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A determination of the correlation between glycemic levels and the estrous cycle in the white rat

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A DETERMINATION OF THE CORRELATION BETWEEN GLYCEMIC LEVELS AND THE ESTROUS CYCLE IN THE WHITE RAT

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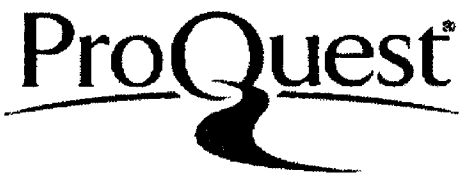

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INTRODUCTION

The blood sugar level is of fundamental importance in the study of an animal for many reasons. It is of primary importance in the physiology of the organism because it is the 'food' of the animal on the cellular level. Glycemic levels may also be used as an indicator of the condition of the animal, and may be diagnostic of abnormalities as in some hypoglycemic and hyperglycemic conditions. These latter conditions may be partially indicative of the state of many of the organs of the body; especially the liver, anterior lobe of the pituitary, pancreatic islets of Langerhans, adrenal cortex, adrenal medulla and the thyroid, (Cori, '40; Folgia et al, '47; Greene et al, '39; Long et al, '40; Soskin et al, '38; Soskin et al, '36).

The question that then arises is, what are the normal values for blood sugar, and what causes fluctuations in these values? Cori ('31) gives the figure of 92 mg per 100 cc as the true value for blood sugar for mixed venous-arterial blood of the rat. However, this figure is not a constant, and the endogenous and exogenous forces that may influence it are many. Among the intrinsic factors of the body that influence blood sugar, we know that some of the

steroids, including the adrenal cortical extracts and possibly progesterone may cause variations in blood sugar values in ferrets and rats (Gaunt et al, '39; Greene et al, '39).

The primary interest in this investigation is concerned with whether or not a blood sugar cycle exists that may be correlated with the estrous cycle. This leads to inspection of the estrous-activity and metabolic cycles in so far as they affect blood sugar levels.

REVIEW OF THE LITERATURE

It has been shown by many authors that multitudes of cyclic phenomena are existent in animals. These include ingestion of food (Richter, '27), adrenal cortex activity (Halberg and Visscher, '52), liver glycogen deposition (Boutwell et al, '48; Higgins et al, '32, 33; Soskin et al, '38), basal metabolism (Lee, '28), diurnal blood sugar (Boutwell et al, '48; Pitts, '43) activity (Slonaker, '24; Wang, '23) and reproductive cycles (Blandau and Soderwall, '41; Boling et al, '41).

The many cyclic variations are of primary interest to those who would understand the whole organism from the psychological and physiological points of view. New information is being sought and accrued about the rhythms and their ramifications, but the end is not yet in sight. As an example of the possible ramifications, Peterson ('47) even suggested that "one might consider the possibility that the human group selected the seventh day of rest because of an existing underlying biochemical rhythm." Be that as it may cyclic phenomena are of fundamental importance in the animal.

Kleitman ('49) stated that, "a physiological rhythm, specifically the diurnal rhythm is essentially a metabolic cycle synchronized with the external periodicity of day

and night through the influence of variations in illumination, temperature and other environmental factors on nervous and endocrine systems."

Variations, up to a point, may be induced in these rhythms by laboratory manipulation of such things as feeding times (Boutwell et al, '48; Halberg and Visscher, '52; Pitts, '43) and by alterations of periods of light and dark and of temperature (Browman, '44).

Normal blood sugar, as we know it, is primarily affected by digestion and absorption of foodstuffs from the gut (Boutwell et al, '48; Pitts, '43) and the dynamic sugar-glycogen balance which is largely maintained by the liver.

The homeostasis of blood sugar is, evidently, maintained in large part by the dynamic balance between it and liver glycogen as (Soskin, '40; Soskin et al, '38) determined by experiments which measured blood sugar entering and leaving the liver under normal conditions as well as when sugar was being added to the blood stream. The idea of a fundamental hepatic regulation would also seem to be supported by experiments involving hypophysectomized-depancreatized (Houssay) animals which have been maintained for many weeks without insulin (Soskin et al, '36). Muscle glycogen normally plays a relatively insignificant role in maintaining glycemic levels because it is incapable of sufficiently rapid conversion to glucose (Bollman et al, '25). As Soskin ('41)

remarks, "it may therefore be stated as axiomatic that the blood sugar level represents a dynamic balance between the rate at which blood sugar is entering the blood stream from the liver and from an exogenous source and the rate at which it is being removed from the blood by the tissues of the body."

The liver glycogen of rats shows a quantitative, diurnal, cyclic variation (Higgins et al, '33), which may be modified by changing the feeding habits of the animals (Boutwell et al '48). Changing of the normal feeding hours, which occur from 6 P.M. to 12 midnight, to the morning hours caused a reversal of the liver glycogen cycle. The low occurs 10-12 hours after feeding in both cases (Higgins et al, '32; '33). It has been found that blood sugar also exhibits a 24 hour cycle (Boutwell et al '48; Pitts,'43). Pitts made noon-midnight blood sugar determinations on rats fed ad libitum, and found that the highest blood sugar readings occured at midnight, presumably because of the feeding habits of rats under normal colony conditions. He also discovered that animals trained to feed in the morning hours showed a reversal of the blood sugar curve, even though they retained the same 24 hour activity distribution pattern as animals allowed to feed normally.

It should not, however, be assumed from the preceding discussion that the homeostatis maintained by the liver is

the only factor to be reckoned with in the investigations of blood sugars. Also to be considered are the action of the pancreatic islets of Langerhans, the adrenal medulla, the adrenal cortex, the anterior lobe of the hypophysis, as well as the fundamental enzyme systems discussed by Cori ('40). There is still uncertainty as to the precise function of the endocrines in normal metabolism, but it is believed that they are secondary to the liver. Likewise the possi- of influence by organs other than those generally accepted, should not be overlooked. The ovarian secretions, for instance, may play some part for it has been shown (Gaunt et al, '39; Greene et al, '39) that progesterone may have some 'cortin' like effects.

We might, here, divert our attention from blood sugar and turn to the other phase of the physiology of the animal which interests us in this paper; namely, the reproductive cycle.

The reproductive cycle of animals and the many changes which accompany it, have been of interest to many zoologists. There are both external and internal differences among the various species. Those such as nesting behavior in birds and increased activity of laboratory rodents in estrus are easily seen. The internal changes of which these are the manifestations are not so easily observed. Stockard and Papinicalaou ('17) devised a vaginal smear technique for

determining the stage of the estrous cycle in guinea pigs, and Long and Evans ('22) applied this in their classic work to the estrous cycle of rats. The rat has proved to be, except for the persistance of the corpora lutea through several cycles, an excellent laboratory subject for the study of sexual and associated phenomena since its estrous cycle is of relatively short duration.

The estrous cycle of rats as indicated by vaginal smears is described as passing through five different stages by Long and Evans ('22). Stage I or proestrous, is characterized by small round epithelial cells of uniform size and appearance. The smear of stage II or estrus is found to contain large, thin, transparent, non-nucleated scale like elements. This is the picture found at the time coitus is usually permitted by the female. The duration of this stage is approximately 12 hours which is somewhat longer than the time usually attributed to stage I. Stage III, which takes from 15-18 hours, is not readily distinguishable from stage II. It is an exaggeration of the preceding stage with sheets of cornified elements, and is the stage in which ovulation usually occurs. A vaginal smear in the next six hours would be found to contain leucocytes and remnants of cells from II - this is stage IV or metestrum. The major portion of the cycle - 57-60 hours - is taken up by the dioestrous interval, or stage V. It is characterized by

leucocytes and small irregularly shaped free epithelial cells contained in thin stringy mucus. According to Long and Evans ('22) ovulation occurs about 21 hours after the first cornified cells occur, or as other authors (Boling et al, '41) describe it, ovulation occurs 10 hours after the start of heat or sexual receptivity. After ovulation, corpora lutea form at the site of ovulation, the functional life of which may be extended by pregnancy or pseudopregnancy.

We have now arrived at the point where we may well ask: Is there any indication of a possible connection between the estrous cycle and blood sugar? There would seem to be in that Greene and his co-workers ('39) showed that progesterone, a secretion of the corpus luteum of the ovary, is 'cortin' (adrenal cortex)-like in that it will maintain life and weight gain in adrenalectomized rats. Gaunt et al, ('39) reported that cortical extracts and progesterone both elevated liver glycogen and blood sugar values in the ferret. This would seem to leave room for speculation on the possibility of ovarian influence on blood sugar. They applied similar experimental procedures to rats. They also found that cortical extracts elicited similar responses, but the influence of progesterone, if any, was extremely slight.

Some more information about the possibilities of a connection between the estrous cycle and metabolic processes was provided by Lee ('28), who made a total of 282 basal metabolic rate determinations on 9 female rats during all

stages of the estrous cycle. In all of the animals an increased basal heat production was observed during the last 10 hours of stage V and the first six hours of stage I. A difference of around 13% above that of stages II, III and IV was found. Lee said that the main event coinciding with the rise in basal heat production was a rather precipitous degeneration of the corpus luteum, and he concluded that "the corpus luteum may have a metabolic sparing or anabolic function, and upon removal of its influence a period of increased katabolic activity begins."

The possibility of a connection between ovarian secretion, of which the estrous cycle is a manifestation, was increased by the work of Folgia, Schuster and Rodriguez ('47) which showed sex differences in the responses of rats to a 95% pancreatectomy. Their female rats became diabetic less frequently and exhibited a higher rate of survival. They also noted that castration in males had an ameliorating effect, while in females, castration sensitized the animals to diabetes. Later (Lewis et al, '50) it was shown that estrogenic substances decrease the incidence of diabetes, while androgenic ones increase them.

We may pursue the problem at hand - the question of a correlation between the estrous cycle and blood sugar - still further by assuming, highly suppositional at this stage, that a possibility of some ovarian influence on blood sugar

does exist. The question which would then come to the fore is: Does a period of change in secretion of substances by the ovary exist which could conceivably cause a change in blood sugar?

Astwood ('39), using uterine changes as indicators of ovarian change, took the results of some of his experimental work to mean that estrogen acts unopposed during a brief period just prior to vaginal cornification. He also stated that the corpus luteum hormone is released from the follicles during their pre-ovulatory swelling, and ceases to be produced at approximately the time of ovulation. This, in itself, would seem to provide support for the thesis that a rather radical change in ovarian secretion does occur.

Boling and Blandau's ('39) results contradict this for their results showed that estrus induced in a spayed female rat by the synergistic action of estrogen and progesterone more nearly approximated that exhibited by a normal female than when estrogen alone was used.

The dominance of estrogen at some times and progesterone at others in the estrous cycle is generally accepted. The amount of hormones present is probably a relative thing with some of each being present all the time (Astwood, '39; Boling and Blandau '39; Hisaw et al, '34). Up to date no clear cut relationship between the estrous cycle and blood sugar in the rat has been reported.

Materials and Methods

In this experiment, the object of which was to see whether or not a correlation exists between glycemic levels and the estrous cycle in rats, four different regimes were set up to facilitate the study. They were:

1. Light 8 A.M. to 8 P.M., dark 8 P.M. to 8 A.M.; rats allowed to feed ad libitum.
2. Light 8 A.M. to 8 P.M., dark 8 P.M. to 8 A.M.; rats allowed to feed from 8 A.M. to 1 P.M. only.
3. Light 8 A.M. to 8 P.M., dark 8 P.M. to 8 A.M.; oophorectomized animals allowed to feed ad libitum.
4. Dark 8 A.M. to 8 P.M., light 8 P.M. to 8 A.M.; rats allowed to feed ad libitum.

The experimental procedure, in all cases, allowed for free access to water. Individual cages were provided for all rats. The animals were fed B no. 21 (Browman, '44) to which a mineral supplement was added. Lettuce was fed two to three times a week; in the case of animals in regime 2 the lettuce was left in the cages from 8 A.M. to 1 P.M. only.

The period through which this experiment was carried on extended from May 1953 to November 1953. This was necessitated by a limited number of activity cages. In this time a total of 56 nulliparous female rats of a nearly homogenous genetical composition were used. The animals were in their forty-second inbred generation. The selection of rats

was made on the basis of age, weight and overall condition. The rats used were approximately 90 days old - plus or minus 10 days, which falls well within the two month range set up by Shirley ('28) for relative homogenity of activity.

The rats were selected for the groups so that where possible litter mates were put into more than one group, otherwise, no conscious selection was exercised in placing them in one group or another. Sixteen animals comprised the experimental group of regime 2, while twenty-four were in group number 1. The animals in the latter group served a twofold function in that they were controls for activity and weight gain, while at the same time serving as experimentals for blood sugar. Group 3 and 4 each contained eight animals.

Following the procedure of Shirley ('28) the rats were all allowed a 15 day pre-experimental period in which to become acclimatized to the individual wheel type recording exercise cages. Introduction to modified feeding regimes (Group 2 only) to frequent handling, and to smear taking was done in this 15 day period. The estrous cycle, as indicated by vaginal smears, was recorded for individual rats, but probably the most important function of this period was to familiarize the animals with the handler. The animals became quite tame and would climb onto the hand by the end of the orientation period. The experimentor did all cage

cleaning, feeding and watering during the 15 day acclimatization period and 30 day experimental period.

The eight animals used in experimental regime 3 were bilaterally ovariectomized after being anesthetized by sodium pentobarbital injected intra-peritoneally. The dorsal approach, which necessitated two incisions, was used. The incisions, however, were quite small and healing was rapid.

The animals were housed in two different rooms. Those in groups 1, 2 and 3 were isolated in the control room in a corner of the greenhouse. This room is lined with fiber board, has a concrete floor, and a wooden door. Three benches, standing approximately three feet off the floor, completely line three walls. The activity cages were placed on these. Two feet above the benches are fluorescent lights giving relatively even illumination on the benches. They are automatically controlled by a Telechron time switch. Temperature control was maintained by a manually operated thermostat, which held the temperature at 75°F - 1°F. Relative humidity was not controlled, nor were the sounds normally associated with activities in the greenhouse.

The animals of group 4 were kept in individual cages in dead storage room. This room is accessible only by a ladder from the stockroom of the Zoology Department. The temperature here is quite constant but uncontrolled. The

lights in this room are manually operated. These animals were exposed to light once every three days during the dark period for about 5 minutes during the time when they had to be removed from this room to take blood samples.

During the experimental period, blood sugars were taken by tail cutting once every three days. This allowed time for the rats to return to normal physiological condition and to allay fear of the investigator induced by the tail cutting (Halberg and Visscher, '52). Blood sugar determinations were made at 8 A.M., 1 P.M. and 8 P.M. If a particular rat was sampled at 8 A.M. on one day the next sample would be taken at 8 P.M. three days later, and then at 1 P.M. after an additional three days.

A vaginal smear of each rat was made daily, in the evening unless a blood sugar sample was made that day, in which case the smear was made at the same time. The smears were obtained by introducing a moistened pledgelet of cotton into the vagina of the animal. The cells which adhered to the cotton were rubbed off on a clean glass slide and examined. Long and Evans ('22) criteria were used to determine the stage of the estrous cycle. Oophorectomized animals also had vaginal smears taken in order to duplicate experimental conditions.

Animals from which samples for blood sugars were to be taken were allowed to enter a specially constructed restrainer.

This consisted of a block of wood 8 inches long and 3½ inches wide to which a rectangular piece of stiff wire mesh had been attached to form a cage open at both ends the approximate length and diameter of the animal's body. The tail was then placed and held in a beaker of water at 35°C for 30 seconds. The tip of the tail was then dried and amputated and the blood collected in a small paraffin lined dish. A Kahn pipette was used to draw up 0.1 cc of this blood which was then delivered into a small flask containing 10 cc of a dilute tungstic acid solution. The pipptte was flushed with this solution, after which the flask's contents were thoroughly mixed and then filtered. All determinations were made in the evening of the day in which sampling took place. In the interval between the sampling and the actual determinations the samples were stored on the shelves of a household type refrigerator.

The Folin-Malmros blood sugar method ('33) was used in the determinations. This colormetric micro-method depends on the fact that when a glucose solution is heated with an alkaline ferricyanide solution, the ferricyanide is reduced to ferrocyanide. The ferrocyanide will react with a ferric iron solution to produce a blue color which is measurable in a colorimeter. Two different colorimeters were used in the study. One was a Baush and Lomb Duboscq type, the other a Bausch and Lomb monochromatic, direct reading, single beam one. When the switch was made from one type of colorimeter

to another blood sugars were run with both instruments and it was found that they gave similar readings. The standard glucose solution, against which the unknown was read, was prepared fresh every week. It was maintained in a sterile flask in a refrigerator.

Sampling is the most critical point in the whole experiment. The sample must be acquired rapidly, in less than three minutes, or the blood becomes hyperglycemic. Clotting may also occur in the collecting dish if the blood flow is slow. All blood sugar samples which required more than three minutes of bleeding were discarded because of the hyperglycemic factor.

Statistical significance throughout this paper was based on the *t* test as described in Snedecor ('40). This test is designed specifically for testing the significance of differences between means. If the probability that the difference was due to chance alone was greater than 5% it was assumed that there was no difference.

Results

The probability is very high that a correlation does exist between the estrous-activity cycle and fluctuations in mean blood sugar values. The blood sugar readings from the 48 rats on regimes 1, 2 and 4 were used in this phase of the study. Combined estrous cycle stages I and II (mean blood sugar of 104.5 mg %) when compared with combined estrous cycle stages III, IV and V (mean blood sugar of 103.4 mg %) gave a t value of 5.213. There were 332 degrees of freedom in this comparison. For 300 degrees of freedom the t value at the 1% level is 2.592; since the sample value far exceeds even the 1% level we may assume a significant difference between the grouped selected estrus stages.

A statistical significance, even at the 5% level, does not exist when individual estrous cycle stages are compared one with the other.

Summaries of the data used in comparing the blood sugars and estrous cycle stages may be seen in Tables 11 and 12.

A comparison of the mean blood sugar values was made for all rats on regimes 1, 2, 3 and 4 by the time of day sampling occured, and by the particular regime (Table 5). The raw data for this phase of the study may be found in Tables 1-4. The twenty-four animals on regime 1, which

were fed ad libitum, did not show significant differences in mean blood sugar values when these values were compared by the time of day the sample was taken. The mean glycemic values for the 8 A.M., 1 P.M. and 8 P.M. sampling periods were 103.7 mg %, 104.2 mg % and 104 mg % respectively.

In regime 2, however, a statistical significance based on the time of sampling periods for blood sugars was obtained. The 8 A.M. readings (102 mg %) when compared with 8 P.M. readings (106 mg %) with 69 degrees of freedom gave a t value of 2.587. The t value for 70 degrees of freedom at the 5% level is 1.994, while the t value at the 1% level is 2.648. The 1 P.M. readings (103 mg %) when compared with the 8 P.M. value with 75 degrees of freedom gives a t value of 2.106. For 80 degrees of freedom the t values at the 5% and 1% levels are 1.990 and 2.638 respectively. A significant difference between the blood sugar means based on feeding times is quite probable in both these cases, that is, 8 A.M.-8 P.M. and 1 P.M.-8 P.M. A difference is not exhibited between 8 A.M. and 1 P.M. mean glycemic levels.

The blood sugars of the 8 oophorectomized rats on regime 3 did not vary significantly with the time of the sampling period. Mean blood sugars of 106.4 mg %, 106.8 mg % and 105.3 mg % were obtained at 8 A.M., 1 P.M. and 8 P.M. respectively.

The estrous cycle as determined by vaginal smears and activity records on all intact animals was found to be completed in 4½ to 5 days. That is, estrus occured every 4½ to 5 days at the time of maximum spontaneous activity. A typical activity cycle may be seen in Graph 1.

TABLE 1

BLOOD SUGAR VALUES, INDIVIDUAL RATS REGIME 1

8 A.M.

Rat no.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	86	120	101	107	92	114	107	103	97	102	108	119	113	95	94	101	107	104	101	107	108	103	X	107
mg %		107	101	100	106	101	104	100	107	109	103	102	103		113	100	102	119		97	99	105		
			92			93		105	104		113					105	100					98		

1 P.M.

Rat no.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	146*	103	100	101	91	105	103	102	114	105	107	109	107	127*	95	103	108	102	105	105	104	104	X	
mg %	106	118	105	102	100	158*	87	106	107	99	99		115		100	99	101	112				104		
		102	99	116	103				104	108						98								
		105		106	105				104	111														
										110														

8 P.M.

Rat no.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
	103	102	106	107		108	166*	97	111	103	97	108	106	100	102	90	104	96	107	110	109	99	X	112
	100		102	110		105		160*	106	103	107	113	104	105	101	104	101	103		111	103	96		103
	103			101		105					104			117	103	98	104	98		104	106			101
											139*									107	103			101
											102													

X All readings dropped because of irregular estrous cycle.

*Readings dropped because of hyperglycemia.

TABLE 2

BLOOD SUGAR VALUES, INDIVIDUAL RATS, REGIME 2

8 A.M.

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	103	100	102	89	101	97	98	103	102	101	119	103	101	105	149*	102
mg %	110	160*	94		102	103		109		100		106	91			104
	166	103			109	104				107		99	111			
						89				95			104			

1 P.M.

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	97	98	89	109	101	94	101	106	99	100	107	102	103	96	100	111
	102	99	107	98	103	101	103	105		109	119		108	104		108
mg %			99	102	102		103			110	101		104			
			101	113			101									
			105													

8 P.M.

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	104	106	107	93	109	109	119	95	111	105	113	108	162*	105		103
mg %	94	110		106		119	96	108	114	107	112	101	102	111		
		114		88			115	99	106	106				107		
							106		108	104						

*Readings dropped because of hyperglycemia.

TABLE 3

BLOOD SUGAR VALUES, INDIVIDUAL RATS REGIME

8 A.M.

Rat no.	49	50	51	52	53	54	55	56
	107	120	103	107	110	92	113	101
mg %	109	110	100	107	103	108	109	104
			103		109			

1 P.M.

Rat no.	49	50	51	52	53	54	55	56
	107	111	108	105	107	105	97	94
mg %	109	117	105	109	115	107	107	106
		107						

8 P.M.

Rat no.	49	50	51	52	53	54	55	56
	108	99	107	97	114	97	101	114
mg %	99	103	105	104	107	104	110	114
	105		104	107	108		107	
					105			

TABLE 4

BLOOD SUGAR VALUES, INDIVIDUAL RATS REGIME 4

8 A.M.

Rat no.	41	42	43	44	45	46	47	48
	99	102	97	103	102	103	111	108
mg %	101	96	107	101	104	105	105	108
	102	113	104	104	112	93	109	102

1 P.M.

Rat no.	41	42	43	44	45	46	47	48
	106	105	115	95	105	107	107	104
mg %	103	104	114	108	97	106	96	104
	100	103	100		98	110	103	113

8 P.M.

Rat no.	41	42	43	44	45	46	47	48
	104	110	96	93	101	110	106	102
mg %	100	113	94	100	119	106	111	105
	103	99	102	104	102	100	98	99
							101	

TABLE 5

COMPARISON OF DATA ACCORDING TO REGIME AND TIME OF DAY SAMPLE TAKEN

Experimental conditions	Sample Time	Freq.	Range	Median	Mean	Mean Diff	S.D.	S.E.	Sig.
I. Regime 1 (Light 8 A.M.- 8 P.M. Dark 8 P.M. - 8 A.M.) Allowed to feed ad lib. Animals 17-40 (Initial age 90 days 10 days)	8 A.M.	50	86-120	103	103.7	0.5	7.011	1.284	No
	1 P.M.	49	91-119	104	104.2		5.714		
	8 A.M.	50	86-120	103	103.7	0.3	7.011	1.176	No
	8 P.M.	56	90-117	103.5	104		4.734		
	1 P.M.	49	91-119	104	104.2	0.2	5.714	1.033	No
	8 P.M.	56	90-117	103.5	104		4.734		
II. Regime 2 (Light 8 A.M.- 8 P.M. Dark 8 P.M.- 8 A.M. Food available 8 A.M.-1 P.M. only). Animals 1-16 (Initial age 90 days 10 days).	8 A.M.	34	89-119	101.5	102	1.0	6.058	1.340	No
	1 P.M.	40	89-119	102	103		5.357		
	8 A.M.	34	89-119	101.5	102	4.0	6.058	1.546	Yes
	8 P.M.	36	88-119	106	106		6.870		
	1 P.M.	40	89-119	102	103	3.0	5.357	1.424	Yes
	8 P.M.	36	88-119	106	106		6.870		
III. Regime 3 (Light 8 A.M.- 8 P.M. Dark 8 P.M.- 8 A.M.) Allowed to feed ad lib.) Oophorectomized animals (49-56). Initial age 90 days 10 days.	8 A.M.	18	92-120	107	106.4	0.4	5.754	1.767	No
	1 P.M.	17	94-117	107	106.8		4.670		
	8 A.M.	18	92-120	107	106.4	1.1	5.754	1.707	No
	8 P.M.	22	97-114	105	105.3		4.858		
	1 P.M.	17	94-117	107	106.8	1.5	4.670	1.535	No
	8 P.M.	22	97-114	105	105.3		4.858		
IV. Regime 4 (Dark 8 A.M.- 8 P.M. Light 8 P.M.- 8 A.M. Allowed to feed ad lib.) Animals 41-48. Initial age 90 days 10 days.	8 A.M.	24	93-113	103.5	103.3	1.2	4.883	1.487	No
	1 P.M.	23	95-115	104	104.5		5.381		
	8 A.M.	24	93-113	103.5	103.3	0.1	4.883	1.541	No
	8 P.M.	25	93-119	102	103.2		5.996		
	1 P.M.	23	95-115	104	104.5	1.3	5.381	1.660	No
	8 P.M.	25	93-119	102	103.2		5.996		

TABLE 6

BLOOD SUGAR VALUES, ESTROUS CYCLE STAGE I

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
mg %		103		98	101	101	103	103	106	110		102		111		102
					109	119						108				

Rat	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
	146*	103	101	116	91	114	166*	100	107		97			100		
mg %				106				106	104		107					
				110												

Rat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
mg %		103	105	108					104	104				110		
			110													

TABLE 7

BLOOD SUGAR VALUES, ESTROUS CYCLE STAGE II

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	103		107	102	102	97	98	109	108	105	119	103	104	105	149*	104
mg %	110		99	93		119	103	106			113	99				
			101									101				
			105													

Rat	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
	86	118	105	107	92	101	104	106		105	103	113	103	95	113	105
mg %	103	102			100	93				99	113		107	127		99
	100	102				158*				108	104		106			98
						105				111	139					104

Rat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	108	119		107	104	105		107		102	114		102	103	111	108
mg %					106	104		101		110			104	105	107	104
								101					105	106	106	104
													97	110	111	
														106		

*Dropped because of hyperglycemia.

TABLE 8

BLOOD SUGAR VALUES, ESTROUS CYCLE STAGE III

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	104	98				103	96	105		101			104	104		
mg %						89	115			106			162*	107		
						104	106			104			102			

Rat	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
mg %	106				101	105			107	103	102		115	105	101	
	103								106						103	

Rat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
mg %	102	98	105							113	97	103				102
	101		107								96	101				

TABLE 9

BLOOD SUGAR VALUES, ESTROUS CYCLE STAGE IV

Rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	106	114		89	109		101	99	99	107	119	106				111
mg %	102			113												108
	94			106	102					95	101					
				88						107	112					

Rat	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
		107	101	100	106				104	109	107	110	104	117	100	98
mg %		105	92		103				104	110	99					
			99		105					103						
			102													

Rat	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	107	117		97	109	103		112	99	96	107	104	112	107	96	114
	104	102			103	104		103	103	99	104	108			98	105
		112							100		115	93				
									100							

*Dropped because of hyperglycemia.

TABLE 10

BLOOD SUGAR VALUES, ESTROUS CYCLE STAGE V

Rat	1	2	3	4	5	6	7	8	9	10	11	12
		100	102	109	101	94	101	95	102	100	107	
		99	107						114	109		
mg %		160*	94		103	109		108	111	100		
		106	89									
		110										

Rat	13	14	15	16	17	18	19	20	21	22	23	24
	101	96	100	103		120	100	101		105	107	103
mg %	91	105					106	102		108	103	102
	111							107			87	160*
	103											97
	108											

Rat	25	26	27	28	29	30	31	32	33	34	35	36
	97	102	108	119	113		94	101	100		101	111
mg %	114			102			95	100	101			104
	111			109			102	103	104			107
				108				90				

Rat	37	38	39	40	41	42	43	44	45	46	47	48
	99	98			101	113	100	95	98	100	105	108
mg %	103	99			102	105	94	100	119		109	102
		96			106	103	102	104	102		103	99
					103						101	

*Dropped hyperglycemia.

TABLE 11

COMPARISON OF DATA ACCORDING TO ESTROUS CYCLE STAGE

Estrous cycle stage	Number of Samples	Animals	Range	Median	Mean	Mean Diff	S.D.	S.E.	Sig
I	35	1-48	91-119	104	104.6	0.1	4.699	0.993	No
II	89	1-48	86-119	104	104.5		5.799		
I*	35	1-48	91-119	104	104.6	1.2	4.699	1.090	No
III	39	1-48	89-115	103	103.4		4.697		
I*	35	1-48	91-119	104	104.6	0.8	4.699	1.075	No
IV	74	1-48	88-119	104	103.8		6.326		
I*	35	1-48	91-119	104	104.6	1.5	4.699	0.993	No[1]
V	99	1-48	87-120	102	103.1		6.067		
II*	89	1-48	86-119	104	104.5	1.1	5.759	0.969	No
III*	39	1-48	89-115	103	103.4		4.697		
II*	89	1-48	86-119	104	104.5	0.7	5.759	0.956	No
IV*	74	1-48	88-119	104	103.8		6.326		
II*	89	1-48	86-119	104	104.5	1.4	5.759	0.863	No[1]
V*	99	1-48	87-120	102	103.1		6.067		
III*	39	1-48	89-115	103	103.4	0.4	4.697	1.057	No
IV*	74	1-48	88-119	104	103.8		6.326		
III*	39	1-48	89-115	103	103.4	0.3	4.697	0.958	No
V*	99	1-48	87-120	102	103.1		6.067		
IV*	74	1-48	88-119	104	103.8	0.7	6.326	0.955	No
V*	99	1-48	87-120	102	103.1		6.067		

*Data repeated.

[1]Approaching significance.

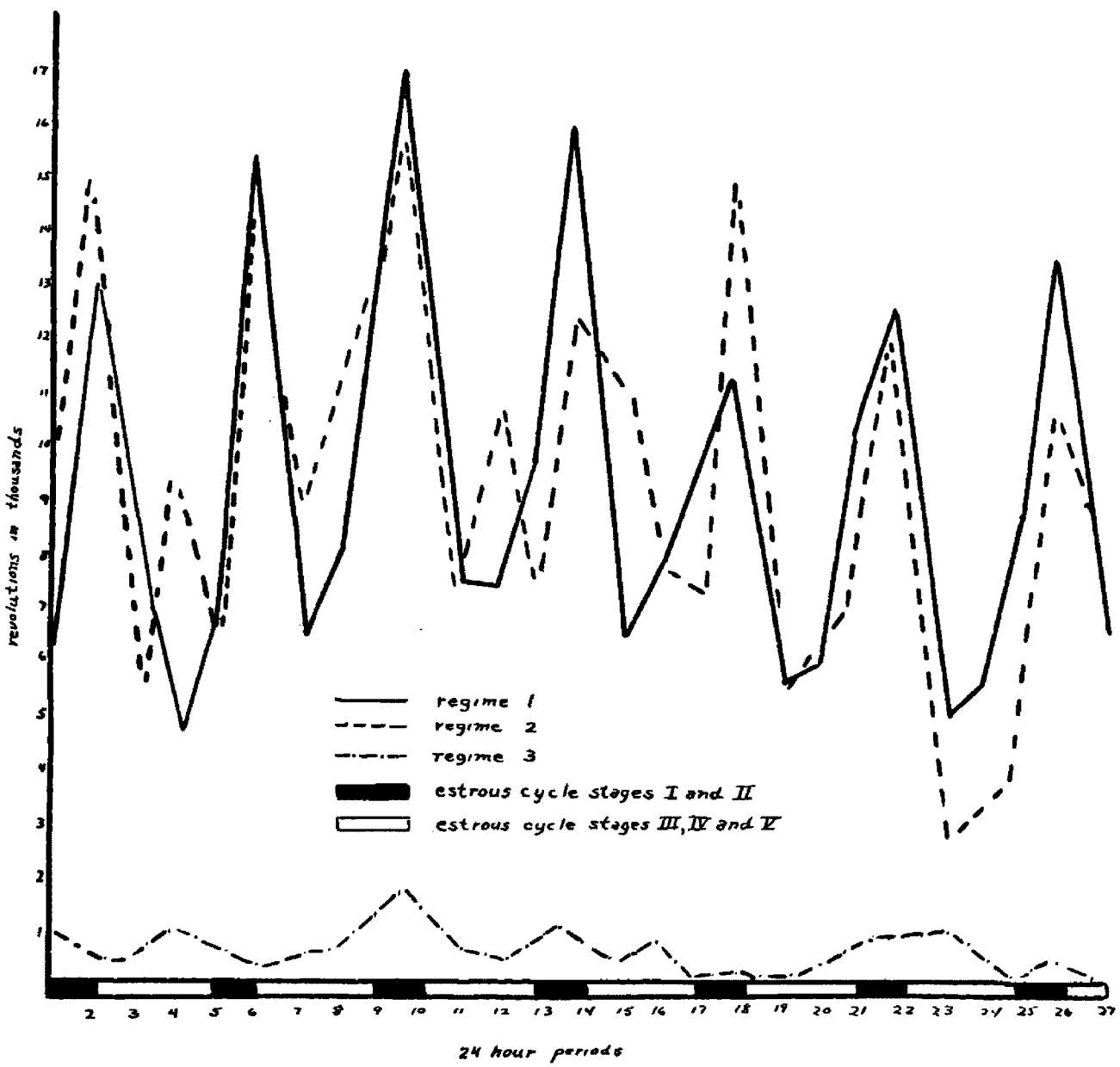
TABLE 12

COMPARISON OF COMBINED MEAN BLOOD SUGAR VALUES REGARDLESS OF TIME OF DAY TAKEN OR REGIME

Stage	Number of Samples	Mean mg %	Range	S.D.	Combined Samples Total Samples	Combined Samples Mean mg %	Combined Samples Range mg %	Combined Samples S.D.	S.E.	Mean Diff	Sig
I	36	104.6	91-116	4.699	121	104.5	86-119	5.786			
II	89	104.5	86-119	5.759							
									0.211	1.1	Yes
III	39	103.4	89-115	4.697							
IV	74	103.8	88-119	6.326	214	103.4	88-120	5.979			
V	99	103.1	94-120	6.066							

FIGURE 1

ACTIVITY CYCLES OF THREE TYPICAL RATS ONE EACH FROM REGIMES 1, 2 AND 3



Discussion

It can be seen from the results that the five stages of the estrous cycle were divided into two groups; namely, one group including the Long-Evans ('22) stages I and II, and the other including the stages III, IV and V. The reasons for combining the stages were the rather obvious similarities of the means within the two groups and the fact that although differences were observable between the estrous cycle stages, they were of too small a magnitude to exhibit significance in the limited number of samples obtained in the experimental period. The division into two groups is not necessarily an arbitrary nor artificial one because we can see similarities in the activity pattern as well as in mean blood sugar values.

Activity, which is a manifestation of internal changes has been shown by many authors (Richter, '27; Shirley, '28; Slonaker, '24; Wang, '23) and by the activity graph (Graph 1) to be in ascendance and at its peak during stages I and II and to be declining and at its ebb in stages III, IV and V. Correlated with activity we find in the ovary itself preparation for ovulation in stages I and II and ovulatory-post-ovulatory changes in stages III, IV and V (Boutwell et al, '48; Hisaw et al, '34; Long and Evans, '22). In the uterus we find vascular engorgement, and marked distention in stages I and II. In stage III the uterine

fluid disappears and the state of distention is replaced by one of flaccidness. The latter conditions are maintained through stages IV and V (Long and Evans, '22).

It is extremely interesting to consider the blood sugar values for the various stages of the estrous cycle while keeping in mind the concomitant secretory phases of the ovary. The lowest mean glycemic value (103.4 mg percent) occured during combined stages III, IV and V at the time when the corpus luteum hormone, progesterone, was at or reaching its highest titers. The higher mean glycemic value (104.5 mg percent) occured during stages I and II while estrogen was reaching its maximum titers (Hisaw et al, '34). The interest in this is rather obvious if one refers to the work of Gaunt et al ('39) who found that injection of progesterone in ferrets elevated both liver glycogen and blood sugar. These experimental results, however, did not hold true for rats.

Inductive reasoning would lead to the conclusion that if rats did have a period of ovarian influenced high blood sugars it would occur at the time of high progesterone titers. Further support for this hypothesis accrues from the work of Foglia et al ('47) in their work with 95% pancreatectomized animals. They found that estradiol exerted a protective effect against diabetes incidence. They also noted a sex difference in cases of

diabetes, with females being less susceptible than males. Lewis et al ('50) in a continuation of the above study found in male and female castrates, 95% pancreatectomized, that estrogens reduced the number of diabetic cases in both populations; progesterone and desoxycorticosterone did not modify the number of incidences; and that androgens increased the susceptibility to diabetes.

In my results, the speculation that the highest blood sugar titers would occur during the progesterone secretory phase of the ovary did not hold true; in fact the converse of this situation was found to occur. The differences between the mean blood sugars were not great, but were rather constant. The highest values were found in the reproductive cycle phase where estrogen was dominant. The only support for these findings that the literature would seem to offer comes from the basal metabolic rate determinations of Lee ('28) in which he found increased metabolic rates in the period of corpus luteum degeneration. He suggested the possibility that the corpus luteum might have an anabolic function, and that upon removal of its influence increased catabolic activity occurs.

The possibility of some other intrinsic factor giving rise to a cyclic variation in blood sugar readings, which might mistakenly be attributed to the ovarian cycle was

recognized. To partially guard against this error the eight oophorectomized animals, of regime 3, were employed. These animals were allowed to feed ad libitum, and they were maintained under a 12 hour day-12 hour night schedule, which corresponded to the solar day. A blood sugar cycle whose duration coincided with the estrous cycle was not observed. The glycemic readings generally paralleled those of the normal rats of regime 1, which lived under the same environmental conditions. It should be noted, however, that the mean blood sugar values consistently exceeded those of the normal females by approximately 1.5 mg percent. The weight gain in the oophorectomized animals also exceeded that of the normals, and the former were much more lethargic than the latter. The relative inactivity of the ovariectomized animal has been noted by Richter ('27), and is borne out by the present work as may be seen in the activity graph (Graph 1). The higher readings might be explained by lowered tissue consumption in the absence of stimulating hormones, or by erratic increased food consumption. If the readings are true, and the glycemic levels are increased in oophorectomized animals, then room for conjecture arises. Speculation on the role of the ovary would seem to present at least three possible alternatives, one direct and two indirect. The hormones of the ovary might effect the glycemic levels <u>per se</u>, that

is as direct controlling agents. The blood sugar levels may be indirectly altered by ovarian induced activity, or conversely by inactivity. The third alternative, again indirect, is that the ovarian hormones might exert influence on the organs which we normally think of as controlling blood sugar levels; namely, the liver, pancreas and adrenals. In this connection it should again be noted that Folgia *et al* ('47) found that castration sensitizes 95% pancreatectomized females to the incidence of diabetes, and that estradiol has an ameliorating effect.

The results, as they were interpreted, also showed significant differences due to feeding regimes. The experimental conditions were set up taking cognizance of the fact that rats normally feed during the evening hours. The maximum mean blood sugar titers, as well as liver glycogen deposition, usually occurs 10 to 12 hours after feeding (Boutwell *et al*, '48; Pitts, '43). It was thought that any evidence for possible ovarian control of blood sugar values would be inconclusive without altering some of the other factors influencing control. For this reason the feeding time, in some of the experimentals (regime 2), was shifted to the morning hours so that the maximum blood sugar values, food induced, would be advanced to early evening instead of midnight (Pitts, '43). The diurnal

blood sugar cycle, and probably the liver glycogen cycle, must have been shifted in regime 2. A significant difference was found when 8 A.M. and 1 P.M. mean values were compared with 8 P.M. mean values in rats fed from 8 A.M. to 1 P.M. only (Table 5).

The rats in regime 4, that is the animals with a day-night environment the reverse of the solar day-night, were employed to see whether or not a reversal of the feeding hours could be effected, as indicated by blood sugar, when food was available constantly. This situation, if it prevailed, would be expected to cause the highest food induced blood sugar values to occur at the artificial midnight (solar noon). This expected situation did not occur. In general the readings were spread over the entire range for this group. This is rather difficult to explain in that the rats used were rather young.

Summary

A total of 56 nulliparous female rats of approximately the same age were used in this series of experiments, the object of which was to see whether or not a correlation existed between the estrous cycle and blood sugar values. Tail blood was collected from an individual every three days and the sample was analyzed by the Folin-Malmros ('33) colorometric blood sugar method. Data were kept so that they could be analyzed from either the time of day the sample was taken, or by the stage of the estrous cycle in which it occured.

The animals were all housed separately, fed the same type of food and allowed free access to water. The individual rats, after being acclimatized for fifteen days, were each subjected to experimental procedures for a total of thirty days. The 56 rats were divided into 4 groups to facilitate the study.

A total of 24 animals comprised group 1. The animals in this group were fed ad libitum, and were on a 12 hour day-12 hour night schedule corresponding to the solar day. Blood sugar readings from this group, when analyzed by the time of day they were taken showed no significant differences in mean blood sugar values.

The second group of rats, 16 individuals, were only

allowed to feed from 8 A.M. to 1 P.M. This group was also on a controlled 12 hour day-12 hour night. The analysis, when made without regard for the estrous cycle stage, showed significant differences in mean blood sugar values between 8 A.M. and 8 P.M., and between 1 P.M. and 8 P.M. A significant difference was not found when 8 A.M. and 1 P.M. readings were compared.

The rats making up the third group were eight ovariectomized females. They experienced the same environmental conditions as the first group and like the first group, did not exhibit significant differences in mean blood sugar values when these values were analyzed by the time of day they were obtained. The mean blood sugar values for these rats were slightly higher than those encountered in normal rats.

Group 4 consisted of 8 normal females, which were allowed to feed ad libitum. Their environment differed from the environment of group 1 in that they lived in a reversed day-night situation. Significant differences based on blood sugar sampling times were not evident.

The rats from groups 1, 2 and 4 were combined to investigate blood sugars from the viewpoint of a correlation with the estrous cycle. Readings from estrous cycle stages I and II were combined and analyzed by comparison with

readings from estrous cycle stages III, IV and V. The comparison showed a highly significant difference, with the highest mean blood sugar readings occuring in the combined stages I and II.

Conclusions

1. Mean blood sugars, from restrictedly fed animals, show a significant increase 10 to 12 hours after feeding is initiated.

2. Rats fed ad libitum do not exhibit significant changes in blood sugar values during the daylight hours under the conditions described in this experiment.

3. There appears to be a correlation between the estrous cycle and mean blood sugar values. The higher mean value occurs in the combined estrous cycle stages I and II, while the lower mean blood sugar value occurs in combined stages III, IV and V.

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