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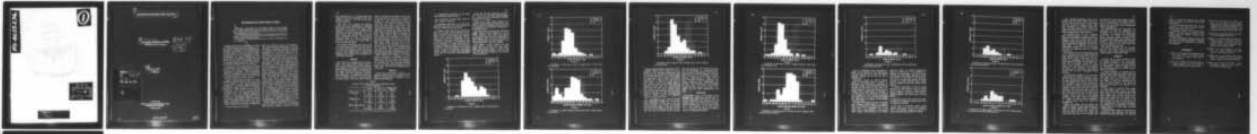
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**THE INCIDENCE OF HYPOGLYCEMIA IN FLIGHT**

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Department of Aviation Medicine

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Air University  
**SCHOOL OF AVIATION MEDICINE, USAF**  
RANDOLPH AFB, TEXAS  
February 1959

## THE INCIDENCE OF HYPOGLYCEMIA IN FLIGHT

Observation of 193 subjects, pre- and postflight, failed to reveal any clinical cases of relative hypoglycemia. There were 13 cases in which there was an in-flight drop in blood glucose of over 60 mg. percent; this was found to be correlated with a high carbohydrate-low protein breakfast.

Incidental findings demonstrated that the poor social hygiene of many of the pilots studied included not only poor dietary habits but also insufficient sleep and the consumption of alcohol the night before flight.

A description of the microtitration method used is included. Standardization data are also appended.

Hypoglycemia has been implicated as a cause of accidents involving pilot error. Lawton (1, 2) has reported several cases in which near misses, incidents, and accidents in the Flying Training Air Force appear to have been caused by pilot incapacitation due to hypoglycemia. Further investigation of relative hypoglycemia led Lawton (2) to the conclusion that this may well have been the cause of the marked increase in aircraft accident rates in the latter half of the morning as compared with the early half.

Improvement in the dietary habits of students was associated with a marked decrease in the accident rates. However, changes in training, supervision, maintenance, and indoctrination occurred simultaneously with the enforcement of adequate nutrition; hence, the association cannot be regarded as proof of causation.

Relative hypoglycemia produced by a sugar breakfast has been shown by Brent et al. (3) to be additive to the common physiologic disturbances of moderate hyperventilation and accelerative forces (3 g's for 5 seconds), leading to a high incidence of loss of consciousness of 10 to 15 seconds' duration in normal pilots. The remaining question is whether or not this relative hypoglycemia occurs in a normal pilot population, and, if so, what is the incidence? There are several ways of answering this question: (1) to determine how many, if any, eat a true carbohydrate breakfast; (2) to measure in-flight blood

sugar in a massive sample of the population, with suitable clinical observation; or (3) to investigate all reported near-misses, incidents, or accidents which might have been caused by hypoglycemia in the pilot. Reports are accumulating which indicate that at least a few such episodes may have been due to hypoglycemia, in conjunction with other imbalances.

Several studies have been made on blood sugar levels of various pilot populations. One investigation (4), made on a group similar to the one used in this study, was hampered by the nonavailability of research laboratory facilities; the use of a clinical laboratory for research purposes is undesirable. Two larger studies reported by Robbins et al. (5, 6) were made under conditions in which flight stress, times of take-off and landing, and preflight eating times could not be controlled because of the nature of the flying mission. Further difficulties were encountered by the relatively small samples obtained from each population at risk. Even if the entire pilot error accident rate described by Lawton (1) for 1955 were due to hypoglycemia, this would constitute one case of hypoglycemia causing an accident in every 8,620 flights of 2 hours' duration. As a result of this consideration, one can hope to arrive at a meaningful concept of the role of hypoglycemia only after obtaining a large sample based on a uniform population at risk.

Another difficulty encountered in these projects was that the technic used for measuring blood glucose required a quantity of blood

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that demanded the use of venous blood, thus adding the variables of peripheral blood flow, tourniquet effect (7), and local tissue carbohydrate metabolism.

Thus these very excellent and intensive studies had to be supplemented by additional field studies which were oriented more toward determining the incidence of relative hypoglycemia. A population was needed that was well-controlled and homogeneous as to in-flight stress, flight duration, time of take-off, flying experience, and age. Of even greater advantage would be a flying population in which the risk of flying at various times of day was already determined.

The method of determining blood sugar levels was to be selected on the basis of two criteria: the technic must require no more than 0.1 cc. of blood, thus allowing the use of capillary blood, and the method must be reliable after equipment and reagents were transported for considerable distances.

#### METHOD

The study was accomplished at two single-engine (T-33) basic flying training bases in Texas. The investigator took blood specimens from students and instructors each morning, before and after each flight. Participation was not strictly enforced but most of the officers and cadets gave full cooperation. Capillary blood specimens were obtained by finger puncture of sufficient depth to avoid squeezing the finger unduly. The blood glucose concen-

tration was measured according to the micro-method of Miller and Van Slyke (8) described in appendix I and standardized as shown in appendix II. After the last blood specimen was taken, each individual completed a form, giving his name, rank, serial number, total and jet flying experience, marital status, type of quarters, time in and out of bed the preceding night, time of take-off and landing, and complete dietary intake during the preceding 24 hours. Some subjects made two flights the same morning; three specimens were obtained from these persons, one before the first flight, one between flights, and one after the second flight. Each subject was used only one day. The purpose of the experiment was not explained to the subject until the experimentation was completed. While the postflight blood specimens were being obtained, the subjects were observed for signs of hypoglycemia, and appropriate questions were asked as to symptoms.

Data obtained were tabulated for a mathematical description of preflight and postflight blood glucose values and differences between the two. These data were examined for correlation with diet (especially breakfast of the day of flight), flying experience, marital status, rank, and time in bed during the preceding night.

#### RESULTS

For the 193 person-flights in which pre- and postflight blood sugars were obtained, no in-flight or postflight signs or symptoms of hypoglycemia were observed.

TABLE I  
Summary of blood glucose values, given in milligrams percent, observed in the study

	Flight 1	Flight 2 only	Flight 2, after Flight 1
Preflight mean	132.3	122.0	119.4
$\sigma$	$\pm 22.3$	$\pm 16.7$	$\pm 23.5$
N	87 persons	102 persons	23 persons
Postflight mean	114.4	114.0	115.1
$\sigma$	$\pm 17.5$	$\pm 17.1$	$\pm 18.0$
N	91 persons	87 persons	23 persons
Mean in-flight change	-17.6	-10.3	-3.48
$\sigma$	$\pm 28.3$	$\pm 22.0$	$\pm 22.0$
N	84 persons	86 persons	23 persons

A mathematical description of the blood sugars obtained is shown in table I.

The distribution of blood sugar values is shown in figures 1 through 9.

No significant differences, other than those associated with diet, were found between instructors, student officers, and cadets.

A change of more than 60 mg. percent was found in 15 cases; 2 were increases and 13 were decreases of more than 60 mg. percent. Eleven of the 13 had returned their questionnaires with sufficient information for the evaluation of diet. It was found that 4 of the 11 had had a breakfast of considerable sugar with no protein, or a sugar (and other carbohydrate) breakfast incidence of 36.4 percent. In addition, 2 more had consumed a very large amount of sugar for breakfast along with a minimal amount of protein. Only one denied eating sugar; his meal was of high carbohydrate content.

Among the 134 other respondents, however, only 13 had had no protein preceding flight, or a sugar and other carbohydrate breakfast incidence of 9.7 percent.

Among those whose blood glucose level changes had decreased less than 60 mg. percent, no significant differences could be found in the protein or carbohydrate consumption on the morning of flight.

Diet was directly correlated with commission status. Cadets almost invariably ate an excellent breakfast, since they were required to eat at the cadet mess hall. Student officers and instructor pilots were less consistent in their eating habits, but most managed to get milk and some other protein for breakfast.

The amount of sleep obtained the night before did not correlate with rank, marital status, diet, alcohol intake, or blood sugar. The mean number of hours in bed was 6.86 hours (6 hours and 50 minutes), with extremes of 4½ and 9½ hours.

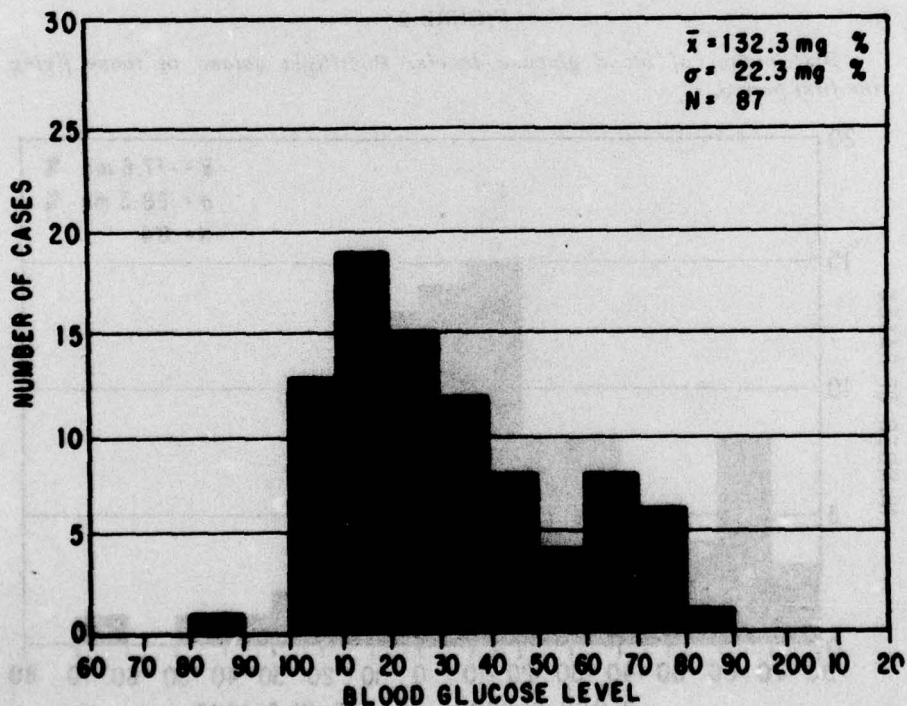


FIGURE 1

*Distribution of blood glucose levels. Preflight values in those flying the first period.*

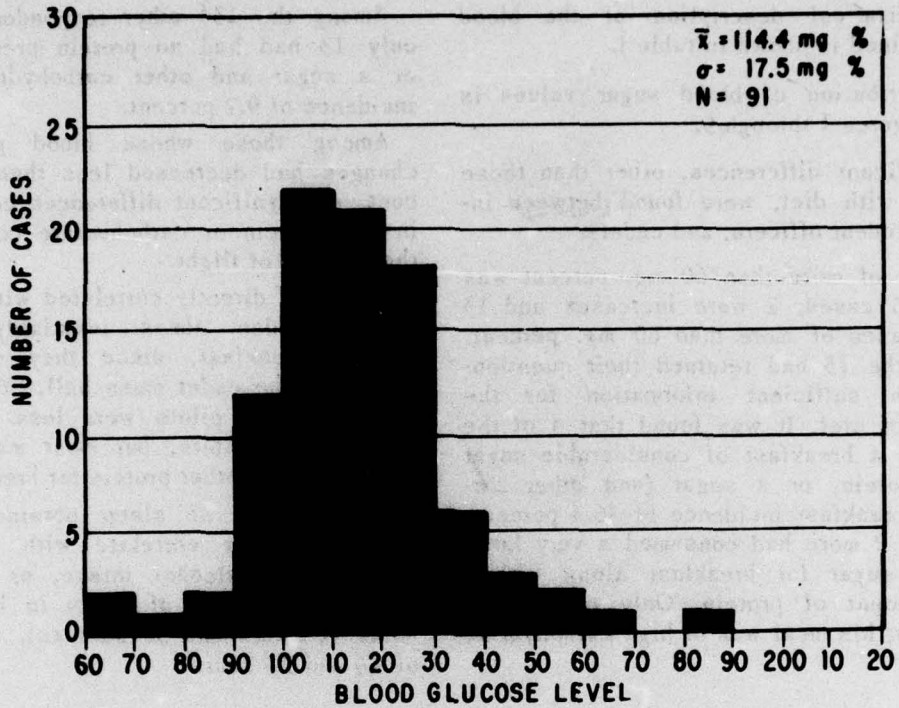


FIGURE 2

*Distribution of blood glucose levels. Postflight values in those flying the first period.*

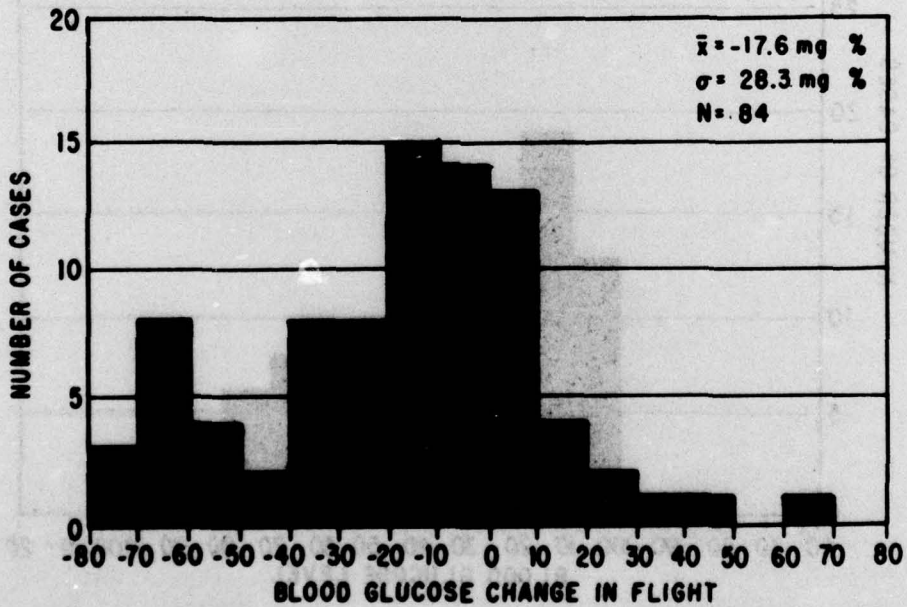


FIGURE 3

*Distribution of in-flight changes in blood glucose levels. Changes during the first period.*

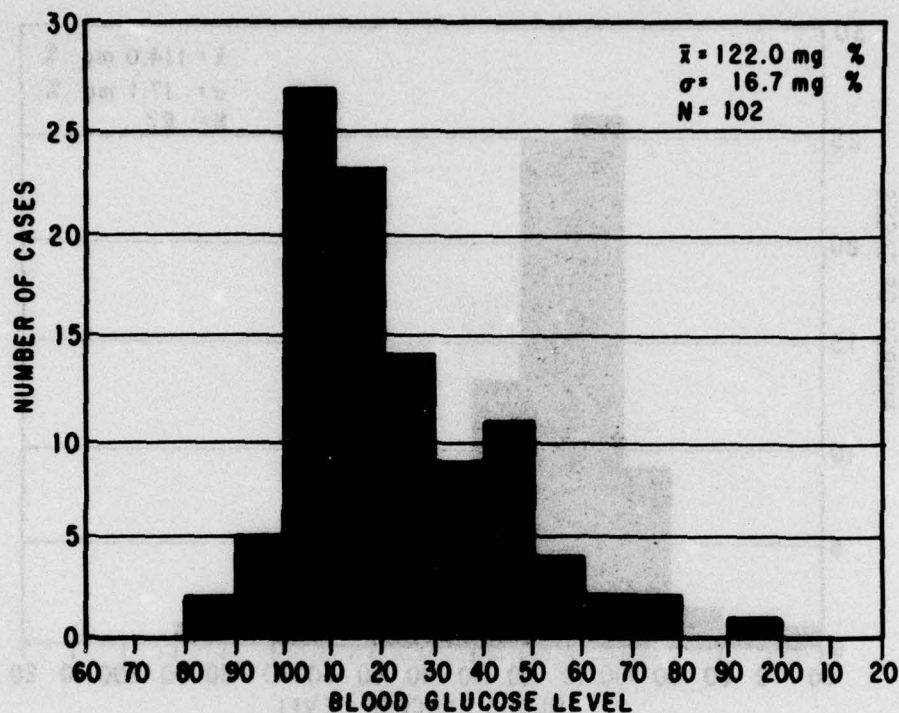


FIGURE 4

*Distribution of blood glucose levels. Preflight values in those flying the second period only.*

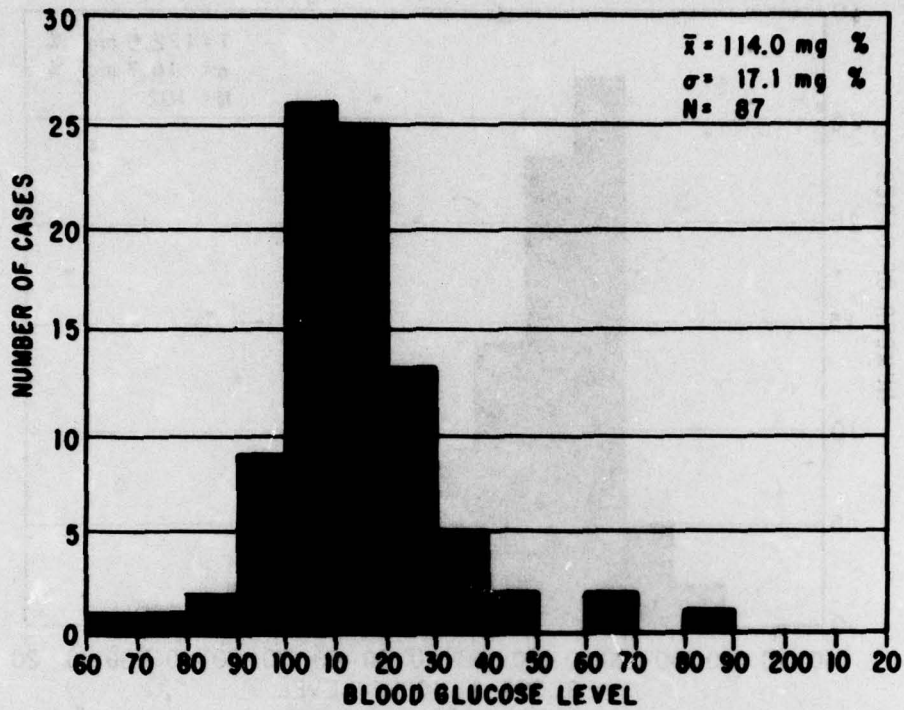
Alcohol intake during the preceding 24 hours accidentally proved to be a check on the reliability of the answered questionnaires. On one occasion the observation followed a night on which the officers ate at the Officer's Club and drank many toasts. Despite the possible consequences of admitting the intake of alcohol within 12 hours of flying, most of the instructor pilots acknowledged moderate drinking—in most cases not more than two drinks and these early in the evening. Forty-four percent of all student officers and instructor pilots made this admission; the cadets indicated virtually no intake of alcohol. The intake of alcohol showed little correlation with diet or blood sugar values.

The experimental design did not require field modification. Weather was the main difficulty encountered; several days in the field were practically lost because low ceilings prevented training flights or delayed take-offs so that only one flight period could be used. This time was not totally wasted, however.

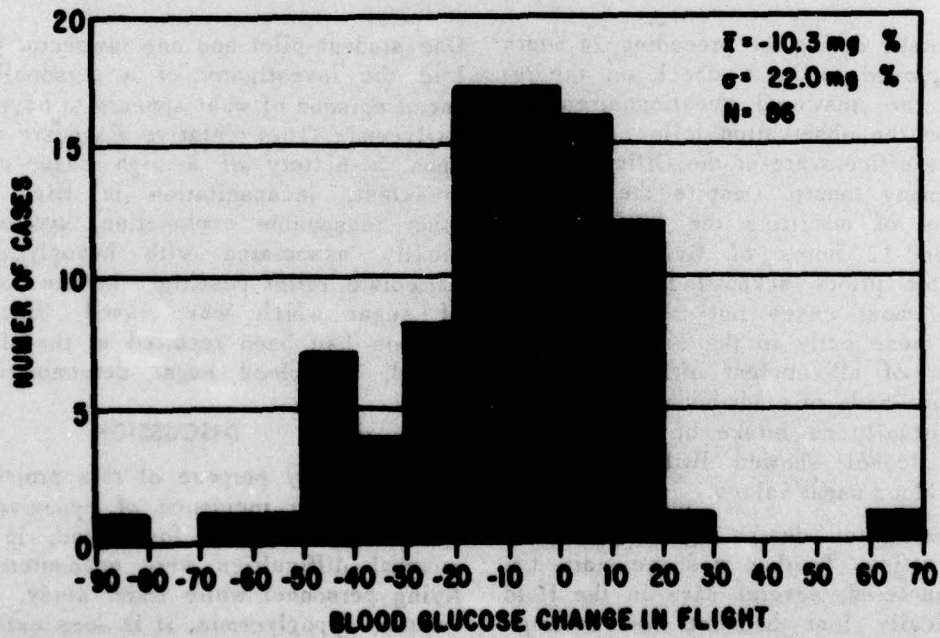
One student pilot and one instructor pilot each told the investigator of a personally experienced episode of what appears to have been hypoglycemia. This tentative diagnosis was based upon a history of a high sugar-no protein breakfast, incapacitation in flight with no other reasonable explanation, symptoms classically associated with hypoglycemia, and immediate relief postflight by the consumption of sugar which was craved. Since neither episode had been reported at the time it occurred, no blood sugar determinations were made.

#### DISCUSSION

The primary purpose of this project was to determine the incidence of hypoglycemia. No clinical cases were found and, in fact, no unusual difficulties were encountered by the flying personnel while under study. Therefore, relative hypoglycemia, if it does exist in fact, has a true incidence ( $P$ ) of 0.00 percent  $< P \leq 1.54$  percent, with 95 percent confidence limits, in the population sampled.



**FIGURE 5**  
*Distribution of blood glucose levels. Postflight values in those flying the second period only.*



**FIGURE 6**  
*Distribution of in-flight changes in blood glucose levels. Changes in those flying the second period only.*

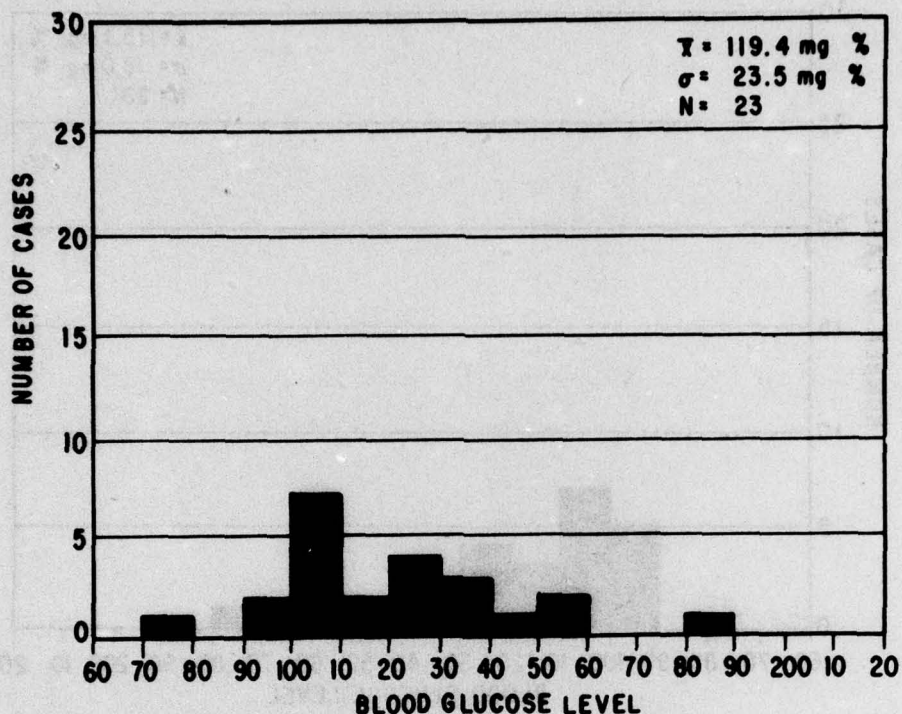


FIGURE 7

*Distribution of blood glucose levels. Preflight values for the second flight in those flying both periods.*

Since no difficulties were encountered in-flight, by the subjects of this investigation, the data cannot be used to prove that various episodes in-flight are or are not due to hypoglycemia.

Present-day studies in aircrew members cannot be interpreted either as proving or disproving that hypoglycemia contributed to the high accident rates experienced by Flying Training Air Force prior to July 1955; it is certain that now, in the single-engine jet basic training plane, hypoglycemia is uncommon if it exists at all. The importance of eating a good breakfast has been strongly emphasized for several years, and the correction of cadet diet deficiencies has probably already greatly altered the picture.

In spite of the emphasis placed on teaching pilots adequate dietary habits, it is apparent that once the pilots have completed this training, many revert to an undesirable diet. If one grants that a sugar breakfast, in association with moderate hyperventilation and aircraft

maneuver during the relative hypoglycemic phase can lead to loss of consciousness or difficulty in coordination, then the incidence of sugar (and other simple carbohydrate) breakfasts shown in this study is of importance. As long as an appreciable number of aircraft members are eating improperly these persons must be sought out and their dietary habits reformed.

A definite relationship of diet to marked decreases in blood glucose levels was found in this study, in contrast to similar studies done by Robbins and others at the School of Aviation Medicine. This may be due to several factors:

1. The use of arterial blood minimizes the effect of changes in the peripheral vascular system which are readily seen postflight.

2. The study of a population flying a regular schedule means there will be less clouding of data by the important variables of time interval between eating and flying, and duration of flight.

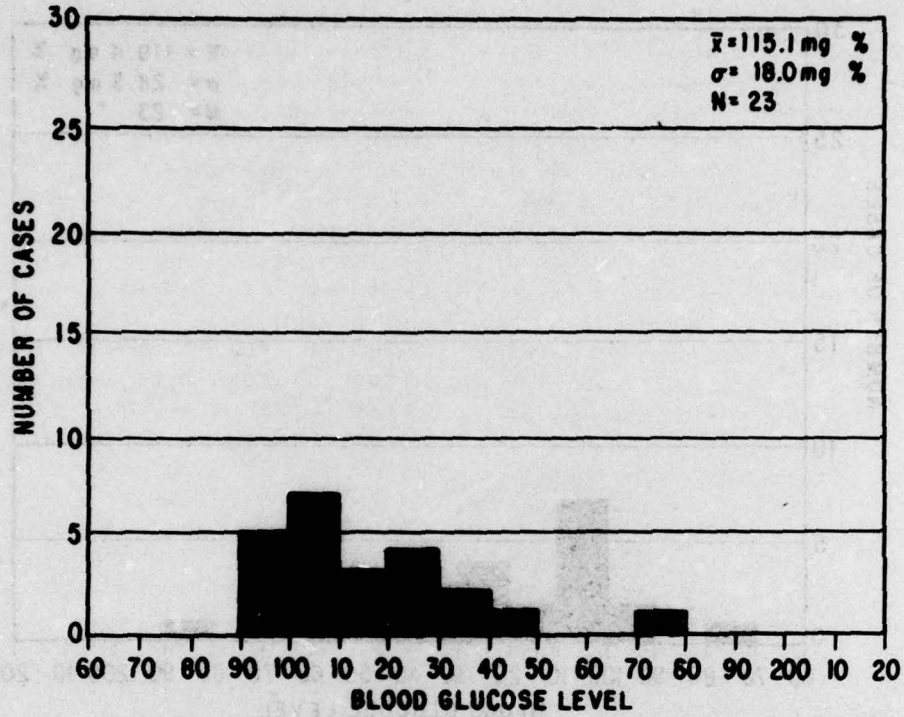


FIGURE 8

*Distribution of blood glucose levels. Postflight values for the second flight in those flying both periods.*

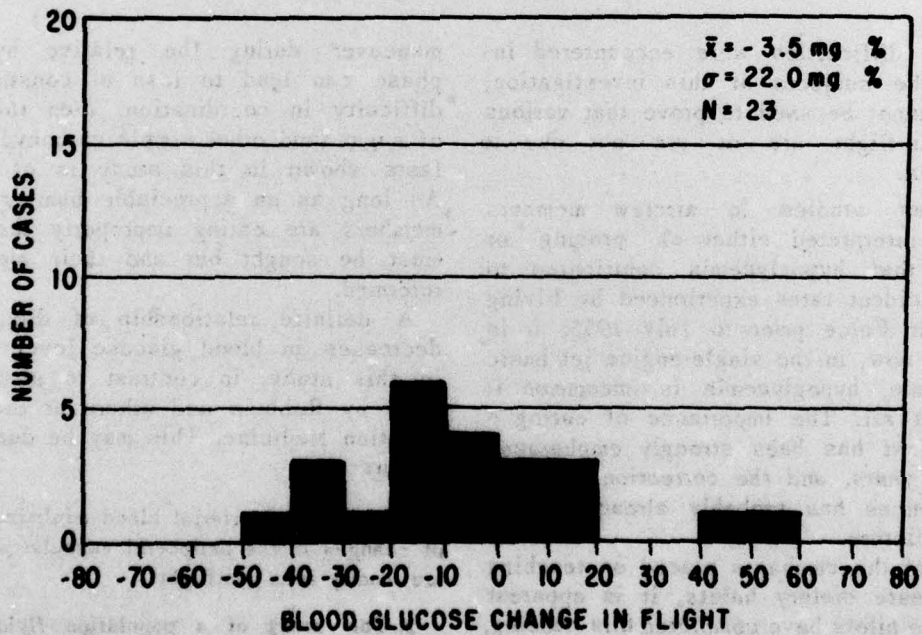


FIGURE 9

*Distribution of in-flight changes in blood glucose levels. Changes during the second period in those flying both periods.*

3. The essentially homogeneous nature of the population was such that the students and instructors fell within very narrow ranges of age, flying experience, physiologic training, personal equipment maintenance, and attitudes toward flying safety and general hygiene. Every individual, for example, had heard the lectures given by Colonel Lawton on hyperventilation and hypoglycemia.

As mentioned above, the use of capillary blood for measurement of glucose levels removes certain variables in blood glucose levels inherent to the use of venous blood. These variables include peripheral vascular changes, local tissue glucose metabolism, and the tourniquet effect. It is suggested, therefore, that considerable caution be used in comparing data from this experiment with that of similar experiments.

One possible flaw in the experimental design is the assumption that the blood obtained by finger puncture before flight, which is essentially arterial blood in appearance and composition, is comparable to the blood taken by finger puncture postflight. The postflight specimen may, for example, consist of a more venous blood, although there was no marked difference in color. Because of the difficulty in obtaining true arterial blood, this particular question has not been investigated during this study.

Two incidental findings of the study merit consideration: the general deficiency in sleep and the excessive numbers consuming alcohol the night before flying. Almost 7 hours in bed may be sufficient rest for many persons, but it is difficult to believe that persons having had only  $4\frac{1}{2}$  to 6 hours of rest are as capable in-flight as they would have been if they had received a full 8 hours of sleep. It does not seem likely that the half of the sample group who had received less than 6 hours and 50 minutes of time in bed were in top physical condition. It may well be that more accidents are caused by falling asleep, drowsiness, or simple fatigue, than are caused by hypoglycemia.

Among the officers, the 44 percent who consumed alcohol the night before flying also appear to warrant special effort for improvement of aircrew effectiveness. Those who consumed more than one or two drinks, and those who drank later in the evening, could

not have been in the best possible condition. It is not the purpose of this report to discuss crew rest and avoidance of alcohol, but it is easily seen that these basic considerations must be re-emphasized.

The microtitration technic was found to be satisfactory for field use. Difficulties encountered in the use of cotton filters were as follows: (1) Filtration was considerably slower than was desired. (2) Occasionally enough precipitant would run through to ruin the sample. Very slight amounts of precipitant suffice to elevate the final reading markedly. (3) Repeat filtration due to filter failure was a source of delay and confusion.

One further difficulty with the procedure was that varied light sources for observing indicator color change compromised the accuracy of titration, especially at night.

#### SUMMARY

1. By the use of clinical criteria no cases of in-flight hypoglycemia were found among 193 subjects studied at two single-engine jet basic training bases. The true incidence of hypoglycemia occurring in-flight in this type of population study is therefore between 0.00 and 1.54 percent.

2. Thirteen instances of a decrease of over 60 mg. percent in-flight were found by measuring capillary blood sugar immediately before and after flight. The 11 in this group that could be evaluated were found to have had a breakfast higher in sugar and carbohydrates and lower in protein content on the morning of flight than was found in the remainder of the population.

3. Mean values of preflight blood sugars were significantly higher in the first flight period than the second; postflight mean blood sugar values were virtually identical. The mean in-flight change in blood sugar concentration was a drop of 17.6 mg. percent in the first flight period, 10.3 mg. percent in the second period, and only 3.5 mg. percent in the second period in those flying both periods. The probability of the differences of in-flight changes being due to chance alone is between 5 and 10 percent.

4. While in-flight changes in blood glucose concentration were correlated with diet and therefore with commission status, no correlation

could be found with preflight rest, marital status of officers, or alcohol intake the night before flight.

5. Rest the night before flight was of a mean duration of 6 hours and 50 minutes, with extremes of 4½ hours and 9½ hours.

6. Forty-four percent of instructor pilots admitted to having consumed alcohol the preceding night; the majority consumed fewer than three drinks.

7. The micro-method of Miller and Van Slyke was found to be useful and reliable in measuring the blood glucose concentration in finger-tip blood samples.

### REFERENCES

1. Lawton, W. H. Physiological investigations in the flying safety program of the Flying Training Air Force. U. S. Armed Forces Med. J. 8:937-944 (1957).
2. Lawton, W. H. Briefing for Advanced Course in Aviation Medicine, Class 57A, School of Aviation Medicine, USAF, 19 October 1956.
3. Brent, H. P., T. M. Carey, T. J. Powell, J. W. Scott, W. G. R. Taylor, and W. R. Franks. Synergism between effects of hyperventilation, hypoglycemia, and positive acceleration. Presented at the 28th Annual Meeting of the Aero Medical Association, Denver, 8 May 1957.
4. Base Aircrew Effectiveness Report, Webb AFB. Report period ending 27 March 1957.
5. Robbins, J. H. In-flight studies of hypoglycemia. Research thesis for Advanced Course in Aviation Medicine, Class 57A, April 1957. Copies on file at the Aeromedical Library, School of Aviation Medicine, USAF.
6. Robbins, J. H., C. H. Kratochvil, J. P. Ellis, and T. R. Howell. In-flight blood sugar studies. J. Aviation Med. (In press)
7. Loughlin, W. C., H. O. Mosenthal, and R. Halpern. Effect of tourniquets on venous blood sugar values. J. Lab. & Clin. Med. 28:1165 (1943).
8. Miller, B. F., and D. D. Van Slyke. A direct microtitration method for blood sugar. J. Biol. Chem. 114:583-595 (1936).

## APPENDIX I

THE MILLER AND VAN SLYKE DIRECT MICROTITRATION METHOD  
FOR BLOOD SUGAR (8)

This is a direct titration method for the sugar in 0.1 cc. of blood. The blood is deproteinized (to less than 1.5 percent nonfermentable reducing material) with cadmium and hydroxide ions, the excess cadmium removed with barium carbonate, and the filtrate heated with a large excess of ferricyanide. The ferrocyanide produced is titrated with ceric sulfate solution, using setopaline C<sup>®</sup> as the indicator for redox potential.

Only two precise measurements are needed—the initial blood volume and the volume of the final titration solution. The ceric sulfate is diluted so that 0.01 cc. of reagent indicates 1 mg. percent of blood sugar.

The following reagents are needed:

1. *Alkaline ferricyanide solution.* A 5.00 gm. quantity of  $K_3Fe(CN)_6$  and 10.6 gm. of anhydrous  $Na_2CO_3$  are dissolved in water and made up to 1 liter. This ferricyanide must be free of ferrocyanide. The solution will keep for months when it is protected from light and dust.

2. *Sulfuric acid, approximately 18 normal.* Add 465 cc. of reagent grade concentrated sulfuric acid (sp. gr. 1.84) to 535 cc. of water. Test the concentrated acid for reducing materials as follows: to 20 cc. of acid and 60 cc. of water add 0.05 cc. of approximately 0.1 normal  $KMnO_4$ . Pink color must persist for 5 minutes.

3. *Setopaline C<sup>®</sup> solution.* Dissolve approximately 25 mg. in 25 cc. of distilled water. Best results are obtained with solutions made fresh daily.

4. *Stock solution 0.1377 normal ceric sulfate.* Approximately 110 gm. of anhydrous ceric sulfate are weighted into an 800-cc. beaker; 35 cc. of concentrated sulfuric acid and 35 cc. of water are then added. Heat, with stirring, and continue to add water until almost all ceric sulfate is dissolved. Filter, cool, and dilute to 1 liter. Standardize with ferrous ammonium sulfate (Mohr's salt), prepared by dissolving 39.214 gm. in 250 cc. of water, adding 25 cc. of the 18 normal sulfuric acid, and diluting to 1,000 cc. for a 0.1 normal solution. Add ceric sulfate, in 15 cc. portions, to 50 cc. of water and 3 cc. of 18 normal sulfuric acid. Titrate with the Mohr's solution until color is almost bleached out. Add 8 drops of setopaline C<sup>®</sup> indicator solution and continue titrating until the color change of golden brown to light yellow color. Normality of the ceric sulfate is calculated as (volume of Mohr's solution/volume of ceric sulfate)  $\times$  0.1000. The ceric sulfate solution is then diluted to 0.1377 normal ceric sulfate, which will keep well as a stock solution stored in a dark bottle. One liter will suffice for about 50,000 determinations.

5. *Dilute ceric sulfate solution for sugar titration.* This solution should be prepared fresh each day. Place 2 cc. of the 0.1377 normal stock ceric sulfate solution into a 100-cc. volumetric flask, add 5 cc. of 18 normal sulfuric acid, and dilute to the mark with distilled water.

6. *Acid cadmium sulfate solution.* A 13-gm. quantity of reagent grade cadmium sulfate ( $3 CdSO_4 \cdot 8H_2O$ ) and 63.5 cc. of exactly 1 normal sulfuric acid are diluted to 1 liter with distilled water.

7. *Sodium hydroxide, 1.1 normal solution.* This must be made from reagent grade sodium hydroxide. Although this solution is not used in the microprecipitation method, it is an easily transported stock solution for making 0.275 normal sodium hydroxide.

8. *Sodium hydroxide, 0.275 normal.* This is prepared by dilution of the 1.1 normal sodium hydroxide.

9. *Sulfuric acid, 0.275 normal, solution.*

10. *Barium carbonate powder.* This should be of the highest purity obtainable.

The procedure is as follows:

Place 4 cc. of the acid cadmium sulfate solution in a 15 x 150 mm. test tube. Add 0.1 cc. of blood from a calibrated pipet. Rinse the interior of the pipet with the solution. After laking has occurred, add 2 cc. of the 0.275 normal sodium hydroxide solution. Specimens at this stage may be stored for 4 hours at room temperature or for 24 hours at 0° C.

Heat the solution in a boiling water bath for 3 minutes; cool in running tap water for 2 minutes. Add approximately 0.3 gm. of the barium carbonate powder, measured in a small spoon. Stopper the tube with the thumb and shake for 10 to 12 seconds. Filter through a small filter plug of washed cotton into a 50-cc. receiving test tube, washing the filter with three 4-cc. portions of distilled water. A graduated 25-cc. pipet may be used for this. For emergency blood sugars, a 7-cm. *washed* filter paper may be used instead of the cotton.

A 2-cc. portion of the alkaline ferricyanide reagent is added and mixed. The mixture should be a perfectly transparent yellow color. Heat evenly in a boiling water bath for 15 minutes; then cool in running tap water for 3 minutes. Add approximately 1 cc. of the 18 normal sulfuric acid, followed by 7 drops of the setopaline C® indicator solution. Titrate with the 0.002754 normal ceric sulfate, using a white light against a white background. The end point is a sharp change from golden yellow to golden brown. A buret is used which permits measuring the delivered solution to within 0.01 cc. The titration should be done fairly quickly, since other slow reactions occurring tend to reoxidize the ceric sulfate.

Calculation is simple; the cubic centimeters of solution delivered, minus the blank, are multiplied by 100 to give the result directly in milligrams of sugar per 100 cc. of blood.

Blank determination is as follows: 2 cc. of 0.275 normal sodium hydroxide, 2 cc. of 0.275 normal sulfuric acid, 14 cc. of water, and 0.3 gm. of barium carbonate are mixed and shaken for 12 seconds. Centrifuge, and pour 15 cc. of the supernatant fluid through a cotton filter into a receiving tube. Add 2 cc. of alkaline ferricyanide solution, and finish as described above. Blanks average about 0.12 cc., and remain constant for weeks.

Cotton for filters should be of good absorbent quality; wash for 2 days in running water, soak for 8 hours in distilled water, and then dry in a place protected from dust.

A mean normal fasting blood glucose level for this method is 83 mg. percent for venous blood and 92 mg. percent for arterial blood, with standard deviations of 4 mg. and 3 mg., respectively.

## APPENDIX II

## CALIBRATION AND STANDARDIZATION OF TECHNIC

After some initial difficulty with the cotton filters, a series of venous blood specimens was run on several blood specimens, as shown in the table below:

Blood specimen	Number of titrations	Mean value (mg. percent)	Standard deviation (mg. percent)
1	12	82.4	4.8
2	12	82.7	4.6
3	12	77.4	3.2
4	12	73.4	7.3
5	12	95.0	6.2
6	12	72.9	4.3

Blood for several titrations was obtained from a single finger-tip wound in each of 2 persons; the results are as follows:

Subject	Number of titrations	Mean value	Standard deviation (mg. percent)
1	6	119.0	4.3
2	5	124.5	4.3

Multiple blanks were run; the mean value for blanks was found to be 13.4 mg. percent, with a standard deviation of 0.3 mg. percent. The mean values of blood sugar determinations were found to be 20 mg. percent less than those obtained by the modified Folin-Wu method currently used by the U. S. Air Force clinical laboratories. A comparison of values showed this difference to have a standard deviation of 5.5 mg. percent.

One great virtue of the microtitrimetric method was discovered early: the main sources of error, which are protein in the filtrate and overshoot during titration, are plainly visible. Therefore, a note may be entered in the record at the time of error, before calculation, allowing for the fair discard of one sample from further computation.