in two parameters (potassium and albumin) and of these five birds, four were observed as having paired. Potassium t values showed a positive correlation and vary from the .03 level to the .005 level for the five statistically significant birds. Albumin t values showed a negative correlation and are significant at

the .01 level for two of the females and are at the .10 level for the remaining three statistically significant females. Potassium levels seem to fluctuate regularly around the mean and when this fluctuation pattern is compared to the pattern of the eggs laid, the possibility of potassium serving as a short-term (24 hour) index becomes evident. Albumin levels seem to show a pattern of general depression as the egg-laying period began.

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APPENDIX A

Tables of Raw Data for the
Six Females Which Did Not Show
Statistical Significance

Variations in five blood chemical constituents for Double Tan.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	173.5	4.93	9.17	259	4.77
April 7	187.5	4.93	11.52	217	4.78
April 10	176.0	4.73	10.06	183	4.63
April 13	167.0	4.52	9.87	168	4.47
April 16	128.0	4.10	9.60	157	4.43
April 19	166.5	3.09	15.00+	124	3.88
April 22	149.0	5.17	15.00+	325	5.45
April 25	152.5	4.82	12.45	147	4.35
April 28	155.5	4.95	9.93	145	4.21
May 1	142.0	4.98	9.87	179	4.39
May 4	177.0	5.90	9.17	186	4.20
May 7	145.5	4.60	10.43	380	4.65
May 10	170.0	5.23	10.71	260	5.38
May 13	170.0	5.62	10.57	254	4.98
May 16	161.0	5.73	9.46	179	4.74
May 19	159.5	5.51	13.25	452	4.09
May 22	175.0	5.67	10.71	331	7.06

Variations in five blood chemical constituents for Red and Green.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4*					
April 7	198.0	5.04	10.85	314	4.30
April 10	159.5	4.46	6.37	200	3.70
April 13	138.5	3.86	8.47	211	3.81
April 16	117.5	3.11	9.17	221	4.16
April 19	172.0	3.30	11.95	170	4.14
April 22	159.5	5.81	6.51	204	3.91
April 25	161.0	4.24	8.35	135	3.95
April 28	177.0	6.77	7.91	219	4.47
May 1	152.5	4.98	9.75	186	3.76
May 4	170.0	5.23	10.57	193	4.00
May 7	145.5	5.43	10.30	176	4.23
May 10	145.5	4.51	7.09	236	4.09
May 13	145.5	4.98	5.82	260	4.47
May 16	152.5	4.51	7.37	204	4.74
May 19	151.0	4.10	5.25	239	3.76
May 22	166.5	5.10	5.69	208	3.54

* No values for this date since bird was not obtained until April 7.

Variations in five blood chemical constituents for Single Red.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	173.5	4.60	7.77	153	3.70
April 7	180.5	5.32	9.87	324	4.17
April 10	178.0	4.82	7.23	273	4.48
April 13	174.0	4.43	6.81	249	4.53
April 16	173.5	4.10	5.55	201	4.67
April 19	175.0	4.16	13.90	221	4.82
April 22	149.0	4.51	6.95	155	5.04
April 25	159.5	5.23	12.77	212	4.74
April 28	173.5	6.21	11.95	168	4.26
May 1	130.0	3.52	6.65	236	3.47
May 4	180.5	6.09	12.77	236	4.09
May 7	152.5	4.93	10.30	179	4.30
May 10	163.0	5.37	11.00	356	4.70
May 13	149.0	4.93	6.37	226	4.39
May 16	145.5	5.05	7.91	231	4.39
May 19	166.5	5.67	10.71	233	4.98
May 22	187.5	6.09	13.90	224	5.21

Variations in five blood chemical constituents for Double Green.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	191.0	5.23	5.25	221	4.86
April 7	201.5	4.71	7.37	268	4.45
April 10	177.0	6.39	6.95	186	4.32
April 13	166.5	5.23	7.50	197	4.17
April 16	135.5	4.19	8.75	211	4.20
April 19	163.5	3.90	9.46	204	4.23
April 22	145.5	5.10	5.12	193	4.47
April 25	145.5	5.23	5.01	189	4.35
April 28	152.5	5.51	7.50	236	4.30
May 1	145.5	4.75	6.08	189	3.64
May 4	187.5	5.23	10.02	189	4.09
May 7	145.5	4.46	7.37	208	4.52
May 10	166.5	4.87	9.03	226	5.15
May 13	145.5	4.66	7.65	223	4.84
May 16	149.0	4.19	8.47	270	4.70
May 19	152.5	4.75	10.30	224	4.65
May 22	161.0	4.71	10.15	264	4.79

Variations in five blood chemical constituents for Double Blue.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	191.0	5.67	8.47	221	4.86
April 7	184.0	4.51	10.15	242	5.71
April 10	159.5	4.98	4.60	306	4.83
April 13*					
April 16	156.0	4.03	8.35	208	4.38
April 19	156.0	3.45	10.30	254	4.30
April 22	142.0	5.17	6.51	189	4.13
April 25	149.0	4.80	8.35	120	4.23
April 28	166.5	5.43	8.07	217	4.30
May 1	149.0	6.03	13.90	274	4.47
May 4	177.0	5.73	11.82	170	4.65
May 7	144.0	4.66	7.77	204	3.95
May 10	166.5	5.58	8.20	368	4.47
May 13	163.0	5.62	8.37	289	5.51
May 16	156.0	4.66	9.17	297	4.98
May 19	163.0	5.10	9.60	181	4.47
May 22	170.0	5.17	8.20	281	4.39

* Bird escaped during sampling.

Variations in five blood chemical constituents for Double Red.

Date	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mEq/L)	Cholesterol (mg%)	Albumin (gm%)
April 4	184.0	5.58	6.95	242	4.46
April 7	205.0	5.04	8.07	324	4.91
April 10	159.5	4.71	6.51	248	4.65
April 13	149.0	4.60	7.65	237	4.53
April 16	142.0	3.45	8.05	231	4.59
April 19	173.5	4.03	11.00	221	4.67
April 22	170.0	4.24	8.75	212	4.74
April 25	145.5	4.60	6.22	208	3.67
April 28	163.0	5.32	6.22	226	3.95
May 1	149.0	5.04	7.65	204	4.02
May 4	173.5	5.04	11.00	176	4.43
May 7	173.5	5.58	12.33	149	4.16
May 10	152.5	4.82	9.32	168	4.35
May 13	149.0	4.93	7.50	168	4.70
May 16	144.0	4.10	5.40	306	4.09
May 19	152.5	4.46	5.95	281	4.47
May 22	166.5	4.46	6.37	229	4.16

APPENDIX B

Tables of Statistical
Data for All Eleven Females Used
in the Study

SINGLE TAN

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	164.088	10.97900700	120.53860000
Potassium	5.101	.79942855	.63908602
Calcium	6.612	1.04023810	1.08209550
Cholesterol	223.647	20.82948900	433.86764000
Albumin	4.222	.52689851	.27762205
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.40947	.14970	.55397	.53540	.04510
.40947	1.00000	.03187	.14156	.47568	.53547
.14970	.03187	1.00000	.00544	.35656	.28616
.55397	.14156	.00544	1.00000	.48238	-.04285
.53540	.47568	.35656	.48238	1.00000	.06719
.04510	.53547	.28616	-.04285	.06719	1.00000

The B Regression Coefficients

B(1) = -.02009
B(2) = 1.07837
B(3) = .46945
B(4) = .00877
B(5) = -.91266

Regression Constant = -2.18035

Standard Error of Estimate = .9259

Multiple Correlation = .71070

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 2.24539 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -.705573 with 11. degrees of freedom
TB(2) = 3.052884 with 11. degrees of freedom
TB(3) = 1.883968 with 11. degrees of freedom
TB(4) = .599568 with 11. degrees of freedom
TB(5) = -1.422544 with 11. degrees of freedom

SINGLE TAN (Continued)

Partial Correlation Coefficients

-.20808
.67724
.49391
.17789
-.39418

SINGLE GREEN

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	158.500	8.16432780	66.65625000
Potassium	4.800	.47268475	.22343088
Calcium	7.222	1.62289340	2.63378300
Cholesterol	238.000	49.32291500	2432.75000000
Albumin	4.251	.47786288	.22835294
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.36075	-.01853	.18942	-.00336	-.21743
.36075	1.00000	-.24212	.30399	.32898	.48431
-.01853	-.24212	1.00000	-.24105	.29077	.04863
.18942	.30399	-.24105	1.00000	.48311	.09636
-.00336	.32898	.29077	.48311	1.00000	.11060
-.21743	.48431	.04863	.09636	.11060	1.00000

The B Regression Coefficients

B(1) = -.07555
B(2) = 1.96786
B(3) = .28549
B(4) = .00546
B(5) = -.94644

Regression Constant = 4.42559

Standard Error of Estimate = .8983

Multiple Correlation = .73093

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 2.52366 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -2.426848 with 11. degrees of freedom
TB(2) = 3.298747 with 11. degrees of freedom
TB(3) = 1.605933 with 11. degrees of freedom
TB(4) = .904195 with 11. degrees of freedom
TB(5) = -1.382609 with 11. degrees of freedom

SINGLE GREEN (Continued)

Partial Correlation Coefficients

-.59051
.70519
.43580
.26302
-.38477

SINGLE BLUE

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	161.941	14.18723200	201.27757000
Potassium	4.852	.73278176	.53696911
Calcium	10.352	2.36851350	5.60985660
Cholesterol	218.588	69.51533800	4832.38230000
Albumin	4.721	.51314270	.26331544
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.03133	.69474	.22171	.17197	-.24527
.03133	1.00000	-.12975	.06497	.11622	.31498
.69474	-.12975	1.00000	-.25930	-.01484	-.23209
.22171	.06497	-.25930	1.00000	.29276	.42725
.17197	.11622	-.01484	.29276	1.00000	-.31995
-.24527	.31498	-.23209	.42725	-.31995	1.00000

The B Regression Coefficients

B(2) = .61255
B(3) = .25696
B(4) = .01338
B(5) = -1.02020

Regression Constant = 6.86409

Standard Error of Estimate = .7183

Multiple Correlation = .83796

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 5.18734 with (5., 11.) Degrees of Freedom
T Test of B(I) = 0. for I=1,2,...10

TB(1) = -2.625377 with 11. degrees of freedom
TB(2) = 2.423316 with 11. degrees of freedom
TB(3) = 1.921577 with 11. degrees of freedom
TB(4) = 3.984069 with 11. degrees of freedom
TB(5) = -2.754342 with 11. degrees of freedom

SINGLE BLUE (Continued)

Partial Correlation Coefficients

-.62066
.58995
.50131
.76854
-.63888

BLUE AND TAN

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	164.117	18.35460500	336.89154000
Potassium	4.844	.82105966	.67413897
Calcium	8.467	1.90646730	3.63461760
Cholesterol	206.117	38.92923300	1515.48520000
Albumin	4.368	.47315274	.22387352
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.40382	.56807	.51224	.61835	.14516
.40382	1.00000	.11632	-.10019	.33302	.60128
.56807	.11632	1.00000	.02489	.49852	.26010
.51224	-.10019	.02489	1.00000	.15622	-.20074
.61835	.33302	.49852	.15622	1.00000	-.03937
.14516	.60128	.26010	-.20074	-.03937	1.00000

The B Regression Coefficients

B(1) = -.01216
B(2) = 1.02392
B(3) = .28416
B(4) = .00097
B(5) = -.97405

Regression Constant = -.08074

Standard Error of Estimate = .8609

Multiple Correlation = .75644

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 2.94266 with (5., 11.) Degrees of Freedom
T Test of B(I) = 0. for I=1.2....10

TB(1) = -.508221 with 11. degrees of freedom
TB(2) = 3.071529 with 11. degrees of freedom
TB(3) = 1.771411 with 11. degrees of freedom
TB(4) = .121989 with 11. degrees of freedom
TB(5) = -1.609257 with 11. degrees of freedom

BLUE AND TAN (Continued)

Partial Correlation Coefficients

-.15146
.67947
.47111
.03675
-.43653

BLUE AND GREEN

The Number of Observations = 16
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	153.718	14.84023200	220.23250000
Potassium	4.487	.72646633	.52775333
Calcium	8.733	2.71674510	7.38070410
Cholesterol	213.187	56.37164600	3177.76250000
Albumin	4.177	.40284818	.16228666
Egg	1.312	1.07819290	1.16250000

Intercorrelation

1.00000	.29968	.04942	-.04531	.26995	.07877
.29968	1.00000	-.34745	.05208	.22695	.47003
.04942	-.34745	1.00000	-.61909	-.22934	-.14449
-.04531	.05208	-.61909	1.00000	.45854	-.15020
.26995	.22695	-.22934	.45854	1.00000	-.55370
.07877	.47003	-.14449	-.15020	-.55370	1.00000

The B Regression Coefficients

B(1) = .00923
B(2) = .89638
B(3) = -.00344
B(4) = .00368
B(5) = -2.18232

Regression Constant = 4.23241

Standard Error of Estimate = .7016

Multiple Correlation = .84717

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 5.08475 with (5., 10.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = .686101 with 10. degrees of freedom
TB(2) = 3.028718 with 10. degrees of freedom
TB(3) = -.036191 with 10. degrees of freedom
TB(4) = .774401 with 10. degrees of freedom
TB(5) = -3.985915 with 10. degrees of freedom

BLUE AND GREEN (Continued)

Partial Correlation Coefficients

.21203
.69169
-.01144
.23785
-.78339

DOUBLE TAN

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	162.088	15.17114300	230.16360000
Potassium	4.969	.68322278	.46679338
Calcium	10.986	1.86617920	3.48262500
Cholesterol	232.117	92.63158300	8580.61020000
Albumin	4.732	.73116998	.53460955
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.28406	-.06369	.01191	.23824	-.21459
.28406	1.00000	-.33957	.40765	.42108	.25751
-.06369	-.33957	1.00000	.24081	-.03576	.05536
.01191	.40765	.24081	1.00000	.39150	.42688
.23824	.42108	-.03576	.39150	1.00000	.28886
-.21459	.25751	.05536	.42688	.28886	1.00000

The B Regression Coefficients

B(1) = -.02194
B(2) = .25445
B(3) = .01599
B(4) = .00338
B(5) = .27309

Regression Constant = 1.27443

Standard Error of Estimate = 1.1141

Multiple Correlation = .53248

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = .87063 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -1.114993 with 11. degrees of freedom
TB(2) = .459250 with 11. degrees of freedom
TB(3) = .089995 with 11. degrees of freedom
TB(4) = .880439 with 11. degrees of freedom
TB(5) = .617151 with 11. degrees of freedom

DOUBLE TAN (Continued)

Partial Correlation Coefficients

-.31865
.13716
.02712
.25657
.18293

RED AND GREEN

The Number of Observations = 16
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	157.000	18.20164800	331.30000000
Potassium	4.714	.91851306	.84366625
Calcium	8.213	2.05628700	4.22831660
Cholesterol	211.000	40.36665200	1629.46660000
Albumin	4.064	.32483777	.10551958
Egg	1.375	1.14746090	1.31666660

Intercorrelation

1.00000	.46844	.25827	.20769	.06263	.05426
.46844	1.00000	-.16010	.13147	.17852	.50247
.25827	-.16010	1.00000	-.17781	.21038	-.20237
.20769	.13147	-.17781	1.00000	.25908	-.15688
.06263	.17852	.21038	.25908	1.00000	-.03152
.05426	.50247	-.20237	-.15688	-.03152	1.00000

The B Regression Coefficients

B(1) = -.00987
B(2) = .74740
B(3) = -.05057
B(4) = -.00581
B(5) = -.19948

Regression Constant = 1.85395

Standard Error of Estimate = 1.1379

Multiple Correlation = .58679

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 1.05028 with (5., 10.) Degrees of Freedom
T Test of B(I) = 0. for I=1,2,...10

TB(1) = -.471335 with 10. degrees of freedom
TB(2) = 1.867025 with 10. degrees of freedom
TB(3) = -.292010 with 10. degrees of freedom
TB(4) = -.711498 with 10. degrees of freedom
TB(5) = -.196041 with 10. degrees of freedom

RED AND GREEN (Continued)

Partial Correlation Coefficients

-.14742
.50840
-.09195
-.21950
-.06187

SINGLE RED

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	165.352	15.53500500	241.33639000
Potassium	5.001	.75237320	.56606544
Calcium	9.553	2.83377990	8.03030880
Cholesterol	228.058	53.75694100	2889.80880000
Albumin	4.467	.45672426	.20859705
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.47801	.44066	.16181	.29361	-.20057
.47801	1.00000	.63329	.11940	.28073	.43711
.44066	.63329	1.00000	.08835	.32700	.50956
.16181	.11940	.08835	1.00000	.04002	-.15471
.29361	.28073	.32700	.04002	1.00000	.15945
-.20057	.43711	.50956	-.15471	.15945	1.00000

The B Regression Coefficients

B(1) = -.04327
B(2) = .58583
B(3) = .19943
B(4) = -.00307
B(5) = .15217

Regression Constant = 3.57750

Standard Error of Estimate = .8350

Multiple Correlation = .77300

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 3.26641 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -2.716605 with 11. degrees of freedom
TB(2) = 1.561685 with 11. degrees of freedom
TB(3) = 2.021974 with 11. degrees of freedom
TB(4) = -.780893 with 11. degrees of freedom
TB(5) = .309222 with 11. degrees of freedom

SINGLE RED (Continued)

Partial Correlation Coefficients

-.63365
.42600
.52054
-.22918
.09283

DOUBLE GREEN

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	160.676	18.88033100	356.46691000
Potassium	4.888	.58326539	.34019852
Calcium	7.763	1.73241900	3.00127570
Cholesterol	217.529	28.20930100	795.76470000
Albumin	4.454	.35861047	.12860147
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.34758	.05703	.16896	.11152	-.39947
.34758	1.00000	-.38501	-.36713	-.08596	-.15466
.05703	-.38501	1.00000	.34978	.16624	.28347
.16896	-.36713	.34978	1.00000	.53415	.20682
.11152	-.08596	.16624	.53415	1.00000	.13432
-.39947	-.15466	.28347	.20682	.13432	1.00000

The B Regression Coefficients

B(1) = -.03230
B(2) = .49645
B(3) = .19986
B(4) = .01075
B(5) = .05555

Regression Constant = -.13987

Standard Error of Estimate = 1.0757

Multiple Correlation = .57623

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 1.09366 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -1.974520 with 11. degrees of freedom
TB(2) = .837650 with 11. degrees of freedom
TB(3) = 1.139071 with 11. degrees of freedom
TB(4) = .829013 with 11. degrees of freedom
TB(5) = .061932 with 11. degrees of freedom

DOUBLE GREEN (Continued)

Partial Correlation Coefficients

-.51154
.24487
.32481
.24249
.01867

DOUBLE BLUE

The Number of Observations = 16
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	161.882	13.32001400	177.42279000
Potassium	5.005	.66665263	.44442573
Calcium	8.800	2.04538120	4.18358450
Cholesterol	238.823	60.20614000	3624.77940000
Albumin	4.331	1.20837120	1.46016100
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.25761	.14557	.12630	.14527	-.24947
.25761	1.00000	.19479	.12897	-.14728	.30120
.14557	.19479	1.00000	-.09831	.11076	.14670
.12630	.12897	-.09831	1.00000	-.36312	.05298
.14527	-.14728	.11076	-.36312	1.00000	-.34854
-.24947	.30120	.14670	.05298	-.34854	1.00000

The B Regression Coefficients

B(1) = -.02506
B(2) = .50964
B(3) = .08481
B(4) = -.00069
B(5) = -.26165

Regression Constant = 3.29368

Standard Error of Estimate = 1.1072

Multiple Correlation = .54069

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = .90890 with (5., 10.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = -1.120608 with 10. degrees of freedom
TB(2) = 1.141948 with 10. degrees of freedom
TB(3) = .603641 with 10. degrees of freedom
TB(4) = -.136558 with 10. degrees of freedom
TB(5) = -1.022902 with 10. degrees of freedom

DOUBLE BLUE (Continued)

Partial Correlation Coefficients

-.32009
.32555
.17906
-.04113
-.29471

DOUBLE RED

The Number of Observations = 17
The Number of Variables = 6

	Mean	Standard Deviation	Variance
Sodium	161.882	16.77938500	281.54779000
Potassium	4.705	.56263952	.31656323
Calcium	7.937	1.98067130	3.92305880
Cholesterol	225.294	47.50100500	2256.34550000
Albumin	4.385	.33104224	.10958897
Egg	1.235	1.09141020	1.19117640

Intercorrelation

1.00000	.45435	.37027	.20196	.42149	-.09224
.45435	1.00000	.16718	-.28654	-.20268	.28869
.37027	.16718	1.00000	-.59085	.26074	.10927
.20196	-.28654	-.59085	1.00000	.21798	-.38840
.42149	-.20268	.26074	.21798	1.00000	-.57105
-.09224	.28869	.10927	-.38840	-.57105	1.00000

The B Regression Coefficients

B(1) = .01655
B(2) = -.08269
B(3) = -.00478
B(4) = -.00742
B(5) = -2.02510

Regression Constant = 9.53638

Standard Error of Estimate = .9853

Multiple Correlation = .66301

F Test of B(1)=B(2)=B(3)=....=B(10)=0.
F = 1.72569 with (5., 11.) Degrees of Freedom
T Test of B(1) = 0. for I=1,2,...10

TB(1) = .581155 with 11. degrees of freedom
TB(2) = -.120534 with 11. degrees of freedom
TB(3) = -.020660 with 11. degrees of freedom
TB(4) = -.735274 with 11. degrees of freedom
TB(5) = -2.128110 with 11. degrees of freedom

DOUBLE RED (Continued)

Partial Correlation Coefficients

.17259
-.03631
-.00622
-.21643
-.54003