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**MITOTIC ACTIVITY IN THE HUMAN EPIDERMIS**

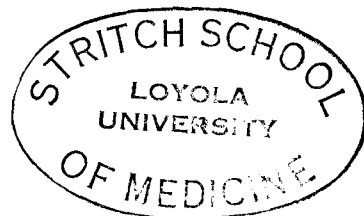
by

**LAWRENCE EINAR SCHEVING**

**A Dissertation Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of  
the Requirements for the Degree of  
Doctor of Philosophy**

**February**

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## LIFE

Lawrence E. Scheving was born in Hensel, North Dakota, October 20, 1920.

He was graduated from Hensel Public High School, Hensel, North Dakota in June 1938. From 1938 to 1940 he attended Askers Business College, Grand Forks, North Dakota. From 1940 to 1946 he was a member of the United States Armed Forces. In 1946 he began undergraduate work at De Paul University and received the Bachelor of Science Degree from there in February 1949. He also received the Master of Science degree from De Paul University in June 1950. He began graduate studies in the Department of Anatomy of Loyola University in June 1951.

From 1950 to the present he has taught in the Department of Biological Science at Lewis College of Science and Technology, Lockport, Illinois.

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To Dr. Arthur J. Gatz, under whose suggestion this problem was undertaken, I wish to express my indebtedness and to sincerely thank him for his constructive supervision and untiring assistance.

I am deeply grateful to the donors and to Dr. Donal O'Sullivan who performed all of the biopsies.



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## MITOTIC ACTIVITY IN THE HUMAN EPIDERMIS

Three viewpoints currently exist regarding the replacement mechanism of desquamated skin. They are; (a) mitotic division of pre-existing cells, (b) amitotic division of epidermal cells and (c) migration and morphological transformation of lymphocytes and/or other types of mesodermal cells into epithelial cells.

This research was undertaken to investigate this problem in greater detail than has been done up to the present time with the view of establishing which method, if any, offers the most feasible explanation. The small number of mitotic figures which have been observed on the usual histological preparation has led many workers to assume that mitotic replacement could not maintain the epidermal layer. Mitotic rhythmicity has been observed in plants and animals. We felt that this might also occur in the human epidermis.

We also observed the numbers of lymphocytes in the epidermis since they, or other types of mesodermal cells, have been suggested to replace the desquamating epidermal cells.

## HISTORY

To give some idea of how many mitotic figures have been counted in adequately preserved material we can cite the work of Thuringer (1924 and 1928) who counted one mitosis for 2,414 cells on the scalp, for 268,275 cells

on the ear, and for 378,325 cells on the leg. Pinkus in 1953 while reviewing work done on this subject prepared a table in the form of mitotic indices (mitoses counted per thousand cells). In this summary, which is a report on the results of eight workers, it is noted that the mitotic index of various regions of the human epidermis is considerably higher than that reported by Thuringer. In the table most of the indices for the adult human epidermis range from 1.6 to 0.1.

The rate of cellular desquamation for the normal human skin has been studied by only one worker. Sutton (1938) applied silver nitrate to the surface of various parts of his body. He found that a period of seven to eleven days elapsed before the stained area was worn off. He concluded that this was the time needed for renewal of the stratum corneum, which he estimated to consist of eight cell layers.

A number of investigators have postulated that the epidermal cells have a mesodermal origin, or a source other than epithelial. Frieboes (1920) considered the epidermis as a syncytium without cell boundaries which is permeated by tonofibrillae systems originating in specialized mother-cells of mesodermal character. In 1928, Bestrom maintained that the epidermis was continually renewed by conversion of connective-tissue cells into epithelial cells. As recently as 1950 a somewhat similar view was proposed by Levander. Cameron (1936) stated that "spindle-shaped" mesodermal cells, migrated into the epidermis of both normal and X-rayed skin. These subsequently differentiated into epithelial cells. Subjecting the animals to X-rays decreased the already low numbers of mitotic figures without changing

the epidermal thickness. However, an increase in the mesodermal cells beneath the epidermis was observed which Cameron postulated would eventually migrate into the epidermis.

Andrew and Andrew in 1949 introduced the hypothesis that a transformation of lymphocytes into epithelial cells occurred in the human epidermis. The migrating lymphocytes became transformed into "clear cells", which later differentiated into the epidermal cells. All of the above viewpoints were perhaps prompted by the supposedly insufficient rate of epidermal mitosis.

The second possibility to account for the paucity of mitotic figures would be explained by the fact that mitotic activity occurs in "bursts" or that there is a periodic rhythm.

Rhythmic mitotic cycles were first investigated in plants. Kellcott, in 1904, found mitotic activity to be at its maximum height at 11:00 P.M. in the onion root tip, and that the least amount of activity was seen at 7:00 A.M. Karsten (1918) reported that the greatest amount of mitotic activity in Spirogyra was at 12:00 midnight. For Zea mais mitotic activity reached a maximum during the night with the least amount of activity during the day. In 1921 Stalfelt reported maximum activity for cellular division in the Pisum sativum cells as occurring at 9:00 to 11:00 A.M. with a minimum amount from 9:00 to 11:00 P.M. It is generally recognized, from the above work and also from numerous other investigations, that a periodic mitotic rhythm does exist in the cell division of the organs of many plants.

Fortuyn-van Leyden (1916 and 1926) was the first investigator to report this phenomenon in animal tissues. Using six young kittens, sacrificed at different hours during the day, she concluded that the greatest amount of mitotic activity in the mesenteries, thymus, spleen, and lymph node was essentially at 2:30 A.M. and the least amount at 2:30 P.M. However, the maximum number of mitoses occurred between 6:30 and 12:30 P.M. in the crypts of Lieberkuhn of the small intestine. The minimum number occurred at 10:30 A.M. On the contrary when using mice instead of cats, she found the maximum mitotic activity in the intestinal glands to occur at 11:00 A.M. The minimum activity was at 3:00 A.M. It is evident that the maxima and minima varied for the two animals, and to a lesser extent for the different tissues of the same animal.

A number of investigators have studied the mitotic activity of the mouse epidermis. Ortiz-Picon, in 1934, found the height of mitotic activity to be at 12:00 noon with lowest activity at 12:00 P.M. In 1940, Cooper and Franklin were generally in agreement as was Bullough, in 1948, when they reported highest activity between 10:00 A.M. and 4:00 P.M. with a peak at approximately 1:00 P.M. Carleton, in 1934, was not in agreement with the above as she contended the highest activity occurred between 8:00 P.M. and 12:00 A.M., being lowest at 12:00 noon. The above results would indicate that considerable variation has been observed on the same tissue by different workers.

Another group working on the epidermis of the rat, notably, Blumenfeld (1939), Babick (1951) and Halberg and co-workers (1954), observed that

the peak of cellular division occurred at approximately 9:00 A.M. with lowest activity at night.

Cooper and Schiff, 1938, reported mitotic activity to be highest at 9:00 P.M. and lowest at 10:00 A.M. in the epidermal portion of the human infant prepuce. Broders and Dublin in 1939 concluded that cellular division in foreskins of new-born human beings was approximately twice as frequent at night as in the daytime. Thirteen and twelve specimens respectively were used for these observations!

A further point of interest was indicated by the fact that the mitotic rhythm varied from organ to organ within the same animal or plant. For example Blumenfeld, (1942), subsequent to his investigation of the epidermis, made a comparative study of the rate of mitotic activity in two additional organs, the renal cortex and the submaxillary gland. In the renal cortex the maximum frequency was during the afternoon and in the submaxillary gland activity was fairly constant during the day and night with the exception of a sharp decline between 2:00 and 4:00 A.M. This of course would indicate different rhythms in different organs. On the contrary, Bullough (1948) in his comparative study of different organs specifically the esophagus, epididymis and duodenal mucosa found that they generally agreed with his findings for mitotic activity in the skin. Milletti (1950), however, reported a definite diurnal rhythm in the multiplication of cells in the bone marrow from the femur of the mouse. The peak of this activity was at 4:00 A.M. with the minimal divisions occurring at mid-day.

Elliott in 1936 found no evidence of mitotic rhythmicity in immature or mature cartilage of cats, dogs, rabbits, mice or rats. This may have been due to the lack of material.

#### MATERIALS AND METHODS

Samples of apparently healthy skin were collected in two ways, first, by surgical procedure from patients undergoing surgery for one reason or another and secondly by biopsy from volunteers. Five hundred samples of skin were collected by the first method and one hundred and eighty by the second. Originally it was planned to utilize the tissue collected by the two methods but later this was abandoned because the samples collected by surgical procedure from persons undergoing surgery were concentrated during the morning hours when most surgery is naturally performed. Too few specimens from night hours were available. The tissue from volunteers was more equally distributed over day and night periods. Furthermore, the tissue collected by the biopsy procedure gave us tissue from healthy, young men of an average age of approximately twenty. In twelve instances the same volunteer subjected himself for biopsy at two or three additional times during the twenty-four hour period.

The volunteers for skin biopsies were scheduled to appear in groups of ten at all hours during the day and evening, except for the hours of 6:00 and 7:00 A.M. The skin was removed from either the right or left shoulder of the volunteer by Dr. O'Sullivan, using a 4 mm skin biopsy punch. (Latter was supplied through the courtesy of Dr. Cleveland J. White.) The tissue sample

was placed in Bouin's fixative for at least twenty-four hours. Dehydration was accomplished by the usual alcohol procedure, it was then cleared in xylol and embedded in paraffin.

Two millimeter strips of the entire depth of the epidermis were carefully measured on the slides made from each sample. All the cells in approximately four millimeters of tissue from each sample were counted in this fashion. All the cells counted, which usually totaled 2,500 to 3,000 for the four millimeter strip, were classified into lymphocytes, clear cells, chromosomal, reconstruction and "resting" or metabolic cells. The measurements of each of these two millimeter strips were made near the margin of the section in order to avoid picking areas indiscriminately from the section. Each of the two counted areas mentioned above as a consequence were widely separated. An average of 9.2 biopsies per hour from 8:00 A.M. through 5:00 A.M. were studied in this project.

Experimental results were subjected to statistical analysis by known reliable methods.

The arithmetical mean by the formula  $\bar{d} = \frac{\sum d}{n}$

where d = the variate or individual sample

where  $\bar{d}$  = the mean of the variable or total of the individual samples for the period divided by n

where n = the number of variates within the variable

The standard deviation by the formula:  $S = \sqrt{\frac{\sum (d - \bar{d})^2}{n(n-1)}}$



The significance of differences were determined according to the method of Fisher by the following expanded formula:

$$t = \frac{(\bar{d} - \bar{y})}{\sqrt{\frac{\sum(d - \bar{d})^2 - \frac{(\sum d)^2}{N_x - N_y - 2}}{N_x - N_y - 2}}} \sqrt{\frac{N_x N_y}{N_x + N_y}}$$

where  $\bar{d}$  = the arithmetical mean of the experimental variable

where  $\bar{y}$  = the arithmetical mean of the control variable

where  $(d - \bar{d})$  = the sum of the extent to which the variates differ from their mean value in relation to the experimental

where  $(y - \bar{y})$  = the same as  $(d - \bar{d})$  except that it pertains to the control

where  $N_x$  = the number in the control variable

where  $N_y$  = the number in the experimental variable

The probability or P values were taken from a standard table based on the value of t and the number of variates.

### EXPERIMENTAL RESULTS

General observation of the epidermal sections showed a considerable variation in the morphology of the epidermal cells. The majority of the cells, peculiar to each layer, were readily identified as the metabolic or "resting cell" stage.

The epidermis was composed of two layers: the stratum germinativum (stratum mucosum) above the dermis and resting on a basement membrane, often called the living component; and the superficial layer, the cornified stratum corneum, commonly referred to as consisting of non-living cells (Figure 8).

The stratum germinativum (or mucosum) ordinarily consisted of three zones. The stratum basale consisted of a single proximal layer of cells which had basal processes embedded in the substance of the basement membrane. The latter membrane demarcated the dermis and epidermis (Figure 9). The stratum spinosum, or "prickle cell layer", was superimposed upon the basal layer. This layer ordinarily had a depth of three to five cells. The identification of this layer was simplified by the abundance of the intercellular bridges between the cells of this zone (Figure 10). The stratum granulosum was the superficial layer of the mucosum. This zone, which was one cell layer in thickness, was readily identified by the basophilic keratohyalin granules which appeared in the cytoplasm (Figure 10).

The stratum corneum of thin skin ordinarily consisted of the superficial desiccated cells of the epidermis. The cells, which are flattened and cornified, had frequently lost a portion of their attachment to the epithelial layer. Cells of this type ordinarily are classified as the scaly layer (Figure 8). The flattened cell layer may be differentiated from the previous area by the fact that the flattened cornified cells were maintained in a relatively smooth layer.

The cells of the basal layer were usually cuboidal or fusiform, sometimes columnar. Those of the stratum spinosum were polyhedral and

cylindrical but became increasingly flattened as the granulosum was approached (Figures 10 and 11). The metabolic cells of the basale and spinosum layers were generally characterized by an essentially finely granular and lightly stained cytoplasm. Tonofibrils were present, especially in the spinosum. Varying amounts of pigment were evident in the stratum basale and occasionally in the stratum spinosum (Figure 12). Supranuclear caps were frequently observed when pigment was not abundant (Figure 10). Connecting the individual cells of the "prickle layer" were cytoplasmic processes which are commonly referred to as intercellular bridges (Figure 10). The "resting cells" of the granulosum, varied in quantity, were flattened and angular and contained coarse basophilic keratohyalin granules. They possessed ill-defined nuclei and intercellular bridges as both were gradually becoming obliterated (Figure 10).

The nuclei of the basale and spinosum strata were usually vesicular in appearance. The nuclei of the basal zone were smaller and more elongated than those which appeared in the spinosum, which were usually spherical to oval (Figure 11). The nuclei of both layers were characterized by a prominent nuclear membrane, clearly differentiating it from the cytosome. Usually two strongly basophilic nucleoli were present, very often located at opposite poles of the nucleus. Multiform karyosomes situated among the finer granules of chromatin were observed (Figure 10).

The epidermal cells in various stages of mitotic activity varied considerably from the typical metabolic cells described above. The cells which showed chromosomes were readily identified as undergoing mitosis. It

was possible to identify prophase, metaphase, anaphase and telophase stages (Figures 10, 11, 12, 13, 14, and 16). These cells have a clear area about the chromosome mass, which can also be observed previous to the advent of chromosomes as well as after their dissolution in the reconstruction phase. Those which showed the above stages are classified as the "chromosomal stages" in the tables.

A second variation in the morphology of the dividing epidermal cell was apparent. These cells were the earlier prophase stages in the process of mitotic division. Typically they exhibited a more condensed, deeply stained, or sometimes fibrillar nucleus which was well demarcated by a clear area of the cytoplasm (Figures 15 and 17). Immediately about the diaphanous central cytoplasm was a thin layer of finely granular cytoplasm which appeared to join the adjacent cells with numerous intercellular bridges. These are the cells which have been designated as the "clear cells", in the tables.

Still another modification was the appearance of cells which were seen in duplicate. These were continuous along one margin or were separated by a fine plasma membrane which did not show any evidence, at least in the early stages, of intercellular bridges. All other margins of these cells showed numerous fine cytoplasmic bridges which joined adjacent cells (Figure 17). The nucleus was variable in structure appearing as a dense basophilic mass, rather large reticulated chromatin clumps, or approaching what could be termed a vesicular nucleus. A clear or diaphanous zone was still apparent about the nucleus in the earlier stages of this phase. These cells were classified as the "reconstruction cells".

The possibility of confusion between "clear cells" and "reconstruction cells", because of their morphological similarity at certain stages, exists (Figure 17). However, this was minimized by establishing the criterion that "clear cells" were obviously single elements, whereas all cells classified as reconstructing were present in duplicate. It was still possible to mistakenly identify one member of a reconstruction pair for a "clear cell" if the other member was present in a different plane and could not be seen.

"Clear cells" were not numerous during the day (Figures 1 and 4; Table I), with the exception of a 2:00 P.M. deviation at which time  $4.46 \pm 0.81$  percent of all cells counted were classified in this category. The second peak of activity occurred at 12:00 midnight and showed  $4.49 \pm 0.49$  percent of the cells in this stage. The peak of "clear cell" activity usually preceded the peak of the "chromosomal stage" (Figure 4).

It is apparent (Figures 2 and 4; Table I) that the "chromosomal stages" of activity were infrequent from 8:00 A.M. to 10:00 P.M., with the exception of a small increase at 4:00 P.M. At this time  $2.89 \pm 0.12$  percent of the cells was observed in the "chromosomal stages". The pronounced increase began to occur at 10:00 P.M., and reached a summit at 12:00 midnight with  $6.05 \pm 0.33$  percent of all cells in the typical stages (prophase, metaphase, anaphase and telophase) of mitotic activity until 5:00 A.M. when the daily average activity was again approached.

The maximum increases in the "reconstruction cells" were attained approximately three hours after the primary (12:00 midnight) and secondary (4:00 P.M.) "chromosomal stages". At 7:00 P.M. it may be noted that

$3.29 \pm 0.48$  percent of all cells were in this phase. At three o'clock in the morning  $8.32 \pm 0.91$  percent of the cells observed were classified in this category (Figures 3 and 4; Table I).

The data indicated that the primary was approximately twice the magnitude of the secondary cycle. (Figure 7). The same relationship can also be observed for the 7:00 P.M. and the 3:00 A.M. reconstruction peaks (Figure 4). This would indicate that the accumulation of "reconstructing cells", after each peak of mitotic activity, is rather constant.

The time necessary for the complete cycle of cell division, from the advent of the "clear cell" to the appearance of the "reconstruction cells", was estimated from the evidence obtained. Figure 4 indicates that "clear cell" activity showed a gradual progressive increase at 5:00 P.M. Two hours subsequent to the beginning of this increase a similar phenomenon was evident for the "chromosomal phases". At 9:00 P.M. the beginning of an increment in "reconstruction cells" may be observed. This would indicate a lapse of two hours from the inception of "clear cell" activity to the advent of "chromosomal stages" and two additional hours for the advent of the reconstruction increase or a total of four hours from the beginning of the first phase (clear cell) to the beginning of the third phase (reconstruction cells).

Further evidence to support this is manifested by the "clear cell" rise seen at 2:00 P.M., followed two hours later (4:00 P.M.) by the secondary "chromosomal phase" cycle and still three hours later (7:00 P.M.) a peak of "reconstruction cell" activity is attained (Figure 4). The time intervening between the 2:00 P.M. "clear cell" peak and the 7:00 P.M. reconstruction peak

was five hours. However, the time lapse between the beginning of the increase in "clear cells" at 2:00 P.M. to the beginning of the 6:00 P.M. increase in "reconstruction cells" was again four hours.

The 12:00 P.M. (midnight) peak in "chromosomal phase" activity (Figure 4) is not preceded by a two hour previous "clear cell" predecessor but it does have a reconstruction peak following three hours later as was seen during the day. The time elapsing between the beginning of the sharp rise in "chromosomal cells" at 10:00 P.M. and the beginning of the rise in "reconstruction cells" at 12:00 P.M. is again two hours.

It is postulated from the above evidence that the total time required for the complete cycle of cell division is from four to five hours.

The data, thus far examined, has considered the fluctuations of the categories into which the process of cell division has been divided; for example "clear", "chromosomal" and "reconstructing cells". By combining the data for each of the above categories the complete mitotic activity for any given hour may be obtained.

The major and minor peaks of mitosis which have been referred to previously again are apparent (Figure 7). In this instance one cannot only observe the increased activity but also the period of time over which the increased activity extends. The minor cycle has a duration of three hours and represented an increase of 100 percent over the average daily mitotic activity. The major cycle, which represents an increased activity of almost 200 percent, extends over a period of six hours.

The initial increase in the major and minor period is due to increased numbers of cells in early stages of division - the "clear cells" (Figures 1 and 4). The maintenance of peak of activity is associated with the increased numbers of cells in "chromosomal stages" (Figures 2 and 4). The peak of the period of reconstruction of the daughter cells (Figures 3 and 4) was primarily responsible for the maintenance of the last portion of the cycle.

Another interesting and significant phenomena was the localization of cellular divisions into what were designated as "nests". These were especially prevalent during the periods of great activity. It was not uncommon to find relatively inactive, in so far as cellular division was concerned, adjacent regions for quite some distance from these active regions. In general, however, various stages of division were very easy to recognize, though not as numerous as in the "nest areas", throughout the epidermis during peaks of activity. This is perhaps the same phenomenon described by Flemming in 1884 while working on the epidermis of adult rabbits, guinea pigs and cats, when he described the occurrence of cells growing "schubweise", for example, in shifts. It is also quite probable that this "nest effect" is the same idea which Thüringer (1928) described while working on epidermis of the prepuce. He stated, "It was possible to count as many as ten to fourteen figures under high power ( X 400 ) in a single field through the center of a "growth wave", while toward the periphery of these proliferation centers the number of mitotic figures would taper off to zero."

Cells undergoing division were not confined to the basal layer, as was once thought, but were scattered throughout the stratum germinativum,



predominating in the middle and lower regions of the stratum spinosum and basal layers. During periods of relative inactivity it was observed that the majority of the few dividing cells were confined to the basal layer.

There was no evidence of lymphocyte or of any other mesodermal elements transforming into epithelial cells. Lymphocytes, usually confined to the basal layer, constituted less than one percent of all cells in the epidermis. In only one instance was a rate as high as four percent observed. They did resemble the "clear cell" in that the nuclei of both were frequently dark and appeared pycnotic. The nucleus of the lymphocyte was surrounded by an agranular, clear cytoplasm. The epithelial "clear cell", however, showed the presence of an additional rim of finely granular cytoplasm which was continuous with adjacent cells by means of numerous intercellular bridges. There was also a size variation, lymphocytes being  $1/3$  to  $1/4$  the diameter of the epithelial "clear cell".

There appeared to be a minor and major peak of cell division. In order to determine whether the same individual followed the rhythmic cycle of cell division or not, second and third biopsies were subsequently taken at variable periods during the day. The results of this study were tabulated in Table II. The cell multiplication in practically all instances paralleled that of the overall activity obtained for the specific hourly period. For example if the hour, from which the biopsy was obtained, was rather inactive for the group the same would hold true for the individual and vice versa. The difference in the mitotic cell counts was frequently insignificant.

## DISCUSSION

The traditional concept of indirect cell-division (mitosis) which includes the prophase, metaphase, anaphase and telophase stages has a definite pedagogical value. However it does not emphasize the cytoplasmic modifications which precede, accompany and follow the chromosomal divisions. Observations in this laboratory on the human epidermis, as well as on tissues derived from various other sources, show a "halo" (or clear area) developing about the nucleus in the early phases of indirect cell division. As the karyosomal changes progress through the spireme to the "chromosomal stages", the clear area surrounding the nucleus becomes larger and more distinct. Therefore in our study, the cells, which are classified as "clear cells", represent the early stages of the prophase.

Figure 18 illustrates the clear zone effect in the epidermis of the *Amblystoma* larva. Here a mitotic figure (metaphase) is seen surrounded by a clear zone. It can be postulated that the clear zone is necessitated by the fact that there will be turbulent nuclear reorganization and it would only seem reasonable that some changes in the cytoplasmic viscosity would precede karyokinesis. Sharpe (1934) states that cytoplasmic viscosity varies greatly at different stages of cell-division and differentiation. It is believed that the clearing effect can be observed, even more clearly, when viewing rapid motion pictures taken of mitosis from tissue culture.

Statistical analysis offers evidence to support the validity of the experimental results. The P values for "clear cell" activity during each of the hours, 11:00 P.M., 12:00 midnight, and 1:00 A.M., was .01, which is very significant (Figure 5). A daily average, of 1.68 percent, which was used as the control in the calculation, was derived from the hours of 2:00 A.M. through 10:00 P.M. The least amount of activity was observed at 1:00 P.M. with a  $\bar{d}$  value of 0.40 (Table I). Another analysis relating to the 2:00 P.M. increment in "clear cell" activity manifested a P value of .02 (Figure 6). The daily average, of 1.53 percent, was obtained from the hours of 3:00 P.M. through 1:00 P.M., exclusive of the 11:00 P.M., 12:00 midnight, and 1:00 A.M. hours.

The "chromosomal cell" peaks at 11:00 P.M., 12:00 midnight, and 1:00 A.M., were calculated against a daily arithmetical mean of 1.44 obtained from the same hours as the aforementioned "clear cells", revealed a P value of .05, .01 and .01 respectively (Figure 5). The smallest  $\bar{d}$  value for any one hour in the control was 0.26 (Table I). The secondary mitotic cycle at 4:00 P.M. possessed a P value of .01 (Figure 6). This was calculated against a control with an arithmetical mean of 1.36 obtained from the hours of 5:00 P.M. through 3:00 P.M., exclusive of the peak hours of 11:00 P.M., 12:00 midnight, and 1:00 A.M.

Another significant P value of .01 was obtained for both the 2:00 A.M. and 3:00 A.M. reconstruction increases in activity (Figure 5). The daily average with an arithmetical mean of 2.42 was obtained from the hours between 4:00 A.M. through 1:00 A.M. The smallest  $\bar{d}$  value was seen at 1:00 P.M. and

had a value of 0.51. The 7:00 P.M. increase in reconstruction activity had a P value of 0.2 (Figure 6). This was calculated against a daily average with an arithmetical mean of 2.42 obtained from the hours 8:00 A.M. through 5:00 A.M., exclusive of the 2:00 A.M. and 3:00 A.M. peak hours.

The results of the calculations of the standard deviation are best explained in Table I. The variation that does exist seems to be associated with the degree of activity. Whenever considerable activity occurs the standard deviation is a little higher. This could be due to the difficulties encountered in identification. The data which has been classified as "chromosomal cells" revealed the most significant results, and likewise were the least variable for the specified periods. This may be explained by the fact that the cells containing chromosomes were capable of positive identification whereas the "clear" and "reconstruction phases" could present classification difficulties. For example a cell identified as a "clear cell" could conceivably be a member of a reconstructing pair with the other member missing because of its position in another plane or section. The inference would seem to follow that variation in the "chromosomal cells" would perhaps resemble more closely biological variation whereas the "clear" and "reconstruction cells" would be biological plus the larger error of identification. It must further be noted that the "nest" effect would be conducive to variation as the two mm strips were measured, with no attempt to take into consideration the number of "nests" in the particular strip. If the number of "nests" in all strips were equal then there would be less chance for variation. It is concluded that the standard deviation is quite low when all factors which

could induce error are considered.

It is apparent that there are two peaks of activity insofar as "chromosome stages" are concerned. One occurred during the day at 4:00 P.M. (minor cycle) and the other at 12:00 midnight (major cycle). The major cycle has approximately twice the magnitude of that seen during the minor cycle. The same for the most part, is true of the clear and reconstruction activity. When all three types of cells are combined we notice that the minor cycle had a duration of three hours and shows an increase of 100 percent in mitotic activity over the daily average. The major cycle, which represents an increase in activity of almost 200 percent, extended over a period of six hours. It is obvious that the majority of the activity takes place at night, which Broders and Dublin, as well as Cooper and Schiff, had observed from their sparse sampling. This, of course, is just the opposite to that of the epidermis of the rat or mouse.

Our evidence indicates that the epidermal replacement mechanism is mitotic activity and it further explains why the impression of a relative lack of mitotic figures has been stated in the literature. It is doubtful that much tissue was taken from individuals for routine examination during the hours of maximum mitotic activity. It is more probable that the majority was taken during the late morning hours when activity was at its lowest ebb.

It is not possible, at this time, to offer any explanation as to why a diurnal periodicity exists. Certainly it is safe to assume that any study of physiological factors which are considered to be mitogenic or of mitotic indices, would necessarily be more valid if this periodicity were

given consideration. Several physiological factors have been investigated by Bullough (1948) in relation to the mitotic periodicity in mice. He reported an increase in epidermal glycogen content during sleep, when the mitotic rate increases. With the onset of sleep glucose is deposited from the blood into the tissues where it appears in the form of glycogen. On awakening the reverse process takes place. He considers this glucose or glycogen to be the critical substance affecting mitotic activity in the adult mouse. Bullough further stated that there seemed to be no reason to doubt that the same would be equally applicable to man. If this is the case in man it is not the onset of sleep itself that is responsible for the deposition of the glucose into the epidermis because the majority of the night volunteers had not slept prior to presenting themselves for biopsy. This was especially true for those subjecting themselves to biopsy during the peak period of activity. Bullough also reported that excessive muscular exercise is followed by an abnormal depression in mitotic rate. In this study the evening volunteers were for the most part more active prior to giving tissue than they were during the earlier hours of the day as many of them engaged in recreation in the nearby gymnasium, as they awaited their turn, while during the day the majority spent their time sitting in the classroom.

Certainly an investigation of the glycogen content, effect of environmental temperature, seasonal changes, possible evidence of nuclear protein synthesis plus the possibility of a reversal in this periodicity are worthy of investigation. One contemplated way of approaching the possibility of a reversal is to secure biopsies from individuals who have reversed their

sleeping habits, such as night workers in factories.

One can conclude that the cyclic diurnal periodicity is an ultimate expression of certain mitogenic factors, whether they be hormonal, nutrition, temperature, environmental, physiological state, age or sex.

In any study of mitogenic factors or of mitotic indices the reliability would seem to necessarily depend on the consideration given to the periodicity.

#### SUMMARY

1. The replacement or increase of the epithelial cells in the human epidermis is accomplished by mitosis. This mitotic activity occurred in daily cycles.
2. Three major categories of cells, within this mechanism of cellular division have been described. They are; first the "clear cell" which is characterized by an inner clear zone in the cytoplasm which makes its appearance before major nuclear reorganization takes place and remains until the daughter cell has attained maturity, at which time it disappears; secondly, the typical "chromosome stages" (prophase, metaphase, anaphase and telophase) and finally the "reconstruction cell" which is present in duplicate and is likewise characterized by the clear zone mentioned above.
3. The three types of cells manifest a primary and secondary daily rhythm in their occurrence. The primary rhythm for the "clear cell" attains

its peak at 12:00 midnight and has a P value of .01 which is very significant. The secondary occurs at 2:00 P.M. and, though not as significant, it does have a P value of .02. "Chromosomal cells" are highest at 12:00 midnight and have a very significant P value of .01. The secondary mitotic cycle occurs at 4:00 P.M. and it also has a P value of .01. The "reconstruction cell" attains maximum magnitude at 3:00 A.M. and has a P value of .01 which is very significant. The minor cycle for "reconstruction cells", occurring at 7:00 P.M., because of its P value of 0.2, cannot be considered significant as the level of significance was established at .05.

4. When combining all three types of cells the major cycle manifested an increased activity of almost 200 percent over the daily average and extended over a period of six hours. The minor cycle had a duration of three hours and represented an increase of 100 percent in mitotic activity.
5. The approximate time for a cell to divide, based on the appearance and activity during the twenty four hour period, of the three categories of cells has been estimated at four to five hours. For example, "clear cells" precede "chromosomal cells" by two hours and reconstructions follow the "chromosomal phase" by approximately two hours.
6. Cellular division frequently occurred in localized regions designated as "nests" and often were surrounded by relative inactive adjacent regions. This was especially true during periods of great activity.



7. Cells undergoing division were most frequently observed in the middle and lower regions of the stratum spinosum and basale, during periods of high activity. During relatively inactive periods the majority of the mitotic figures were in the basal layer.
8. There was no evidence of lymphocyte or of any other mesodermal elements transforming into epithelial cells.

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T A B L E S

TABLE I

HOURLY ARITHMETICAL MEAN OF CLEAR, CHROMOSOMAL AND RECONSTRUCTION CELLS WITH STANDARD DEVIATION

HOURLY OF DAY	CLEAR CELLS	CHROMOSOMAL PHASE	RECONSTRUCTION CELLS
8:00 A.M.	$1.05 \pm 0.21$	$1.13 \pm 0.22$	$3.59 \pm 0.54$
9:00 A.M.	$1.43 \pm 0.14$	$0.45 \pm 0.08$	$2.79 \pm 0.41$
10:00 A.M.	$0.65 \pm 0.07$	$0.62 \pm 0.16$	$1.01 \pm 0.18$
11:00 A.M.	$0.61 \pm 0.07$	$0.26 \pm 0.04$	$0.66 \pm 0.15$
12:00 A.M.	$1.19 \pm 0.12$	$0.92 \pm 0.15$	$1.26 \pm 0.16$
1:00 P.M.	$0.40 \pm 0.08$	$0.37 \pm 0.07$	$0.51 \pm 0.08$
2:00 P.M.	$4.46 \pm 0.81$	$1.45 \pm 0.30$	$4.15 \pm 0.66$
3:00 P.M.	$2.20 \pm 0.45$	$1.50 \pm 0.29$	$4.27 \pm 0.83$
4:00 P.M.	$1.06 \pm 0.03$	$2.89 \pm 0.12$	$3.35 \pm 0.11$
5:00 P.M.	$1.33 \pm 0.35$	$1.26 \pm 0.17$	$2.02 \pm 0.58$
6:00 P.M.	$1.35 \pm 0.15$	$0.70 \pm 0.12$	$2.41 \pm 0.32$
7:00 P.M.	$1.65 \pm 0.21$	$1.00 \pm 0.23$	$3.29 \pm 0.40$
8:00 P.M.	$1.27 \pm 0.18$	$1.14 \pm 0.09$	$1.85 \pm 0.43$
9:00 P.M.	$1.68 \pm 0.26$	$1.23 \pm 0.07$	$1.89 \pm 0.23$
10:00 P.M.	$1.18 \pm 0.31$	$2.44 \pm 0.42$	$1.94 \pm 0.67$
11:00 P.M.	$3.19 \pm 0.44$	$2.61 \pm 0.37$	$1.49 \pm 0.44$
12:00 P.M.	$4.49 \pm 0.49$	$6.05 \pm 0.33$	$1.54 \pm 0.49$

TABLE I-continued

HOUR OF DAY	CLEAR CELLS	CHROMOSOMAL PHASE	RECONSTRUCTION CELLS
1:00 A.M.	$3.59 \pm 0.55$	$4.86 \pm 0.39$	$2.91 \pm 0.45$
2:00 A.M.	$3.15 \pm 0.57$	$3.02 \pm 0.36$	$4.75 \pm 0.61$
3:00 A.M.	$3.08 \pm 0.08$	$2.97 \pm 0.25$	$8.32 \pm 0.91$
4:00 A.M.	$2.37 \pm 0.31$	$2.05 \pm 0.26$	$4.18 \pm 0.17$
5:00 A.M.	$1.87 \pm 0.29$	$2.00 \pm 0.33$	$4.12 \pm 0.41$

TABLE II

COMPARISON OF PERCENTAGES FOR BIOPSIES FROM  
SAME INDIVIDUAL WITH HOURLY AVERAGE

HOURS OF BIOPSIES FROM SAME INDIVIDUAL	CLEAR CELLS		CHROMOSOMAL PHASE		RECONSTRUCTION CELLS	
	Percent	Compared with daily average	Percent	Compared with daily average	Percent	Compared with daily average
11:00 P.M.	3.28	3.19	2.66	2.61	.69	1.49
1:00 A.M.	3.55	3.59	7.35	4.86	2.38	2.97
5:00 A.M.	3.05	1.87	2.29	2.00	4.35	4.12
10:00 P.M.	1.02	1.31	2.61	2.44	.41	1.80
12:00 P.M.	5.07	4.49	6.12	6.05	1.24	1.54
5:00 A.M.	2.09	1.87	1.12	2.00	2.09	4.12
9:00 P.M.	1.38	1.63	1.01	1.23	1.04	1.94
1:00 A.M.	3.44	3.59	5.55	4.86	2.78	2.97
4:00 P.M.	.71	2.37	2.95	2.05	3.49	4.18
4:00 P.M.	1.13	2.37	1.95	2.05	3.65	4.10
8:00 P.M.	.84	1.13	1.16	1.14	.69	1.98
1:00 A.M.	3.44	3.59	5.55	4.86	2.78	2.97
12:00 P.M.	3.81	4.49	5.01	6.05	.76	1.54
10:00 P.M.	1.72	1.31	3.02	2.44	1.83	1.80



TABLE II-continued

HOURS OF BIOPSIES FROM SAME INDIVIDUAL	CLEAR CELLS		CHROMOSOMAL CELLS		RECONSTRUCTION CELLS	
	Percent	Compared with daily average	Percent	Compared with daily average	Percent	Compared with daily average
1:00 A.M.	4.40	3.59	4.60	4.86	4.64	2.97
5:00 A.M.	1.53	1.87	1.63	2.00	3.87	4.12
8:00 A.M.	.71	1.05	2.59	1.13	3.24	3.59
1:00 A.M.	2.85	3.59	4.10	4.86	1.60	2.97
7:00 P.M.	1.68	1.65	.61	1.00	2.22	3.29
1:00 A.M.	1.59	2.59	3.08	4.86	.83	2.97
2:00 A.M.	3.62	3.15	3.52	3.02	4.71	4.75
5:00 P.M.	.99	1.87	1.23	2.00	3.39	4.12
7:00 P.M.	1.27	1.65	.25	1.00	3.06	3.29
4:00 A.M.	3.81	2.37	1.99	2.05	2.26	4.18
8:00 A.M.	.67	1.05	1.24	1.13	2.99	3.59
10:00 P.M.	.49	1.31	1.90	2.44	1.67	1.80
10:00 P.M.	.68	1.31	2.74	2.44	2.43	1.80
12:00 midnight	1.42	4.49	5.52	6.05	1.38	1.54

FIGURES AND PLATES

Figure 1. Variation in clear cell activity during the twenty four hour period.

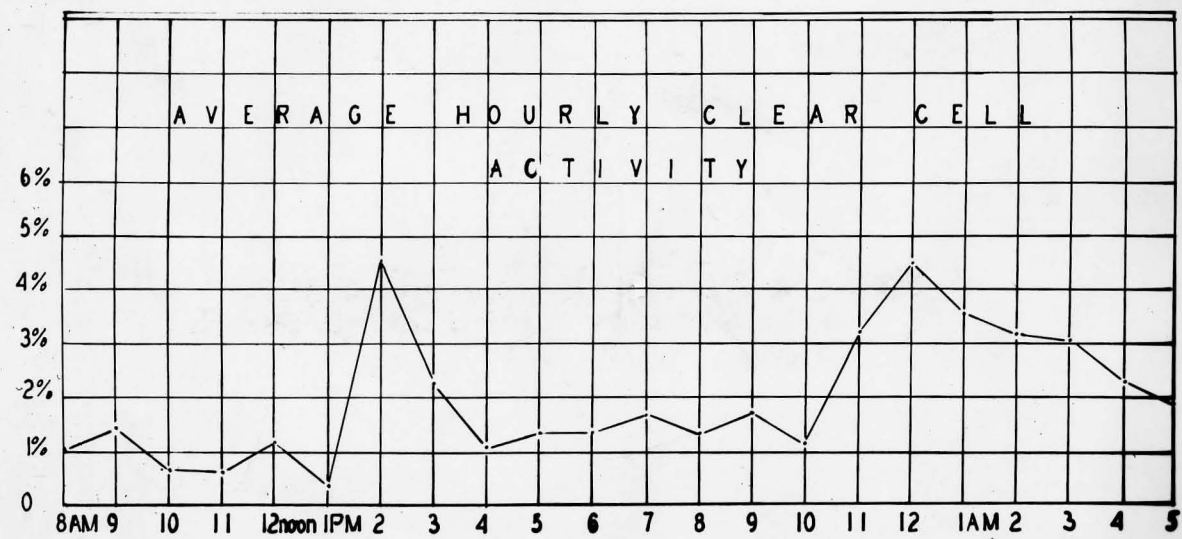


Figure 1

Figure 2. Variation in the chromosome phase activity during the twenty four hour period.

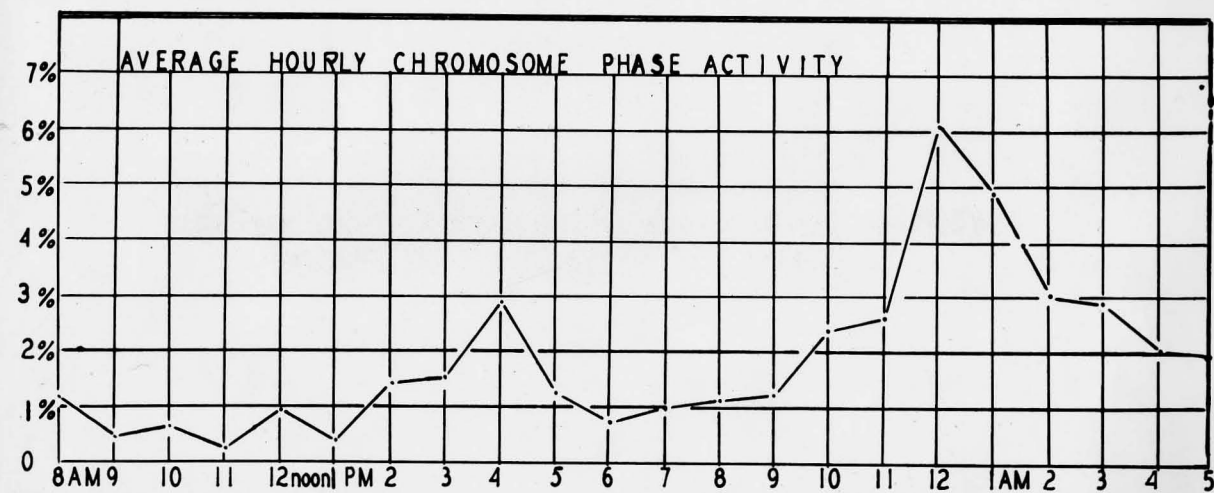


Figure 2



Figure 3. Variation in reconstruction cell activity during the twenty four hour period.

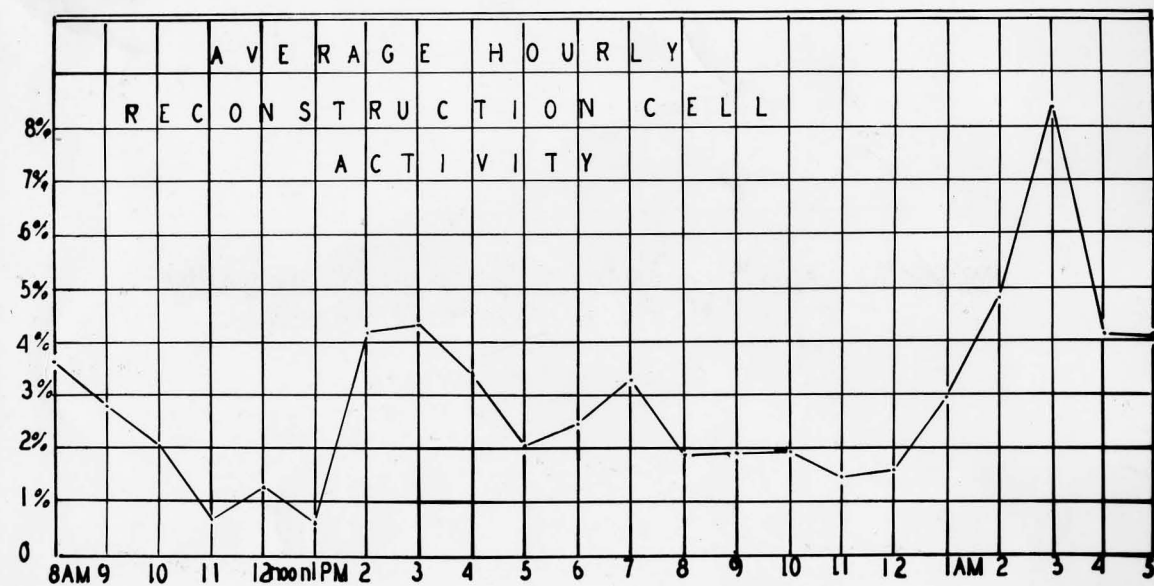


Figure 3

Figure 4. Variation in the activity of clear, chromosomal and reconstruction cells.

----- Clear cells  
 -.-.-.-.- Chromosomal cells  
 ..... Reconstruction

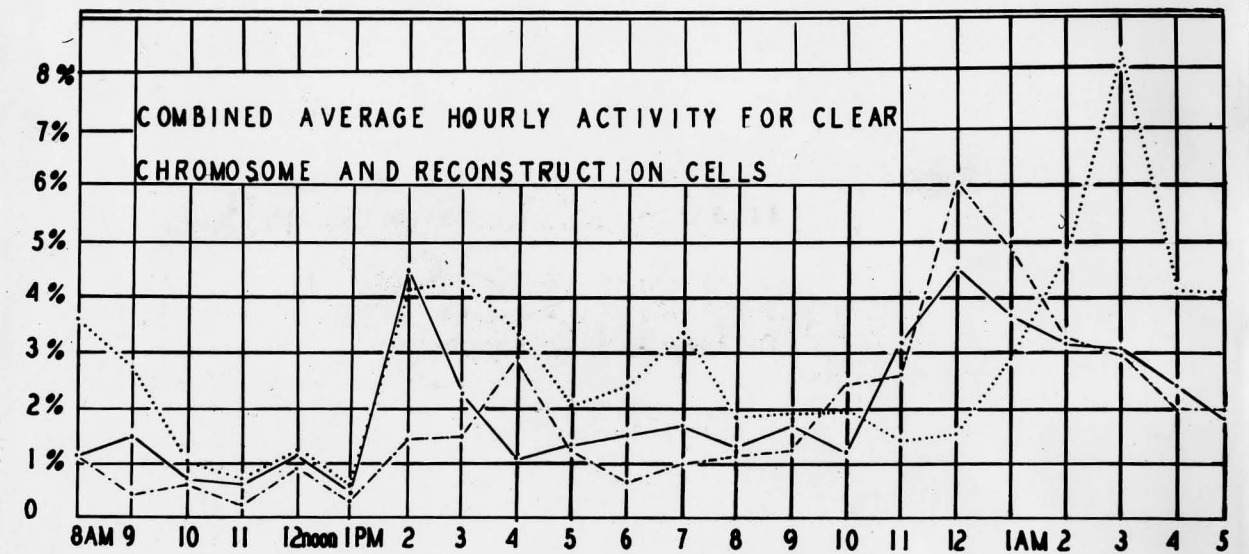


Figure 4



Figure 5. Statistical values of the major peaks of activity for clear, chromosomal and reconstruction cells. The numerical values 3.15, 3.02 and 4.27 indicates the maximum percentage activity for any one hour of the hours used to calculate the daily average. Likewise the numerical values of 0.4, 0.26 and 0.5 indicate the lowest percent of activity during this same period.

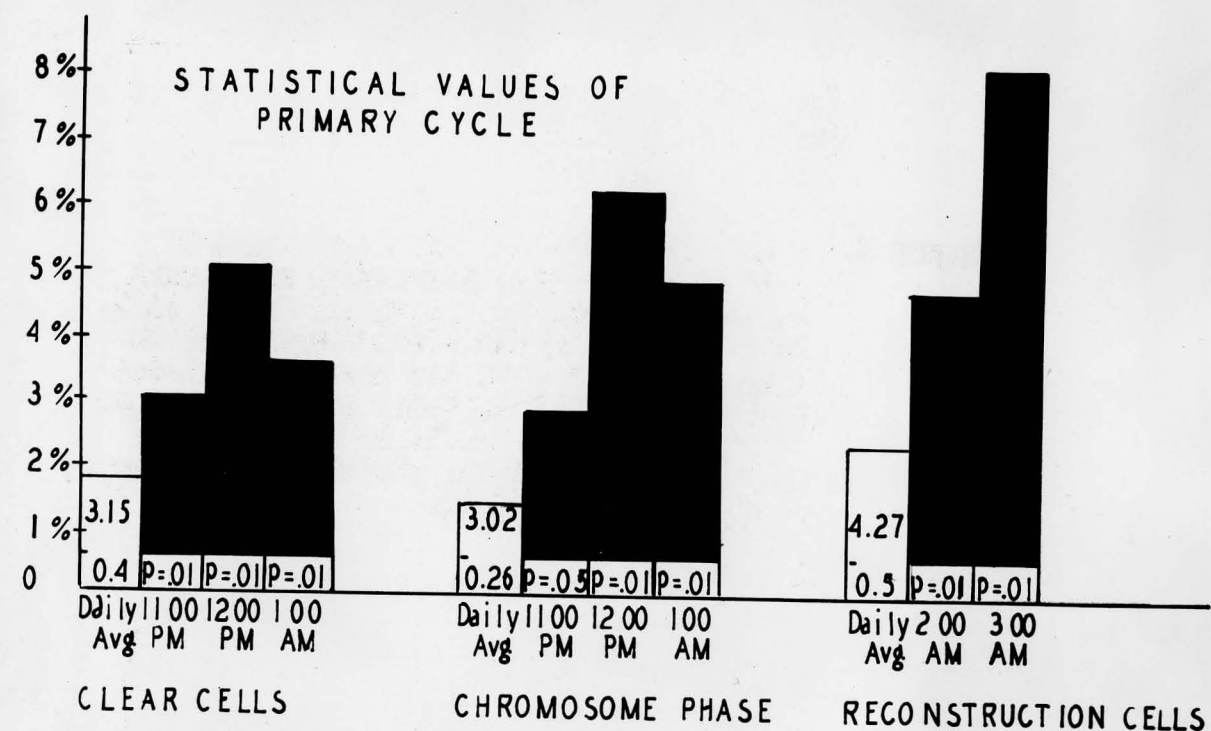


Figure 5

Figure 6. Statistical values for secondary peaks of activity for clear, chromosomal and reconstruction cells.

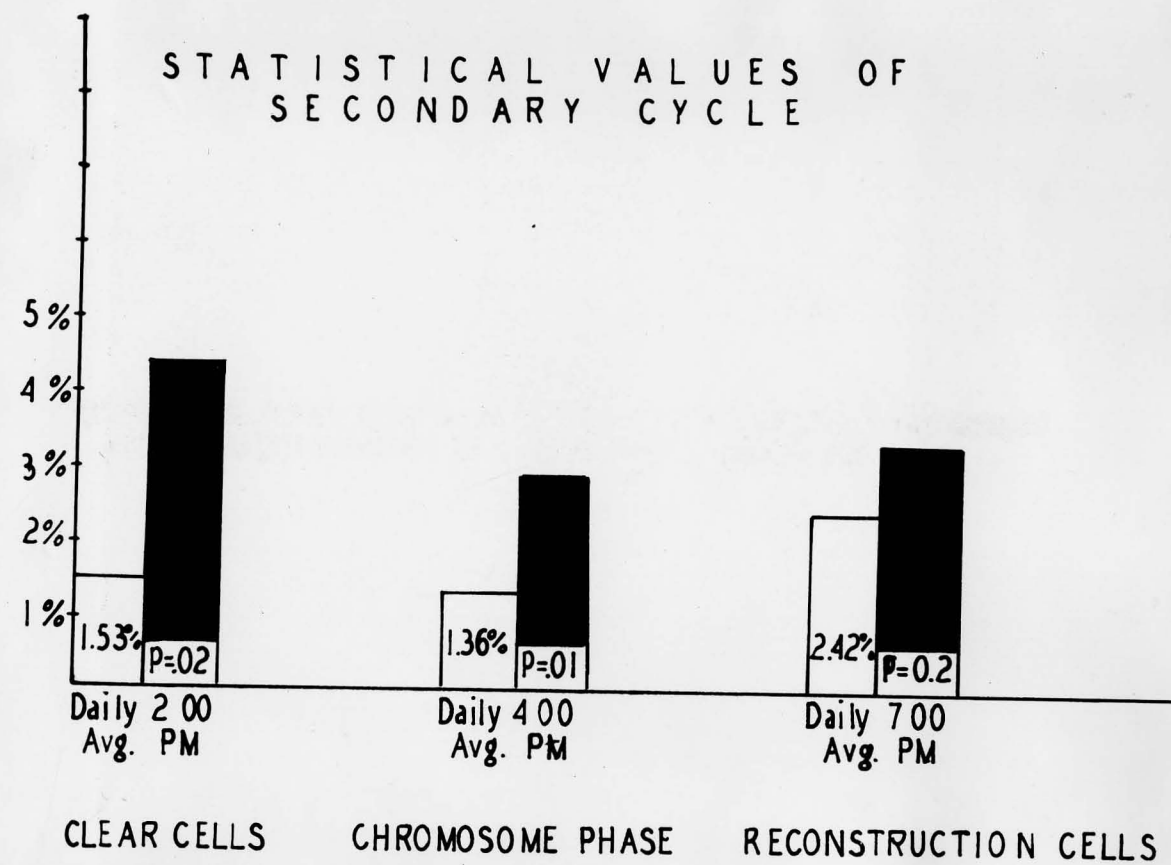


Figure 6



Figure 7. Graph represents total hourly activity for clear, chromosomal and reconstruction cells.

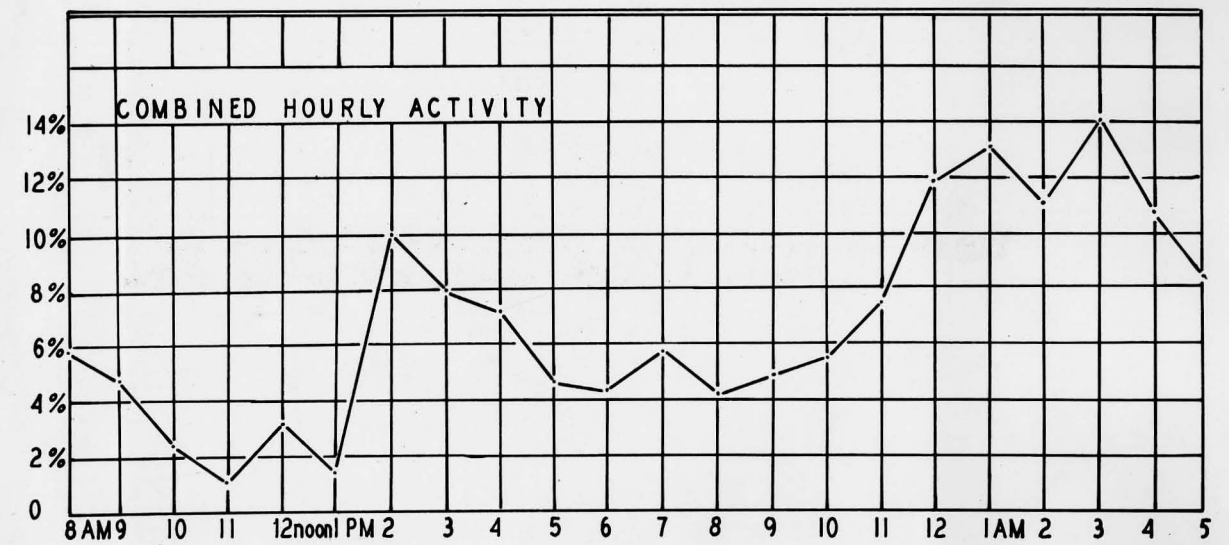


Figure 7

PLATE I

Abbreviations:

- A. - Stratum corneum (scaly layer)
- B. - Stratum germinativum (mucosum)
- C. - Dermis
- D. - Sebaceous gland
- E. - Hair follicle

Figure 8

Human epidermis. Note two main layers of epidermis superimposed on the dermis; the stratum corneum and stratum germinativum (mucosum). Also note hair follicle and sebaceous gland. 75X. All photomicrographs are material fixed in Bouin's fluid and stained with iron Hematoxylin.

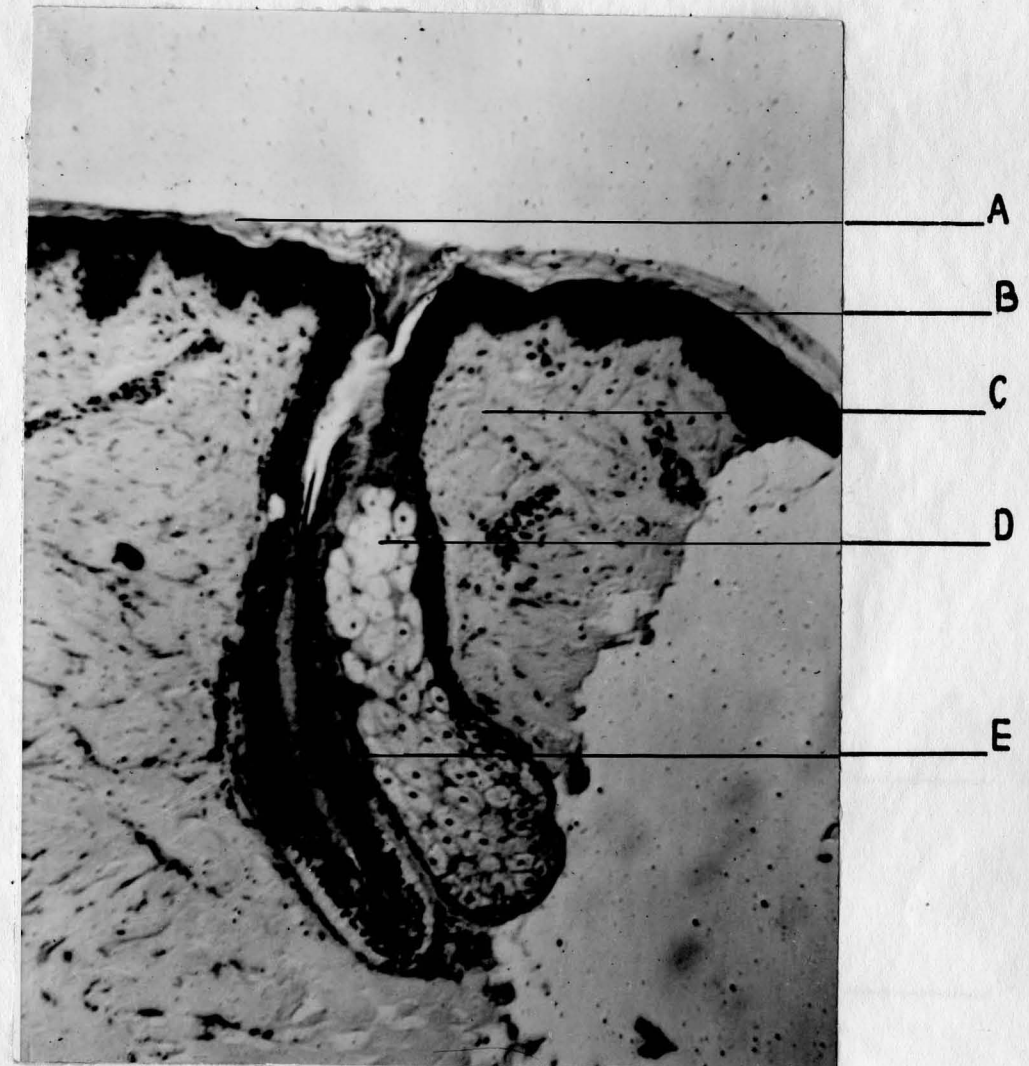


Figure 8



## PLATE II

## Abbreviations:

- A. - Basement membrane  
B. - Dermis

## Figure 9

Human epidermis. Note basement membrane separating the basal processes of the cells of the stratum basale from the dermis 1900X.

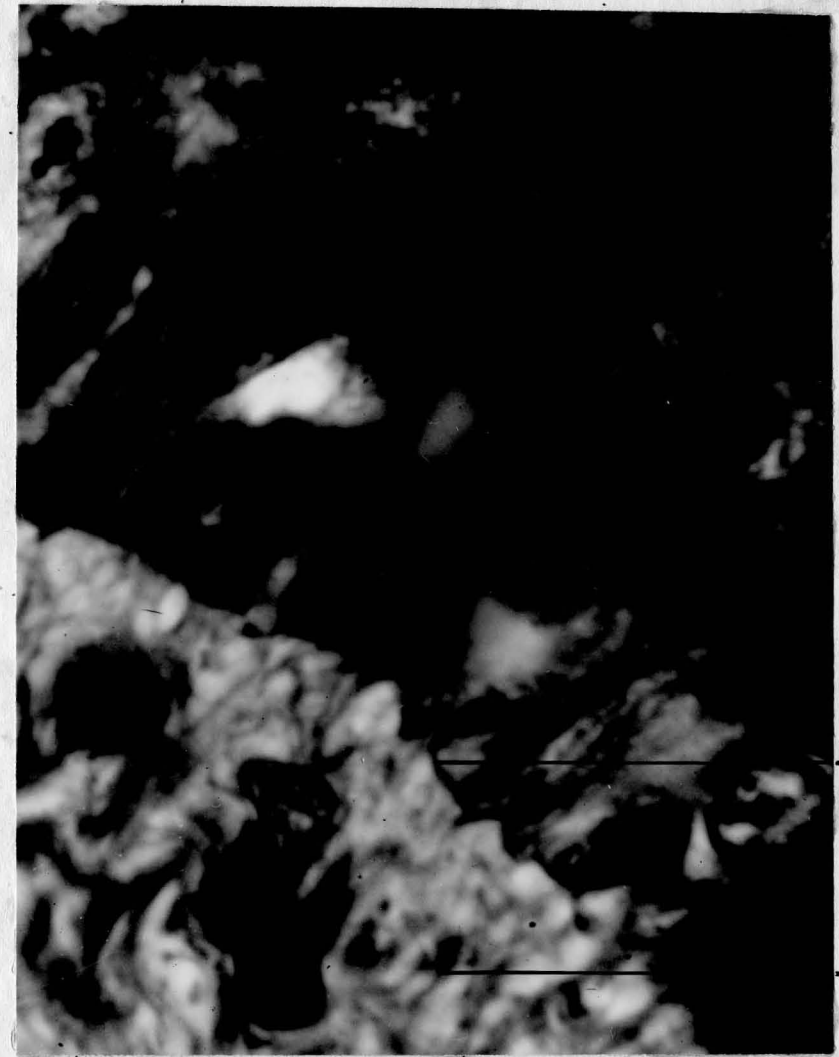


Figure 9

PLATE III

Abbreviations:

- A. - Stratum granulosum
- B. - Nucleoli (two)
- C. - Intercellular bridges
- D. - Supra nuclear caps
- E. - Karyosomes

Figure 10

Human epidermis. Note stratum basale with supra nuclear caps over nuclei, stratum germinativum (mucosum) and stratum granulosum. Also note nuclear membrane, multiform karyosomes, prominent basophilic nucleoli, and intercellular bridges. Note early prophase in lower spinosum showing clear zone and rim of cytoplasm connecting to other cells of the spinosum. 1900X.

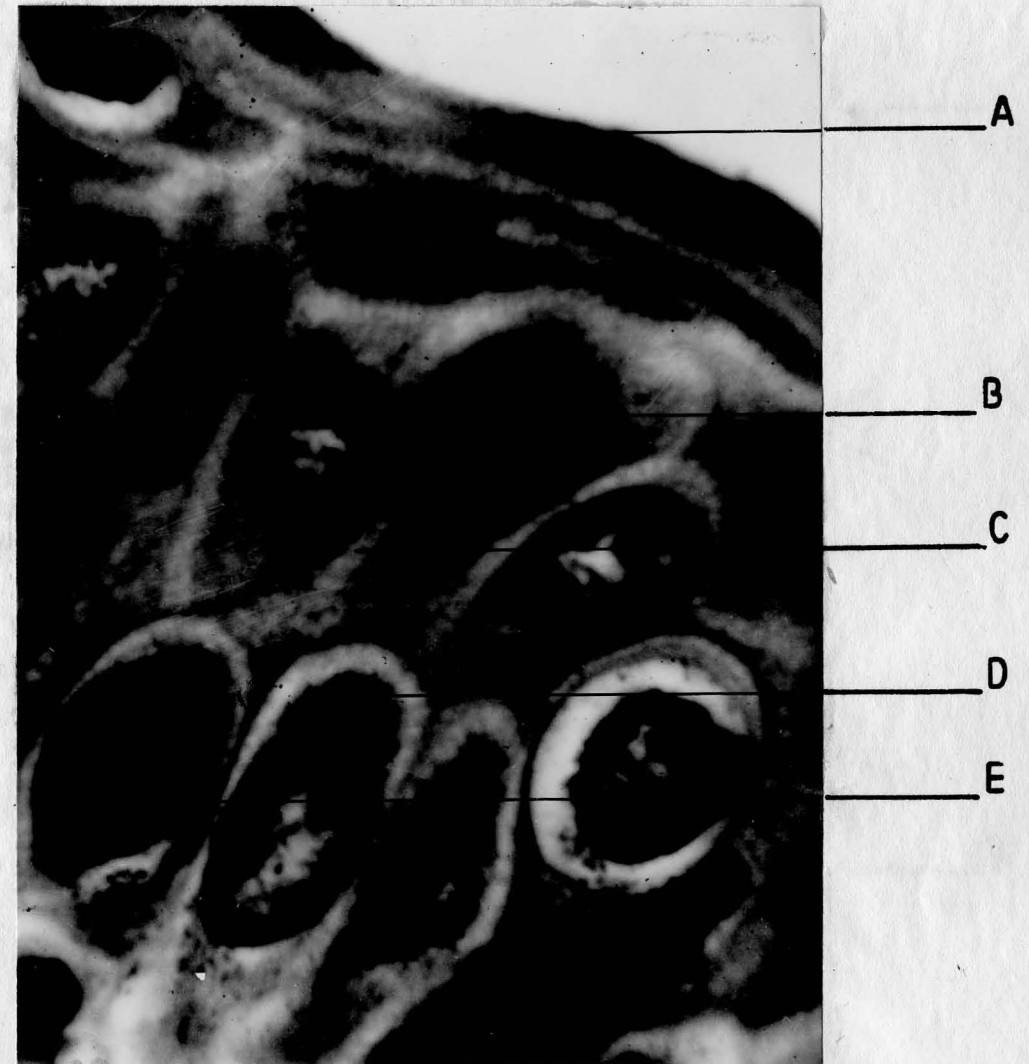


Figure 10



PLATE IV

\* Abbreviations:

- A. - Stratum granulosum
- B. - Fusiform nucleus of basal layer

Figure 11

Human epidermis. Note fusiform nucleus of basal layer and flattened nuclei of stratum granulosum. Also observe that the nuclei of the superficial cells of the spinosum become flattened and angular adjacent to the margin of the stratum granulosum. 1900X.

44

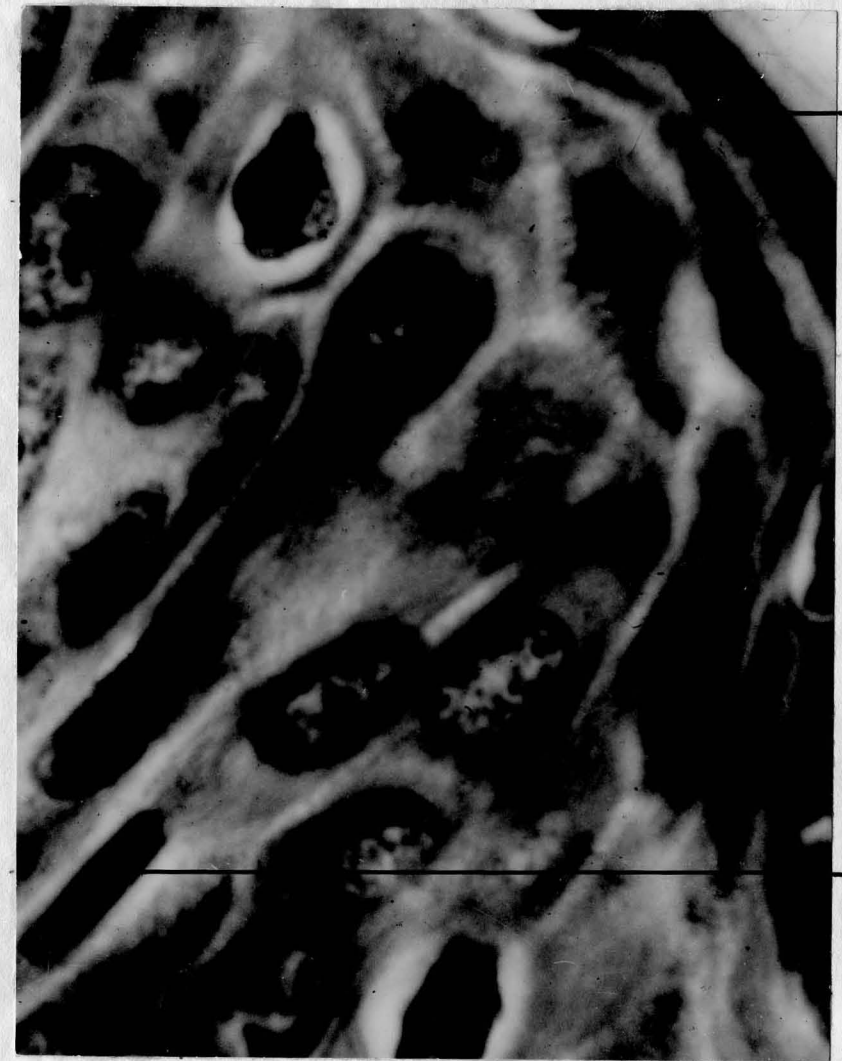


Figure 11

PLATE V

Abbreviation:

- A. - Cell in telophase stage of division
- B. - Diffuse pigment

Figure 12

Note telophase, in lower spinosum, with clear zone surrounding the nuclei, presence of intercellular bridges at periphery of two daughter cells. Intercellular bridges have not yet appeared between the two daughter cells. Also note abundance of pigment. 1900X.

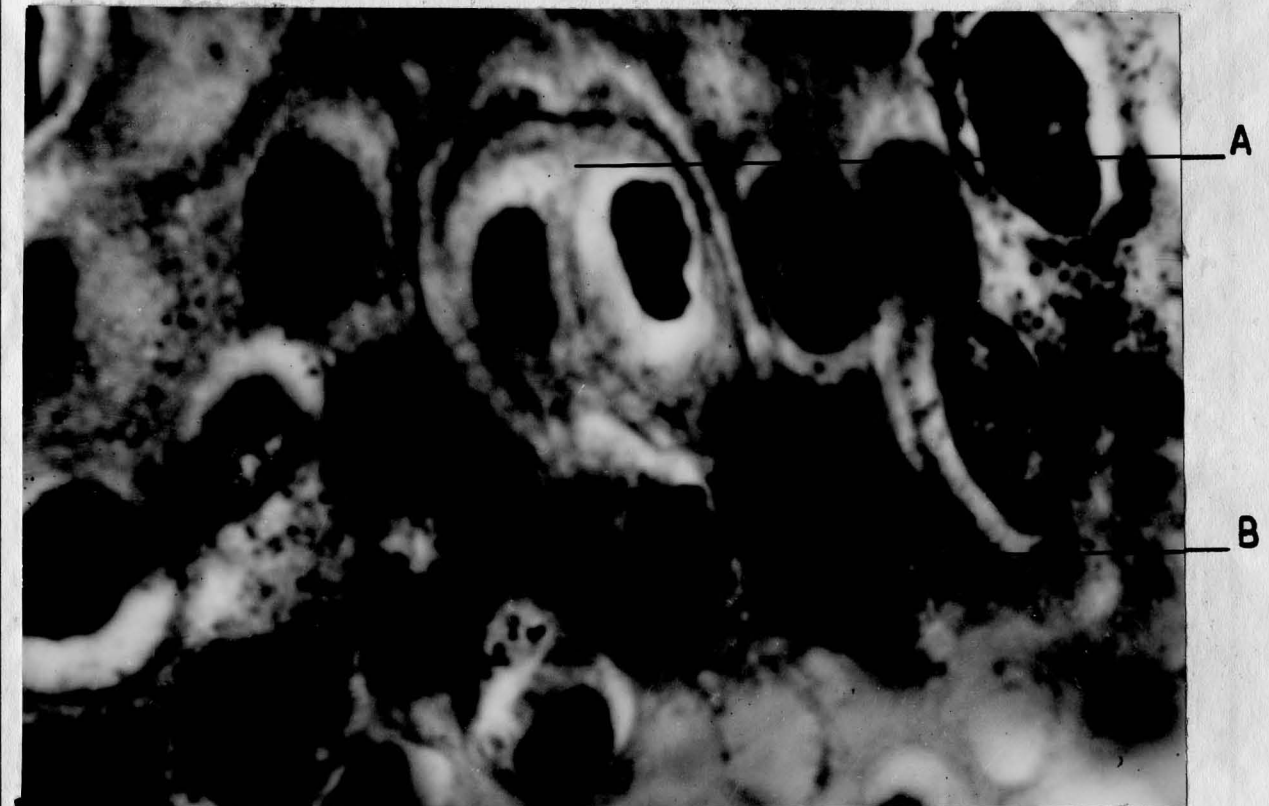


Figure 12



PLATE VI

Figure 13

Human epidermis. Note anaphase in center  
of figure located in middle of spinosum.  
Also note clear zone around nuclear material.  
1900X.

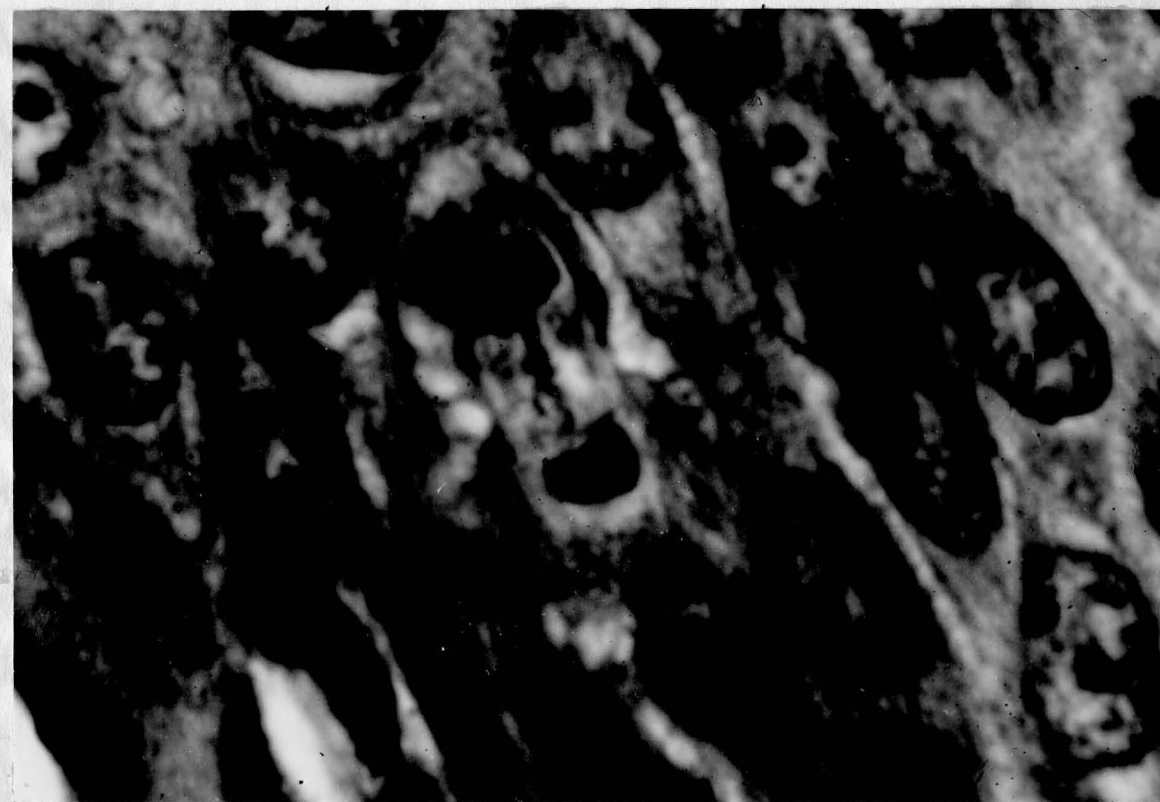


Figure 13

PLATE VII

Figure 14

Human epidermis. Note early prophase, located in spinosum, clear zone around nucleus and rim of cytoplasm attached to adjacent cells. 1900X.



Figure 14



PLATE VIII

Abbreviations:

A. - Typical clear cell

Figure 15

Human epidermis. Note very early clear cell with rim of cytoplasm connected to other adjacent cells by intercellular bridges. 1900X.

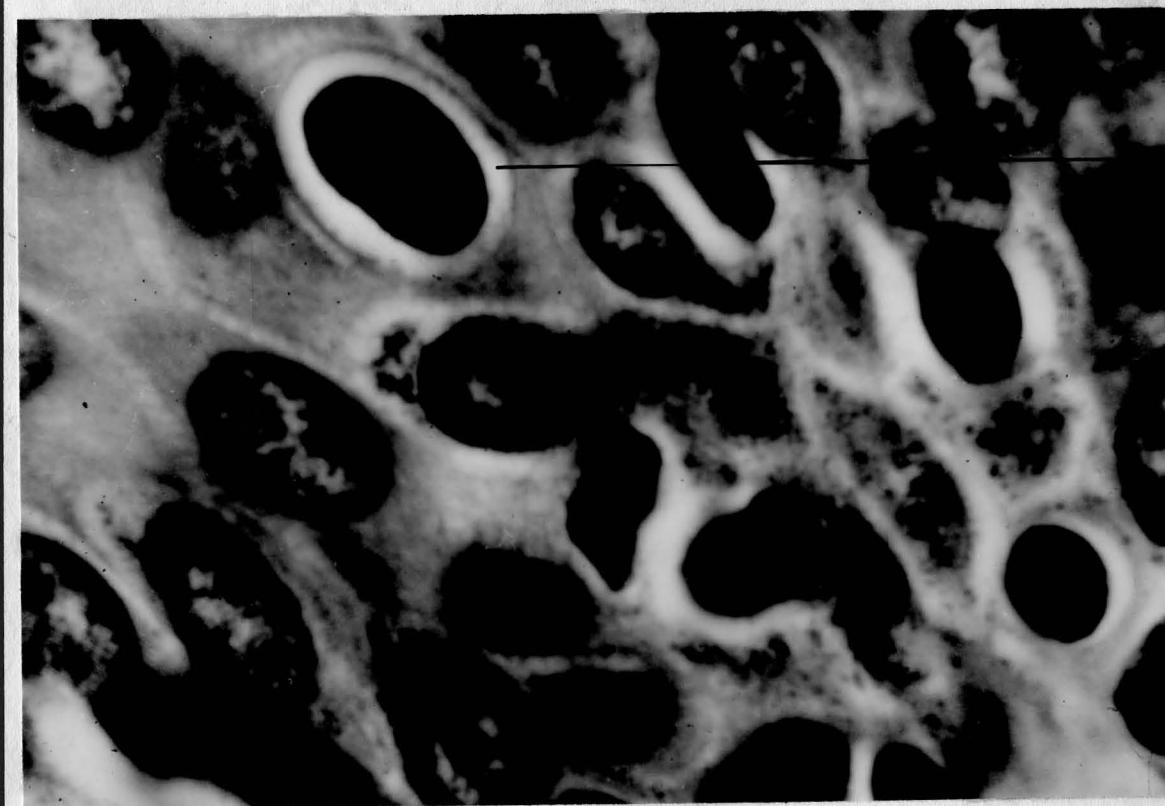


Figure 15

PLATE IX

Figure 16

Human epidermis. Note early prophase showing clear zone and intercellular bridges in stratum spinosum. 1900X.



Figure 16



PLATE X

Abbreviations:

- A. - Typical reconstruction cell
- B. - Typical early clear cell

Figure 17

Human epidermis. Note typical single early cell with rim of cytoplasm connected to cytoplasm of adjacent cells by intercellular bridges. Also note typical duplicate reconstruction cells with large clear zone still present. 1900X.

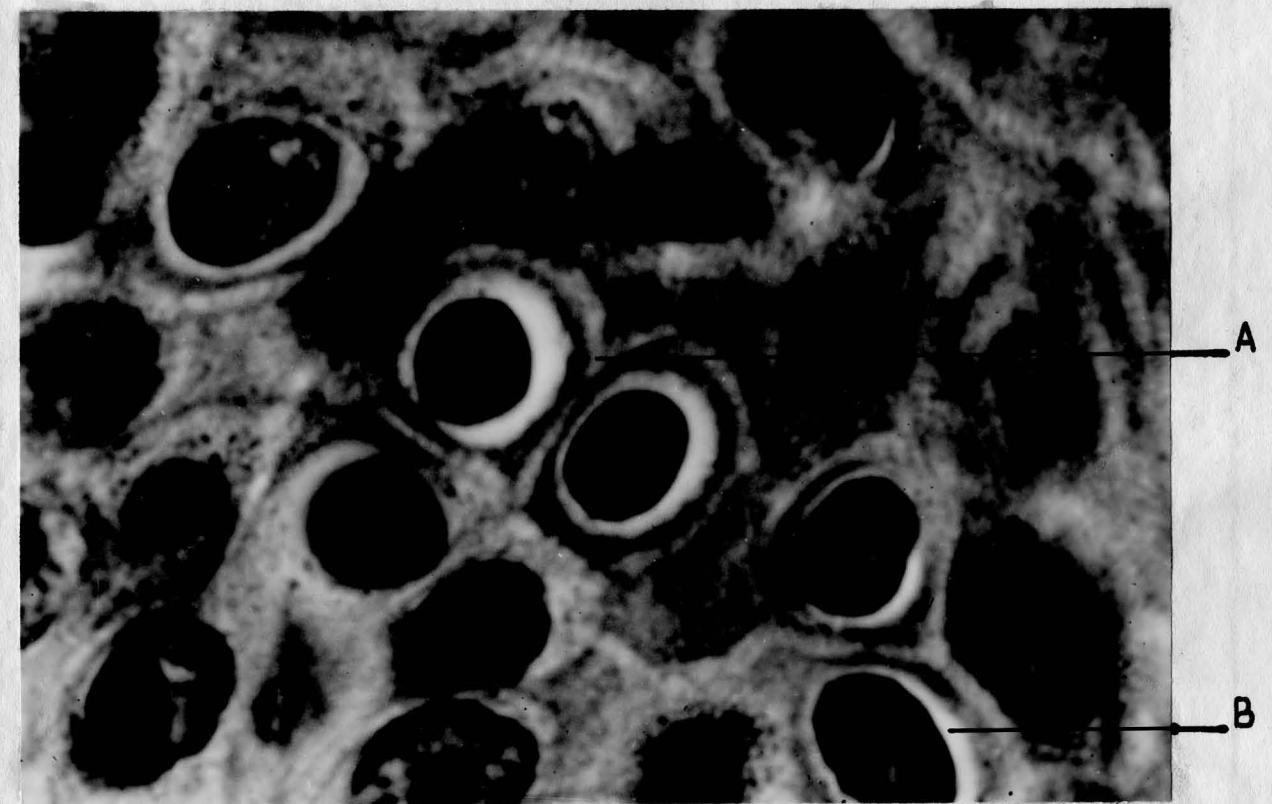


Figure 17



PLATE XI

Figure 18

Epidermis. Amblystoma larva. Note mitotic figure (metaphase) with clear zone around chromosomal mass. 450X.

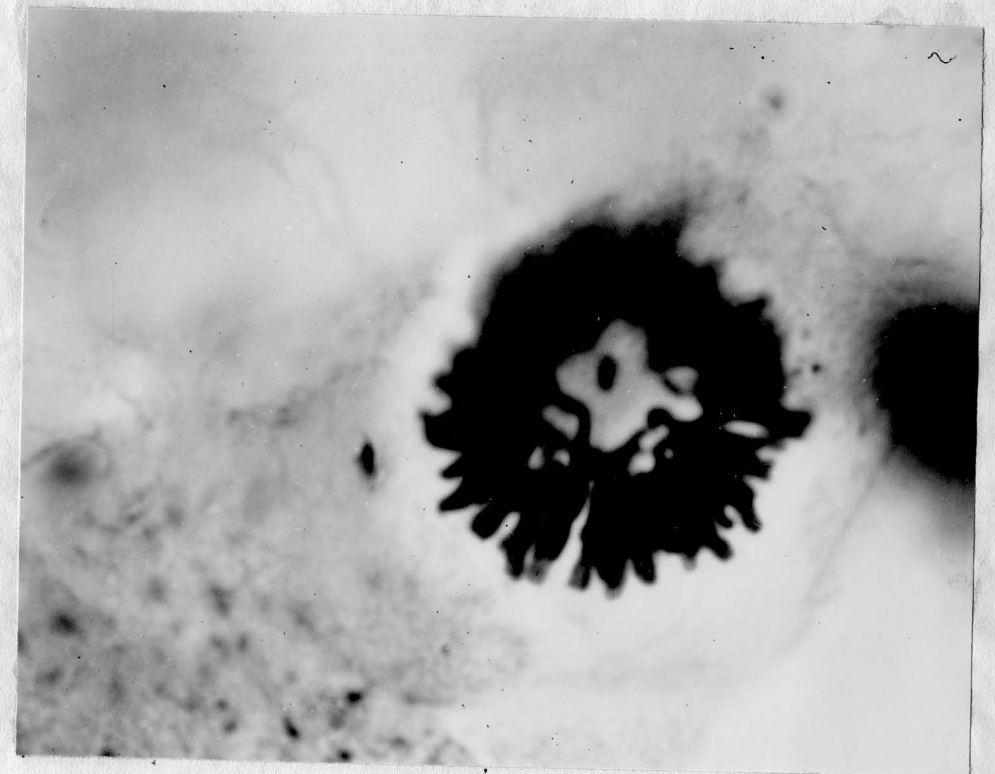


Figure 18

STATISTICAL APPENDIX

## APPENDIX I

## STATISTICAL DATA FOR 8:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.10	0.05	.0125	0.54	.59	.3481	5.14	1.55	2.4025
1.27	0.22	.0484	1.01	.12	.0144	2.13	1.46	2.1316
1.94	0.89	.7921	1.45	.32	.1024	2.74	0.85	.7225
0.81	0.24	.0576	0.52	.61	.3721	4.83	1.24	1.5376
0.76	0.29	.0841	0.35	.78	.6084	2.25	1.34	1.7956
2.57	1.52	2.3104	1.55	.42	.1764	7.59	4.00	16.0000
1.29	0.24	.0576	0.47	.66	.4356	4.46	.87	.7569
0.53	0.52	.2704	1.02	.11	.0121	2.91	.68	.4624
0.67	0.38	.1444	1.24	.11	.0121	2.99	.60	.3600
0.71	0.34	.1156	2.59	1.46	2.1316	3.24	.35	.1225
Total (d - $\bar{d}$ ) <sup>2</sup> = 3.8931			Total (d - $\bar{d}$ ) <sup>2</sup> = 4.2132			Total (d - $\bar{d}$ ) <sup>2</sup> = 26.2916		
Arithmetical Mean = 1.05			Arithmetical Mean = 1.13			Arithmetical Mean = 3.59		
Standard deviation = $\pm$ 0.20			Standard deviation = $\pm$ 0.22			Standard deviation = $\pm$ 0.54		

## APPENDIX I

## STATISTICAL DATA FOR 9:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.14	0.29	.0841	0.17	0.28	.0784	2.78	0.01	.0001
1.74	0.31	.0961	0.44	0.01	.0001	2.42	0.37	.1369
1.82	0.39	.1521	0.48	0.03	.0009	1.83	0.96	.9216
0.86	0.57	.3249	0.29	0.16	.0256	2.58	0.21	.0441
1.39	0.04	.0016	0.19	0.26	.0676	2.30	0.49	.2401
1.90	0.47	.2209	0.95	0.50	.2500	1.12	1.67	2.7889
0.55	0.88	.7744	0.17	0.28	.0784	3.89	1.10	1.2100
1.45	0.02	.0004	0.83	0.38	.1444	5.84	3.05	9.3025
0.94	0.49	.2401	0.38	0.07	.0049	2.27	0.52	.2704
Total (d - $\bar{d}$ ) <sup>2</sup> = 1.8946			Total (d - $\bar{d}$ ) <sup>2</sup> = .6503			Total (d - $\bar{d}$ ) <sup>2</sup> = 14.9146		
Arithmetical Mean = 1.43			Arithmetical Mean = 0.45			Arithmetical Mean = 2.79		
Standard deviation = $\pm$ 0.14			Standard deviation = $\pm$ 0.08			Standard deviation = $\pm$ 0.40		

## APPENDIX I

## STATISTICAL DATA FOR 10:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
0.32	0.33	.1089	0.24	0.38	.1444	0.80	0.21	.0441
0.66	0.01	.0001	0.44	0.18	.0324	1.00	0.02	.0004
1.08	0.43	.1849	0.58	0.04	.0016	0.65	0.36	.1296
0.69	0.04	.0016	0.62	0.0	0.0	1.84	0.83	.6889
1.01	0.36	.1296	0.60	0.02	.0004	0.47	0.54	.2916
0.78	0.13	.0169	0.37	0.25	.0625	1.30	0.29	.0841
0.83	0.18	.0324	0.28	0.34	.1156	0.65	0.36	.1296
0.65	0.0	0.0	0.39	0.23	.0529	1.39	0.38	.1444
0.37	0.28	.0784	0.09	0.53	.2809	1.04	0.03	.0009
0.42	0.23	.0529	0.23	0.39	.1521	0.73	0.28	.0784
0.57	0.08	.0064	2.01	1.39	1.9321	1.15	0.14	.0196
Total (d - $\bar{d}$ ) <sup>2</sup> = .6121			Total (d - $\bar{d}$ ) <sup>2</sup> = 2.7749			Total (d - $\bar{d}$ ) <sup>2</sup> = 3.6113		
Arithmetical Mean = 0.65			Arithmetical Mean = 0.62			Arithmetical Mean = 1.03		
Standard deviation = $\pm$ 0.07			Standard deviation = $\pm$ 0.159			Standard deviation = $\pm$ 0.07		



## APPENDIX I

## STATISTICAL DATA FOR 11:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
0.62	0.01	.0001	0.0	0.26	.0001	1.56	0.90	.8100
0.38	0.23	.0529	0.18	0.08	.0064	0.49	0.17	.0289
0.87	0.26	.0676	0.42	0.16	.0256	1.52	0.86	.7396
0.94	0.33	.1089	0.25	0.01	.0001	0.87	0.21	.0441
0.56	0.05	.0025	0.34	0.08	.0064	1.00	0.34	.1156
0.90	0.31	.0961	0.38	0.12	.0144	0.54	0.12	.0144
0.71	0.10	.0100	0.28	0.02	.0004	0.59	0.07	.0049
0.27	0.34	.1156	0.15	0.11	.0121	0.01	0.65	.4225
0.40	0.21	.0441	0.29	0.03	.0009	0.40	0.26	.0676
0.66	0.05	.0025	0.19	0.07	.0049	0.27	0.39	.1521
0.65	0.04	.0016	0.09	0.17	.0289	0.37	0.29	.0841
Total (d - $\bar{d}$ ) <sup>2</sup> = .5019			Total (d - $\bar{d}$ ) <sup>2</sup> = .1677			Total (d - $\bar{d}$ ) <sup>2</sup> = 2.4838		
Arithmetical Mean = 0.61			Arithmetical Mean = 0.26			Arithmetical Mean = 0.66		
Standard deviation = $\pm$ 0.07			Standard deviation = $\pm$ 0.038			Standard deviation = $\pm$ 0.15		

## APPENDIX I

## STATISTICAL DATA FOR 12:00 Noon

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.17	.02	.0004	1.84	.92	.8464	2.06	.80	.6400
.97	.22	.0484	1.54	.62	.3844	1.13	.13	.0169
.72	.47	.2209	1.28	.36	.1296	1.99	.73	.5329
.79	.40	.1600	1.36	.34	.1156	1.24	.02	.0004
1.62	.43	.1849	1.31	.39	.1521	.24	1.02	1.0404
1.61	.42	.1764	.58	.34	.1156	1.01	.25	.0625
1.61	.42	.1764	.23	.69	.4761	1.49	.23	.0529
1.53	.34	.1156	.20	.72	.5184	.37	.89	.7921
1.37	.18	.0324	.95	.03	.0009	1.47	.21	.0441
1.00	.19	.0361	.40	.52	.2704	1.14	.12	.0144
1.72	.53	.2809	1.06	.14	.0196	1.13	.13	.0169
1.06	.13	.0169	.57	.35	.1225	.78	.48	.2304
.31	.88	.7744	.52	.40	.1600	.62	.64	.4096
Total (d - $\bar{d}$ ) <sup>2</sup> = 2.2237			Total (d - $\bar{d}$ ) <sup>2</sup> = 3.3116			Total (d - $\bar{d}$ ) <sup>2</sup> = 3.8535		
Arithmetical Mean = 1.19			Arithmetical Mean = .92			Arithmetical Mean = 1.26		
Standard deviation = $\pm$ 0.11			Standard deviation = $\pm$ 0.15			Standard deviation = $\pm$ 0.16		

## APPENDIX I

## STATISTICAL DATA FOR 1:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
.20	.20	.0400	.38	.01	.0001	.55	.04	.0016
.09	.31	.0961	.33	.04	.0016	.27	.24	.0576
.88	.48	.2304	.30	.07	.0049	.96	.45	.2025
.52	.12	.0144	.21	.16	.0256	.33	.18	.0324
.31	.09	.0081	.26	.11	.0121	.35	.16	.0256
.31	.09	.0081	.31	.06	.0036	.35	.16	.0256
.21	.19	.0361	.14	.23	.0529	.07	.44	.1936
.58	.18	.0324	.93	.56	.3136	.80	.29	.0841
.18	.22	.0484	.25	.12	.0144	.59	.08	.0064

Total (d -  $\bar{d}$ )<sup>2</sup> = .5140      Total (d -  $\bar{d}$ )<sup>2</sup> = .4288      Total (d -  $\bar{d}$ )<sup>2</sup> = .6294

Arithmetical Mean = .40      Arithmetical Mean = .37      Arithmetical Mean = .51

Standard deviation =  $\pm 0.08$       Standard deviation =  $\pm 0.07$       Standard deviation =  $\pm 0.08$

## APPENDIX I

## STATISTICAL DATA FOR 2:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.74	2.72	7.3984	2.14	.69	.4761	3.54	.61	.3721
3.51	.95	.9025	.99	.46	.2116	2.51	1.64	2.6896
4.17	.29	.0841	1.22	.23	.0529	3.84	.31	.0961
3.34	1.12	1.2544	.79	.66	.4356	4.34	.19	.0361
8.48	4.02	16.1604	2.72	1.27	1.6129	3.95	.20	.0400
7.13	2.67	7.1289	2.42	.97	.9409	5.93	1.78	3.1684
5.88	1.42	2.0164	.42	1.03	1.0609	8.32	4.17	17.3889
5.63	1.17	1.3689	1.09	.36	.1296	4.81	.66	.4356

Total (d -  $\bar{d}$ )<sup>2</sup> = 36.3140      Total (d -  $\bar{d}$ )<sup>2</sup> = 4.9205      Total (d -  $\bar{d}$ )<sup>2</sup> = 24.2268

Arithmetical  
Mean = 4.46

Arithmetical  
Mean = 1.45

Arithmetical  
Mean = 4.15

Standard  
deviation =  $\pm 0.8$

Standard  
deviation =  $\pm 0.3$

Standard  
deviation =  $\pm 0.6$

## APPENDIX I

## STATISTICAL DATA FOR 3:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
2.46	.26	.0676	1.10	.40	.1600	3.84	.43	.1849
2.34	.14	.0196	.87	.63	.3969	5.73	1.46	2.1349
1.03	1.17	1.3689	1.88	.38	.1444	2.84	1.43	2.0449
2.70	.50	.2500	2.52	1.02	1.0404	7.57	3.30	10.8900
1.59	.61	.3721	1.32	.18	.0324	2.81	1.46	2.1316
4.06	1.86	3.4596	1.79	.29	.0841	4.01	.26	.0676
.42	1.78	3.1684	.21	1.29	1.6641	.85	3.42	11.6964

Total (d -  $\bar{d}$ )<sup>2</sup> = 8.7062      Total (d -  $\bar{d}$ )<sup>2</sup> = 3.5223      Total (d -  $\bar{d}$ )<sup>2</sup> = 29.1470

Arithmetical  
Mean = 2.20

Arithmetical  
Mean = 1.50

Arithmetical  
Mean = 4.27

Standard  
deviation =  $\pm 0.45$

Standard  
deviation =  $\pm 0.29$

Standard  
deviation =  $\pm 0.45$

## APPENDIX I

## STATISTICAL DATA FOR 4:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.13	.05	.0025	1.95	.94	.8836	3.65	.30	.0900
1.26	.20	.0400	3.45	.56	.3136	4.20	.85	.7225
.72	.35	.1225	2.95	.06	.0036	3.49	.14	.0196
1.10	.04	.0016	3.75	.86	.7396	2.48	.87	.7569

Total (d -  $\bar{d}$ )<sup>2</sup> = .1666    Total (d -  $\bar{d}$ )<sup>2</sup> = 1.9404    Total (d -  $\bar{d}$ )<sup>2</sup> = 1.5890

Arithmetical Mean = 1.06    Arithmetical Mean = 2.89    Arithmetical Mean = 3.35

Standard deviation =  $\pm 0.03$     Standard deviation =  $\pm 0.12$     Standard deviation =  $\pm 0.11$

## APPENDIX I

## STATISTICAL DATA FOR 5:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
.64	.69	.4761	.81	.45	.2025	.53	1.49	2.2201
.49	.84	.7056	.81	.45	.2035	.72	1.30	1.6900
3.06	1.73	2.9929	1.72	.46	.2116	3.72	1.70	2.8900
1.71	.38	.1444	1.51	.25	.0625	1.12	.90	.8100
1.89	.56	.3136	1.89	.63	.3969	4.25	2.03	4.1209
.66	.67	.4489	1.53	.27	.0729	1.46	.56	.3136
.99	.34	.1156	1.23	.03	.0009	3.39	1.37	1.8769

Total (d -  $\bar{d}$ )<sup>2</sup> = 5.1971      Total (d -  $\bar{d}$ )<sup>2</sup> = 1.1498      Total (d -  $\bar{d}$ )<sup>2</sup> = 13.9215

Arithmetical  
Mean = 1.33

Arithmetical  
Mean = 1.26

Arithmetical  
Mean = 2.02

Standard  
deviation =  $\pm 0.35$

Standard  
deviation =  $\pm 0.17$

Standard  
deviation =  $\pm 0.58$

## APPENDIX I

## STATISTICAL DATA FOR 6:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.62	.27	.0729	1.13	.43	.1849	2.71	.30	.0900
1.54	.19	.0361	.85	.15	.0225	3.63	1.22	1.4884
.82	.53	.2809	.23	.47	.2209	2.92	.51	.2601
1.62	.27	.0729	.50	.20	.0400	2.41	0.0	0.0
1.30	.05	.0025	.32	.38	.1444	1.13	1.28	1.6384
.29	1.06	1.1236	.33	.37	.1369	1.71	.70	.4900
1.45	.10	.0100	.34	.36	.1296	1.46	.95	.9025
2.11	.76	.5776	1.20	.50	.2500	3.75	1.34	1.7956
1.84	.49	.2401	.89	.19	.0361	3.68	1.27	1.6129
.91	.44	.1936	.20	.50	.2500	.44	1.47	2.1609
1.44	.09	.0081	.21	.49	.2401	1.53	.88	.7744
Total (d - $\bar{d}$ ) <sup>2</sup> = 2.6183			Total (d - $\bar{d}$ ) <sup>2</sup> = 1.6554			Total (d - $\bar{d}$ ) <sup>2</sup> = 11.2132		
Arithmetical Mean = 1.35			Arithmetical Mean = .70			Arithmetical Mean = 2.41		
Standard deviation = $\pm$ 0.15			Standard deviation = $\pm$ 0.12			Standard deviation = $\pm$ 0.32		



## APPENDIX I

## STATISTICAL DATA FOR 7:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.27	.38	.1444	.25	.75	.5625	3.06	.23	.0529
1.68	.03	.0009	.61	.39	.1521	2.22	1.07	1.1449
1.44	.21	.0441	.81	.19	.0361	5.13	1.84	3.3856
1.38	.27	.0729	2.18	1.18	1.3924	5.61	2.32	5.3824
1.69	.04	.0016	.84	.16	.0256	1.64	1.65	2.7225
1.11	.54	.2916	.56	.44	.1936	4.34	1.05	1.1025
3.12	1.67	2.7889	.10	.90	.8100	3.07	.28	.0784
1.78	.13	.0169	2.14	1.14	1.2996	2.14	1.15	1.3225
1.61	.04	.0016	1.06	.06	.0036	5.15	1.86	3.4596
.83	.82	.6724	1.67	.67	.4489	1.83	1.46	2.1316

Total (d -  $\bar{d}$ )<sup>2</sup> = 4.0353Total (d -  $\bar{d}$ )<sup>2</sup> = 4.9244Total (d -  $\bar{d}$ )<sup>2</sup> = 20.7829Arithmetical  
Mean = 1.65Arithmetical  
Mean = 1.00Arithmetical  
Mean = 3.29Standard  
deviation =  $\pm 0.2$ Standard  
deviation =  $\pm 0.23$ Standard  
deviation =  $\pm 0.42$ 

## APPENDIX I

## STATISTICAL DATA FOR 8:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.79	.52	.2704	1.46	.32	.1024	1.26	.59	.3481
.71	.56	.3136	1.20	.06	.0036	2.11	.26	.0676
1.01	.26	.0676	.51	.63	.3969	1.33	.52	.2704
1.35	.80	.6400	.93	.21	.0441	1.60	.25	.0625
.84	.43	.1849	1.16	.02	.0004	.69	1.16	1.3456
1.06	.21	.0441	1.09	.05	.0025	3.72	1.87	3.4969
1.36	.09	.0081	1.07	.07	.0049	2.66	.81	.6561
1.20	.07	.0049	.89	.25	.0625	1.07	.78	.6084
2.55	1.28	1.6384	1.56	.42	.1764	4.78	2.93	8.5849
1.09	.18	.0324	1.15	.01	.0001	3.23	1.38	1.9044
Total (d - $\bar{d}$ ) <sup>2</sup> = 3.2044			Total (d - $\bar{d}$ ) <sup>2</sup> = .7438			Total (d - $\bar{d}$ ) <sup>2</sup> = 17.3449		
Arithmetical Mean = 1.27			Arithmetical Mean = 1.14			Arithmetical Mean = 1.85		
Standard deviation = $\pm 0.19$			Standard deviation = $\pm 0.09$			Standard deviation = $\pm 0.43$		

## APPENDIX I

## STATISTICAL DATA FOR 9:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
.84	.84	.7056	.84	.39	.1521	.67	1.22	1.4884
1.46	.22	.0484	1.17	.06	.0036	2.85	.96	.9216
1.10	.58	.3364	1.07	.16	.0256	1.31	.58	.3364
3.15	1.47	2.1609	1.55	.32	.1024	2.30	.41	.1681
2.89	1.21	1.4641	1.37	.14	.0196	1.63	.26	.0676
.84	.84	.7056	1.31	.08	.0064	2.13	.24	.0576
1.38	.30	.0900	1.01	.22	.0484	1.04	.85	.7225
.82	.86	.7396	.98	.25	.0625	1.24	.65	.4225
1.85	.17	.0289	1.01	.22	.0484	2.31	.42	.1764
1.90	.22	.0484	1.40	.17	.0289	2.55	.66	.4356
Total (d - $\bar{d}$ ) <sup>2</sup> = 6.3279			Total (d - $\bar{d}$ ) <sup>2</sup> = .4979			Total (d - $\bar{d}$ ) <sup>2</sup> = 4.7967		
Arithmetical Mean = 1.68			Arithmetical Mean = 1.23			Arithmetical Mean = 1.89		
Standard deviation = $\pm$ 0.26			Standard deviation = $\pm$ 0.07			Standard deviation = $\pm$ 0.23		

## APPENDIX I

## STATISTICAL DATA FOR 10:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
.72	.46	.2116	2.05	.39	.1521	1.22	.72	.5184
.49	.69	.4761	1.90	.54	.2916	1.67	.27	.0729
.56	.62	.3844	.49	1.95	3.8025	.62	1.32	1.7424
1.72	.54	.2916	3.02	.58	.3364	1.83	.11	.0121
.68	.50	.2500	2.74	.30	.0900	2.43	.49	.2401
2.84	1.66	2.7556	4.08	1.64	2.6896	5.65	3.71	13.7641
1.02	.16	.0256	2.61	.17	.0289	.41	1.53	2.3409

$$\text{Total } (d - \bar{d})^2 = 4.3949$$

$$\text{Arithmetical Mean} = 1.18$$

$$\text{Standard deviation} = \pm 0.31$$

$$\text{Total } (d - \bar{d})^2 = 7.3911$$

$$\text{Arithmetical Mean} = 2.44$$

$$\text{Standard deviation} = \pm 0.41$$

$$\text{Total } (d - \bar{d})^2 = 18.6909$$

$$\text{Arithmetical Mean} = 1.94$$

$$\text{Standard deviation} = \pm 0.67$$

## APPENDIX I

## STATISTICAL DATA FOR 11:00 P.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
4.66	1.47	2.1609	3.60	1.01	1.0201	3.86	1.37	1.8769
3.28	.09	.0081	2.66	.05	.0025	.69	.80	.6400
3.42	.23	.0529	1.65	.96	.9216	.87	.62	.3844
3.46	.27	.0729	2.60	.01	.0001	2.38	.89	.7921
3.99	.80	.6400	3.59	.98	.9604	.84	.65	.4225
3.52	.33	.1089	2.04	.57	.3249	1.02	.47	1.0404
2.57	.67	.4489	3.15	.54	.2916	.89	.60	.3600
4.02	.83	.6889	4.58	1.97	3.8809	4.18	2.69	7.2361
.77	2.42	5.8564	1.07	1.54	2.3716	.38	1.11	1.2321

Total (d -  $\bar{d}$ )<sup>2</sup> = 13.8279    Total (d -  $\bar{d}$ )<sup>2</sup> = 9.7737    Total (d -  $\bar{d}$ )<sup>2</sup> = 13.9845

Arithmetical  
Mean = 3.19

Arithmetical  
Mean = 2.61

Arithmetical  
Mean = 1.49

Standard  
deviation =  $\pm 0.4$

Standard  
deviation =  $\pm 0.37$

Standard  
deviation =  $\pm 0.4$

## APPENDIX I

## STATISTICAL DATA FOR 12:00 Midnight

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
7.58	3.09	9.5481	5.06	.99	.9801	1.37	.17	.0289
3.68	.81	.6561	7.22	1.17	1.3689	1.20	.34	.1156
3.38	1.11	1.2321	7.91	1.86	3.4596	2.00	.46	.2116
5.07	.58	.3364	6.12	.07	.0049	1.24	.30	.0900
4.61	.12	.0144	5.22	.83	.6889	1.30	.24	.0576
5.44	.95	.9025	7.15	1.10	1.2100	2.70	1.16	1.3456
3.81	.68	.4624	5.01	1.04	1.0816	.76	.78	.6084
3.85	.64	.4096	5.20	.85	.7225	2.50	.96	.9216
1.88	2.61	6.8121	6.14	.09	.0081	.14	1.40	1.9600
3.07	1.42	2.0164	5.52	.53	.2809	1.38	.16	.0256
Total (d - $\bar{d}$ ) <sup>2</sup> = 22.3901			Total (d - $\bar{d}$ ) <sup>2</sup> = 9.8055			Total (d - $\bar{d}$ ) <sup>2</sup> = 5.3649		
Arithmetical Mean = 4.49			Arithmetical Mean = 6.05			Arithmetical Mean = 1.54		
Standard deviation = $\pm$ 0.49			Standard deviation = $\pm$ 0.33			Standard deviation = $\pm$ 0.49		

## APPENDIX I

## STATISTICAL DATA FOR 1:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
4.40	.81	.6561	4.60	.26	.0676	4.64	1.73	2.9929
3.55	.04	.0016	7.35	2.49	6.2001	2.38	.43	.1849
1.77	1.82	3.3124	3.22	1.64	2.6896	1.79	1.12	1.2544
3.44	.15	.0225	5.55	.69	.4761	2.78	.13	.0169
2.10	1.49	2.2201	4.62	.24	.0576	1.57	1.34	1.7956
4.18	.59	.3481	4.33	.53	.2809	1.93	.98	.9604
1.77	1.82	3.3124	3.69	1.17	1.3689	1.59	1.32	1.7424
2.85	.74	.5476	4.10	.76	.5776	1.60	1.31	1.7161
4.31	.72	.5184	6.21	2.35	5.5225	3.15	.24	.0576
5.18	1.59	2.5281	5.89	1.03	1.0609	5.92	3.01	9.0601
.59	3.00	9.0000	3.08	1.78	3.1684	.83	2.08	4.3264
8.46	4.84	23.4256	4.91	.05	.0025	4.91	2.00	4.0000
2.14	1.45	2.1025	6.28	1.42	2.0164	.94	1.97	3.8809
Total (d - $\bar{d}$ ) <sup>2</sup> = 47.9954			Total (d - $\bar{d}$ ) <sup>2</sup> = 23.4891			Total (d - $\bar{d}$ ) <sup>2</sup> = 31.9886		
Arithmetical Mean = 3.59			Arithmetical Mean = 4.86			Arithmetical Mean = 2.91		
Standard deviation = $\pm 0.55$			Standard deviation = $\pm 0.39$			Standard deviation = $\pm 0.45$		

## APPENDIX I

## STATISTICAL DATA FOR 2:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
3.89	.74	.5476	2.32	.70	.4900	3.52	1.23	1.5129
2.07	1.08	1.1664	3.26	.24	.0576	5.63	.88	.7744
3.62	.47	.2209	3.52	.50	.2500	4.71	.04	.0016
Total (d - $\bar{d}$ ) <sup>2</sup> = 1.9349			Total (d - $\bar{d}$ ) <sup>2</sup> = .7976			Total (d - $\bar{d}$ ) <sup>2</sup> = 2.2889		
Arithmetical Mean = 3.15			Arithmetical Mean = 3.02			Arithmetical Mean = 4.75		
Standard deviation = $\pm 0.57$			Standard deviation = $\pm 0.36$			Standard deviation = $\pm 0.61$		



## APPENDIX I

## STATISTICAL DATA FOR 3:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
2.95	.13	.0169	3.15	.18	.0324	8.86	.54	.2916
3.17	.09	.0081	3.02	.05	.0025	6.35	1.97	3.8809
3.21	.13	.0169	2.80	.17	.0289	9.22	.90	.8100
Total (d - $\bar{d}$ ) <sup>2</sup> = .0419			Total (d - $\bar{d}$ ) <sup>2</sup> = .0638			Total (d - $\bar{d}$ ) <sup>2</sup> = 4.9825		
Arithmetical Mean = 3.08			Arithmetical Mean = 2.97			Arithmetical Mean = 8.32		
Standard deviation = $\pm$ 0.08			Standard deviation = $\pm$ 0.25			Standard deviation = $\pm$ 0.91		

## APPENDIX I

## STATISTICAL DATA FOR 4:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
1.86	.51	.2601	1.83	.22	.0484	3.55	.53	.2809
3.81	.44	.1936	1.99	.06	.0036	2.26	1.92	3.6864
.91	1.46	2.1316	1.36	.69	.4761	3.98	.20	.0400
3.42	1.05	1.1025	2.20	.15	.0225	6.24	2.06	4.2436
1.85	.52	.2704	2.06	.01	.0001	4.32	.14	.0196
2.26	.11	.0121	3.75	1.70	2.8900	2.00	2.18	4.7524
1.54	.83	.6889	1.89	.16	.0256	4.21	.03	.0009
1.47	.90	.8100	2.65	.60	.3600	6.14	1.86	3.4596

Total (d -  $\bar{d}$ )<sup>2</sup> = 5.4692      Total (d -  $\bar{d}$ )<sup>2</sup> = 3.8263      Total (d -  $\bar{d}$ )<sup>2</sup> = 16.4834

Arithmetical Mean = 2.37      Arithmetical Mean = 2.05      Arithmetical Mean = 4.18

Standard deviation =  $\pm 0.31$       Standard deviation =  $\pm 0.26$       Standard deviation =  $\pm 0.17$

## APPENDIX I

## STATISTICAL DATA FOR 5:00 A.M.

CLEAR CELLS			CHROMOSOMAL STAGES			RECONSTRUCTION CELLS		
d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>	d	(d - $\bar{d}$ )	(d - $\bar{d}$ ) <sup>2</sup>
2.09	.22	.0484	1.12	.88	.7744	2.09	2.03	4.1209
3.05	1.18	1.3924	2.39	.39	.1521	4.35	.77	.5929
1.53	.34	.1156	1.63	.37	.1369	3.87	.25	.0625
1.69	.18	.0324	1.79	.21	.0441	4.47	.35	.1225
1.16	.71	.5041	1.40	.60	.3600	3.84	.28	.0784
2.58	.71	.5041	3.32	1.32	1.7424	4.51	.39	.1521
Total (d - $\bar{d}$ ) <sup>2</sup> = 2.5970			Total (d - $\bar{d}$ ) <sup>2</sup> = 3.2099			Total (d - $\bar{d}$ ) <sup>2</sup> = 5.1293		
Arithmetical Mean = 1.87			Arithmetical Mean = 2.00			Arithmetical Mean = 4.12		
Standard deviation = $\pm$ 0.29			Standard deviation = $\pm$ 0.33			Standard deviation = $\pm$ 0.41		

APPROVAL SHEET

The dissertation submitted by Lawrence E. Scheving has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

DATE

Jan 7, 1957

Arthur R. Gatz  
Signature of Adviser