

AN INVESTIGATION OF THE RELATIONSHIP BETWEEN
MENTAL DEFICIENCY AND HYPOPITUITARISM

A Thesis

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CHAPTER I

THE PROBLEM AND DEFINITIONS OF TERMS USED

Medical science began the investigation of mental deficiency; and psychology and education, having adopted and nurtured it for a few years, have realized the necessity for a joint investigation of the problem. For if there is mental impairment, might not some structural impairments also exist?

The thyroid gland became one of the primary targets for such research in the area of mental deficiency. Little has been done, however, to investigate the relationship between the "master" gland (pituitary) and mental deficiency. Since the pituitary functions as a control over the endocrine system and indirectly influences other activities,¹ it would seem logical that research begin here. Because of the gland's location, delicate nature, and complexity, research has avoided it except for some animal studies, for the most part unrelated to humans.

If the accepted role of the pituitary gland as the center of endocrine activity is true, a significant relationship between mental deficiency and the function of

¹Catherine P. Anthony, Textbook of Anatomy and Physiology, p. 489.

this gland might exist. To determine if this was true, a study was needed to determine the correlation between mental deficiency and pituitary insufficiency (hypopituitarism).

I. THE PROBLEM

Statement of the problem. It was the purpose of this study to determine the relationship which exists between pituitary dysfunction and mental deficiency.

II. DEFINITIONS OF TERMS USED

Mental deficiency. Mental deficiency is a term subject to much disagreement in the area of definitions. For the purpose of this study, however, an operational definition was used to supply the basis for examination of the sample. This was achieved by combining the definitions of Heber and Ingram. "Mental retardation refers to subaverage intellectual functioning which originates during the developmental period..."² "approximately 5-75 IQ"³ on a standardized individual intelligence test.

Pituitary function. The pituitary gland is

²Rick Heber, "Definition of Mental Retardation," Mental Retardation, Jerome H. Rothstein (ed.). p. 9.

³Ibid., p. 12.

divided into three lobes: anterior, posterior, and intermediate. Each lobe is responsible for the production of certain hormones which affect certain "target area". This can best be illustrated by the diagrams in Figures 1 and 2.

Hypopituitarism. Several conditions were investigated as a part of the general dysfunction of the pituitary gland referred to as hypopituitarism. For the purpose of this study, hypopituitarism has referred to the collective conditions of the failure of the pituitary gland to function at the normal level.

The 1964 Current Medical Terminology of the American Medical Association indicated the following significant laboratory findings resulting from hypopituitarism: (1) low basal metabolism rate, (2) increased carbohydrate tolerance, (3) insulin sensitivity, (4) abnormal or decreased urine gonadotropin, and (5) hypoglycemia.

Added conditions of panhypopituitarism syndrome as listed to include: (1) anemia, (2) reduced corticoids in urine, and (3) flat glucose tolerance curve.

Kugelmass reports on the general retardation of vital functions in hypopituitarism. Laboratory findings indicate:

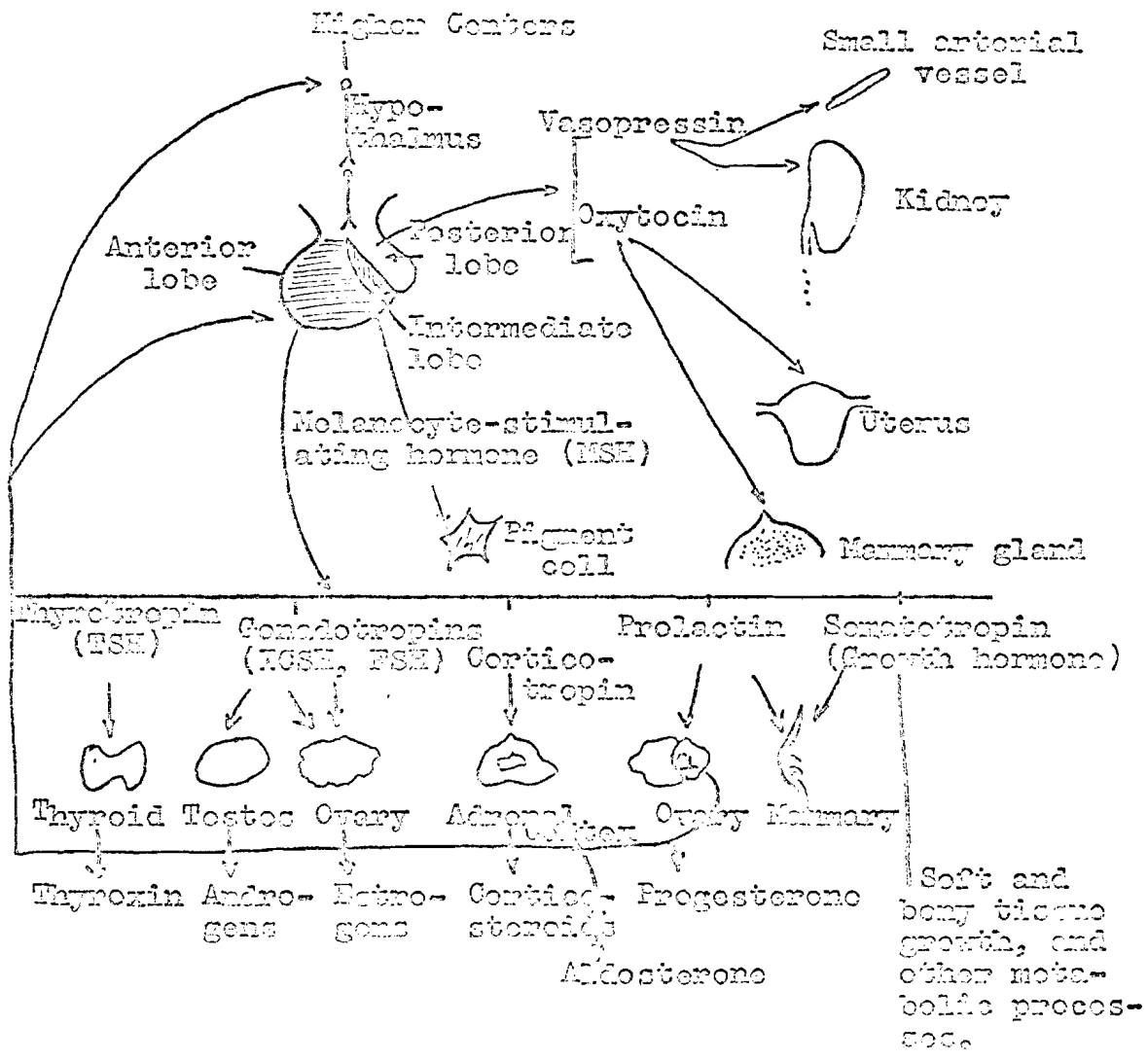


FIGURE 1

RELATIONSHIPS AMONG THE ANTERIOR AND POSTERIOR
 PITUITARY GLANDS AND THEIR TARGET STRUCTURES
 (FROM HARRIS, BE AD., BIOCHEMISTRY)

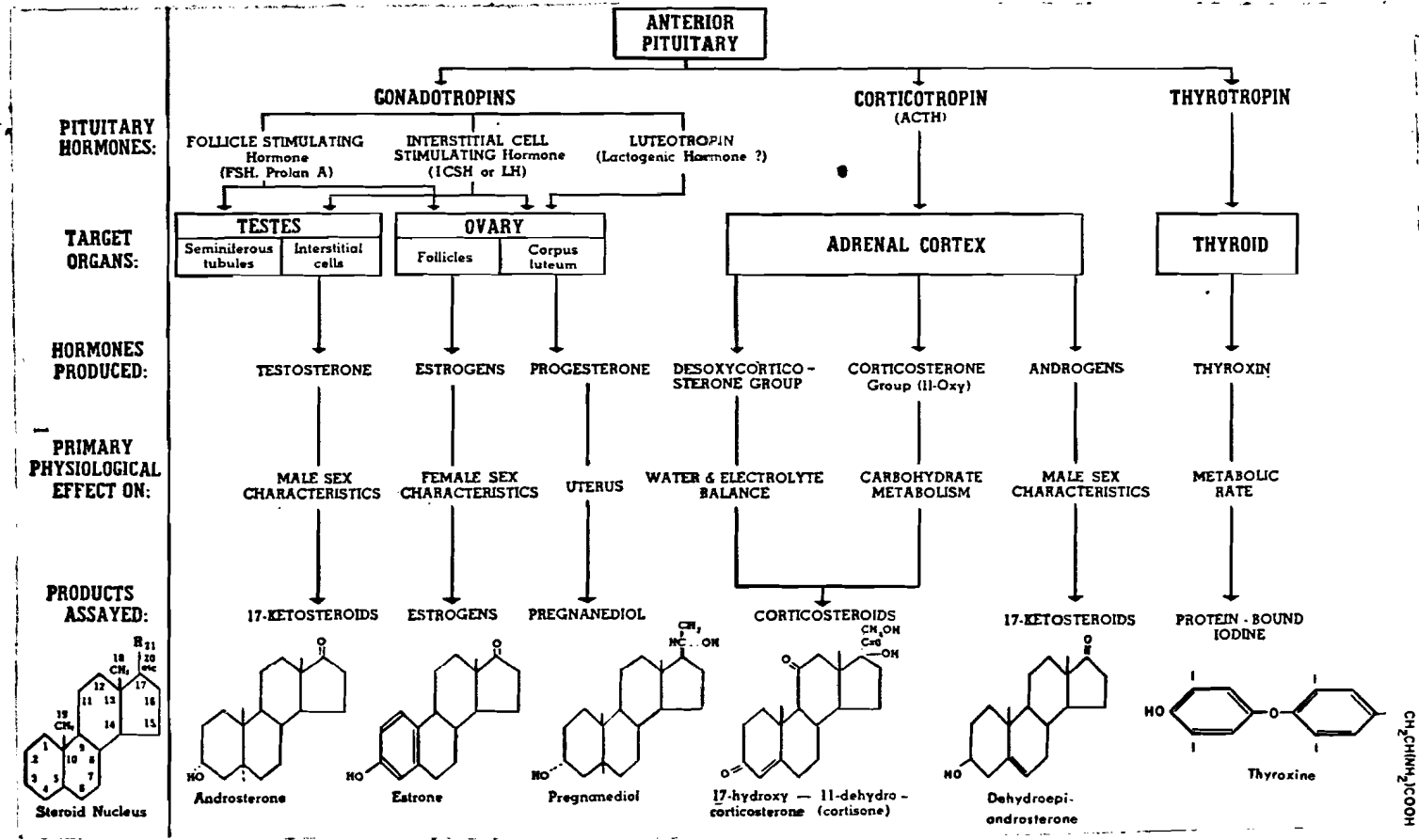


FIGURE 2'
ANTERIOR PITUITARY HORMONES
(FROM BIO-SCIENCE LABORATORIES)

1. Marked reductions of the basal metabolism rate.
2. Some elevation of the serum cholesterol.
3. Moderate decrease in the fasting blood sugar level.
4. Abnormality of the dextrose tolerance curve.
5. Marked sensitivity to insulin.
6. Moderate hypochromic anemia.
7. Leukopenia with relative lymphocytosis and eosinophilia.
8. Decrease in the urinary excretion of the neutral 17-ketosteroids.⁴

⁴J. Newton Kugelmass, "Mental Deficiency of Hypopituitarism in Childhood," The New York State Journal of Medicine, 52:1655-1659, July, 1952.

CHAPTER II

REVIEW OF THE LITERATURE

Research involving hypopituitarism is scarce and sporadic. In 1928, Fox presented a case for glandular therapy for the purpose of increasing the IQ.⁵ Endocrine importance was recognized early because of the Cretin. Here the mental and physical deficiency made an obvious case for hypothyroidism. Other endocrine glands were recognized and their hormones used as therapy on the mental deficient. No prior diagnosis was performed, however, to determine any glandular malfunction. The therapy was, in fact, based on very little research concerning the function and integration of the separate endocrine glands; and the conditions associated with glandular malfunction were hardly known.

Menninger stated that there was "no uniformity of association of any mental picture with any type of pituitary disorder."⁶ Gordon, et al., described the association of mental retardation occurring with endocrine

⁵Edna J. Fox, "An Investigation of the Effects of Glandular Therapy on the Intelligence Quotient," Mental Hygiene, 12:90-102, 1928.

⁶W.C. Menninger, "Mental Disturbances Associated with Pituitary Disorders," Journal of the American Medical Association, 91:951-955, 1928.

disorders in the following order: childhood myxedema, hypothyroidism, adiposogenital dystrophy, and growth deficiency of the anterior pituitary.⁷

Brousseau reports that in the literature of Mongolism, very few reports were found concerning the conditions of the glands other than the thyroid. Where they have existed, "their reports are contradictory."⁸

Mateer reports that hypopituitary cases formed one of the most interesting of the improvable groups of a study involving pituitary acceleration of the IQ's.⁹ A question arises as to how these studies determined the nature and extent of pituitary insufficiency in their sample. Modern science has been slow in giving way to the nature of pituitary hormones and their composition, and even more retarded in determining the nature of their presence and effect in humans. Turner confirms the "confused state" of the biochemistry of the anterior lobe hormones as late as 1855.¹⁰

⁷M.B. Gordon, and L. Kuskin, "Mental Retardation Associated with Endocrine and Non-Endocrine Conditions," Endocrinology, 17:561-571, 1935.

⁸Kate Brousseau, Mongolism, p. 94.

⁹Florence Mateer, "Pituitary Acceleration of IQ's," Psychological Bulletin, 31:686, November, 1934.

¹⁰Donnell C. Turner, General Endocrinology, p. 396.

One of the most advanced studies since 1928 is that of Kugelmass, in 1952, which attempts to express mental retardation as a stage of the condition of hypopituitarism.¹¹ This study examines six cases of mental deficiency of hypopituitarism in children and again supported the theory of hormone therapy. These cases were, however, selective and specialized and relate to other studies which fail to show what relationship exists, generally, overall, in the cases of mental deficiency.

Perhaps the most significant study is the one of Asling et al., which deals with the effects of hypophysectomy of rats at six days of age. The report of the study indicates that hypophysectomy at six days of age was incompatible with life, the cause of which was attributed to brain damage. The same animals, when ~~treated~~ treated with growth hormones, survived and showed no signs of neural damage.¹²

Asling did not make any correlation of these results to brain damage in humans, but the results indicate that an investigation of this nature is merited.

¹¹Kugelmass, op. cit., pp.1655-1659.

¹²Asling, et al., "Deaths in Rats Submitted to Hypophysectomy at an Extremely Early Age and the Survival Effected by Growth Hormone," Anatomical Record, 114:49-65, 1952.

These studies have indicated the importance of glandular therapy in cases of low intelligence; described the association of mental retardation in various endocrine disturbance; evaluated known cases of hypopituitarism and mental retardation, and experimented with the removal of the pituitary gland in an effort to discover the nature of those hormones on the growth of the brain. They have failed, however, to investigate the nature of mental deficiency as a function of hypopituitarism, especially necessary in relation to those cases of mental deficiency of undetermined etiology.

CHAPTER III

PROCEDURE

General method. Several studies have been reviewed which point to a lack of research in the area of mental deficiency as a function of hypopituitarism. To determine the extent of this relationship, this study has compared the level of pituitary function in children of normal mental ability with those recognized as mentally deficient. Conditions relative to hypopituitarism have been reviewed for the purpose of selecting a battery of clinical laboratory tests to determine the extent of pituitary function in the sample.

Sample and data. A random sample of seven (7) students was made from a local school district in Kansas who conformed to the following criteria:

1. Age ranging from 16 to 18 years.
2. IQ between 60 and 75 from a standardized individual intelligence test.
3. Mental deficiency unrelated to specific endocrine or metabolic disorders such as Cretinism, Phenylketonuria, etc.

Permission was obtained from a parent or guardian and an appointment for testing at the student's home was made for prior to the morning meal. The twenty-four hour urine was collected by the students over a convenient twenty-four hour period.

A control group of "normal" students was omitted in favor of the established normals for the various laboratory tests.

From an analysis of the conditions relative to hypopituitarism syndrome, the following clinical laboratory tests were used in identifying a relationship between mental deficiency and hypopituitarism.¹³

1. Complete Blood Count (CBC). The complete blood count was divided into four parts: hemoglobin, red blood count, white blood count, and differential white count. The blood was obtained from a venipuncture at the time all the blood was collected prior to the morning meal. Hemoglobin was determined by the Cyanmethemoglobin colorimetric method.

The specific references to hypopituitarism syndrome available from these results included: anemia, leukopenia, lymphocytosis, and eosinophilia.

2. Fasting Blood Sugar (FBS) and Glucose Tolerance Test. Blood was drawn according to the procedure of Exton and Rose for a two dose, one hour determination of glucose tolerance. Glucose levels were determined by the Somogyi-Nelson method for the purpose of identifying sugar

¹³Detailed procedures for these laboratory analyses are included in Appendix A.

metabolism and possible hypoglycemia (a condition reported of hypopituitarism syndrome).

3. Protein Bound Iodine (PBI). The protein bound iodine was used in lieu of the Basal Metabolism Rate because of its greater accuracy and convenience. Blood was drawn from the subject in the fasting state, and the serum was sent to Bio-Science Laboratories for the protein bound iodine determination by the alkaline ash method.

4. Serum Cholesterol (Chol). Serum was separated from the whole blood from the subjects in the fasting state and evaluated for cholesterol by the Bloor method. An elevated serum cholesterol is indicative of the high carbohydrate tolerance in hypopituitaryism syndrome.

5. 17-ketosteroids (17-KS), Corticosteroids, 17-OH-Corticosteroids, 17-ketogenic steroids (17-KGS). The adrenal cortex produces not only steroid hormones which are unique to this gland, but also those which are classified as C-19 compounds (17-KS), C-18 compounds (estrogens), and C-21 compounds (progesterone). The important C-21 compounds associated with adrenal cortex activity are referred to as Corticosteroids. These tests should indicate low results in hypopituitary cases.¹⁴

¹⁴Bio-Science Laboratories, Specialized Diagnostic Laboratory Tests, (Sixth Edition,) p. 44.

For these tests, a 100 milliliter aliquot of a twenty-four hour urine specimen was sent to Bio-Science Laboratories for total 17-ketosteroid and 17-ketogenic steroids determination by the 17-ketogenic method.

Facilities. Bio-Science Laboratories, Los Angeles, California, division of endocrinology, was responsible for two of the tests: Protein bound iodine, and the 17-OH-Corticosteroids. St. Mary's Hospital Laboratory, Emporia, Kansas, with the permission and cooperation of W.E. Luedtke, M.D., and Anne-Leigh Sampson, M.T. (ASCP), conducted the remaining tests.

CHAPTER IV

RESULTS OF THE INDIVIDUAL TESTING

The conditions relative to hypopituitarism syndrome were reported earlier. These included:

1. Anemia--decrease in the presence of red blood cells.
2. Leukopenia--decrease in the presence of white cells.
3. Eosinophilia--increase in the percentage of eosinophils per total white cells.
4. Lymphocytosis--increase in the percentage of lymphocytes per total white cells.
5. Hypoglycemia--reduction in the blood sugar level.
6. Abnormal glucose tolerance curve.
7. Low protein bound iodine.
8. Increased carbohydrate tolerance--increased serum cholesterol.
9. Decreased total 17-ketosteroids and 17-ketogenic steroids.

The findings for the individual tests are presented with the normal values in Tables I and II,

Individual results. Each student was evaluated to determine evidence of hypopituitary syndrome.

Subject 1. J.C. is a 16 year-old boy with a Stanford-Binet measured IQ of 68. The red count, white count, and hemoglobin were normal. There is no evidence of anemia or leukopenia. The differential white count indicated eosinophilia and lymphocytosis. The fasting blood sugar indicated some hypoglycemia. Poor glucose

TABLE I

RESULTS OF HEMATOLOGY TESTING ON A SAMPLE OF 16-17
YEAR-OLD MENTALLY DEFICIENT STUDENTS
AND THE CORRESPONDING NORMAL VALUES
FOR BOTH MALES AND FEMALES
FOR EACH TEST

Subject	Hemoglobin	Red Cells	White Cells
J.C. (M)	15.5	5.0	8,300
R.L. (M)	15.2	4.9	8,200
J.D. (M)	13.5	4.4	8,300
K.R. (M)	13.5	4.3	7,100
B.J. (F)	13.2	4.3	5,800
S.F. (F)	12.4	4.0	8,100
J.F. (F)	12.0	3.9	6,800

Subject	Differential Leukocyte Count					
	Baso- phils	Eosino- phils	Stabs	Neutro- phils	Lympho- cytes	Mono- cytes
J.C.	1	6	0	33	57	3
R.L.	0	5	0	39	55	1
J.D.	0	6	0	56	37	1
K.R.	0	2	0	49	49	0
B.J.	0	3	0	58	39	0
S.F.	0	9	0	37	53	1
J.F.	0	4	0	48	47	1

TABLE II

RESULTS OF BLOOD CHEMISTRY TESTING ON A SAMPLE
OF 16-17 YEAR-OLD MENTALLY DEFICIENT
STUDENTS AND THE CORRESPONDING
NORMAL VALUES FOR BOTH
MALES AND FEMALES
FOR EACH TEST

NORMAL VALUES	70-90mg/100cc	70-90mg/ 100cc	145-165mg/ 100cc	125-250mg/ 100cc
Subject	F.B.S.	Glucose Tolerance Test		
J.C.	68	68	120	144
R.L.	76	76	172	145
J.D.	68	68	172	122
K.R.	50	50	94	105
B.J.	49	49	150	106
S.F.	58	58	68	113
J.F.	52	52	100	104

NORMAL VALUES	125-250/100cc	4-8micro- grams/100cc	M=9-22* F=6-15*	M=5-23** F=3-15*
Subject	Serum Choles.	P.B.I.	17-KS	17-KGS
J.C. (M)	119	3.7	3.3	2.5
R.L. (M)	145	5.4	4.7	5.6
J.D. (M)	123	4.8	5.2	5.5
K.R. (M)	120	4.9	5.6	6.8
B.J. (F)	122	6.0	15.0	11.0
S.F. (F)	108	6.6	3.9	3.1
J.F. (F)	142	6.5	3.9	3.3

* mg/24 hours.

tolerance was indicated by the glucose tolerance test by a marked increase at both levels. The serum cholesterol showed further evidence of poor carbohydrate tolerance. There was no evidence of hypothyroidism and the 17-OH-Corticosteroids were decreased.

Subject 2. R.L. is a 16 year-old boy with a Stanford-Binet measured IQ of 62. The results of hematological testing indicate an elevated percentage of eosinophils and lymphocytes in the differential count. There is no evidence of anemia or leukopenia. Glucose values were normal and the cholesterol is within the normal range. There is no evidence of hypothyroidism and the 17-OH-Corticosteroids show no abnormality.

Subject 3. J.D. is a 16 year-old boy with a Stanford-Binet measured IQ of 69. Clinical testing shows a lowered red cell count and hemoglobin, which is an indication of anemia. There is no evidence of leukopenia. The percentage of eosinophils and lymphocytes are above the normal range. There is evidence of hypoglycemia but a normal glucose tolerance curve. The serum cholesterol is below the normal range. The protein bound iodine does not indicate thyroid abnormality and the 17-OH-Corticosteroids are not normal.

Subject 4. K.R. is a 17 year-old boy with a Stanford-Binet measured IQ of 75. Testing shows this

student to have indications of anemia, lymphocytosis, and hypoglycemia. The glucose tolerance curve indicates a slow rise at the half-hour and further rise at the hour. A low carbohydrate tolerance was reported. There is no abnormality in the thyroid function test and a normal value was reported in the 17-ketogenic steroids. A low value was reported in the total 17-ketosteroids.

Subject 5. B.J. is a 17 year-old girl with a Stanford-Binet measured IQ of 77. Testing indicates the absence of anemia, leukopenia, eosinophilia, and hypothyroidism. Blood sugar levels show signs of hypoglycemia and an abnormal glucose tolerance curve. A low value carbohydrate tolerance was also reported. The 17-OH-Corticosteroids were normal.

Subject 6. S.F. is a 16 year-old girl with a Stanford-Binet measured IQ of 72. Results show indications of eosinophilia, lymphocytosis, hypoglycemia, and a slow reaction to glucose during the glucose tolerance test. The serum cholesterol was below the normal range. No abnormalities were reported in the thyroid function or Corticosteroids.

Subject 7. J.F. is a 17 year-old girl with a Stanford-Binet measured IQ of 66. The red cell count showed evidence of anemia, but the hemoglobin was within the normal range. There were no abnormalities in the

appearance of the red blood cells. The differential count showed signs of eosinophilia and lymphocytosis. The glucose values reported indicate hypoglycemia and a flat glucose tolerance curve. The carbohydrate tolerance is normal and there were no signs of hypothyroidism. The 17-OH-Corticosteroid values were both low.

Results of the sample as a whole. Several conditions tended to be present in all of the subjects. These included the absence of leukopenia and anemia, presence of at least two per cent eosinophils and thirty-seven per cent lymphocytes, no evidence of increased carbohydrate tolerance (in fact five cases reported decreased carbohydrate tolerance), only one exception of a reduced fasting blood sugar, and only one exception of no evidence of hypothyroidism.

Summary of evidence of hypopituitarism. Of the five conditions listed as significant by the American Medical Association as a part of the hypopituitary syndrome, only hypoglycemia was indicated in the sample. Secondary conditions reported in the sample included eosinophilia and lymphocytosis. No other evidence of hypopituitarism was found.

CHAPTER V

SUMMARY AND CONCLUSIONS

To determine the relationship between mental deficiency and hypopituitarism, this study has compared levels of pituitary function in persons of normal mental ability with those recognized as mentally deficient. A series of nine conditions have been examined and reported of each of seven subjects.

Summary. Studies have indicated the importance of glandular therapy in cases of low intelligence; described the association of mental retardation in various endocrine disturbances; evaluated known cases of hypopituitarism and mental retardation; and experimented with the removal of the pituitary gland in an effort to discover the nature of those hormones on the growth of the brain. It was the purpose of this study, to determine the relationship which exists between mental deficiency and pituitary dysfunction.

In the seven subjects tested, with IQ's ranging from 62-75, and chronological ages from 16-17, a trend was indicated by the presence of several conditions in most of the sample. These included eosinophilia, lymphocytosis, low carbohydrate tolerance, hypoglycemia, and

borderline evidence of anemia and reduced 17-OH-Corticosteroids excretion in the urine.

Of the five significant laboratory findings indicated by the American Medical Association, hypoglycemia was the only condition of the three that were tested by this study. Added conditions of the syndrome that were present included eosinophilia and lymphocytosis. Evidence of decreased carbohydrate tolerance in most of the sample was directly opposed to the hypopituitary increase in the carbohydrate tolerance.

Conclusions. Although there was some indication of a deviation from the normal in the conditions tested, there was insufficient evidence of a relationship between mental deficiency and pituitary dysfunction.

Recommendations. Asling's study clearly points the way for research in the area of brain development and the presence of the growth hormone of the pituitary gland. The failure of this study to demonstrate this relationship between mental deficiency and hypopituitarism does not constitute a recommendation for withdrawal in this area.

The limitations of this study did not permit the further testing indicated by the results. Certain questions remain to be answered:

1. Why was there some evidence of eosinophilia and lymphocytosis in most of the subjects?
2. What significance is there in the high percentage of hypoglycemia and low carbohydrate tolerance in the sample?
3. What results must be further explored for relative weight in determining hypopituitarism?

Further research is necessary to evaluate the conditions of the syndrome more effectively in light of the current data. One of the shortcomings of this present study has been the absence of available diagnostic testing designed specifically for detection of hypopituitarism. Conditions need to be explored to determine the significance of the white count and cholesterol results.

The fact that this sample has shown that no positive relationship exists between mental deficiency and hypopituitarism, suggests that further research is necessary to evaluate this on a much larger sample and should included a greater cross reference ability in the diagnostic measurements to identify causations in conditions which compose the hypopituitary syndrome.

BIBLIOGRAPHY

BIBLIOGRAPHY

- American Medical Association, 1964 Current Medical Terminology. Chicago: American Medical Association, 1964.
- Asling, C. Willet, et al. "Deaths in Rats Submitted to Hypophysectomy at an Extremely Early Age and the Survival Effected by Growth Hormone," Anatomical Record, 114:49-65, 1952.
- Bio-Science Laboratories, Specialized Diagnosed Laboratory Tests. Sixth Edition. Los Angeles: Bio-Science Laboratories, 1963.
- Bray, John D., Golsen Toro, and Philip G. Ackerman. Bray's Clinical Laboratory Methods. St. Louis: The C.V. Mosby Co., 1962.
- Brousseau, Kate. Mongolism. Baltimore: The Williams and Wilkins Co., 1928.
- Comroe, Julius H. Methods in Medical Research. 2 vols. Chicago: Yearbook Publishers, Inc., 1950.
- Ellis, Norman R. (ed.). Handbook of Mental Deficiency. New York: McGraw-Hill Book Co., Inc., 1963.
- Fox, Edna J. "An Investigation of the Effects of Glandular Therapy on the Intelligence Quotient," Mental Hygiene, 12:90-102, 1928.
- Gordon, M.B., and L. Kuskin. "Mental Retardation Associated With Endocrine and Non-Endocrine Conditions," Endocrinology, 19:561-571, 1935.
- Kleiner, Israel S., and James M. Orten. Biochemistry. St. Louis: The C.V. Mosby Co., 1962.
- Kugelmass, J. Newton. "Mental Deficiency of Hypopituitarism in Childhood," The New York State Journal of Medicine, 52:1655-1659, July, 1952.
- Levinson, Abraham, and John A. Bigler. Mental Retardation in Infants and Children. Chicago: Yearbook Publishers, 1960.

- Mateer, Florence. "Pituitary Acceleration of IQ's," Psychological Bulletin, 31:686, November, 1934.
- Menninger, W.C. "Mental Disturbances Associated with Pituitary Disorders," Journal of the American Medical Association, 91:951-955, 1928.
- Perkoff, G.T., et al. "Selective Hypopituitarism with Deficiency of Anterior Pituitary Basophils," Journal of Clinical Endocrinology and Metabolism, 20:1270-1271, September, 1960.
- Rothstein, Jerome H. Mental Retardation. Chicago: Holt, Rinehart and Winston, 1961.
- Turner, C. Donnell. General Endocrinology. Philadelphia: W.B. Saunders Co., 1955.
- Varley, Harold. Practical Clinical Biochemistry. New York: Interscience Publishers, Inc., 1958.

APPENDIX.

HEMOGLOBIN

(Cyanmethemoglobin Method)

Drabkin and Austin (1935)
Crosby, Munn, and Furth (1954)

1. Draw finger-tip oxylated blood into a 0.02 ml. pipette.
2. Expel into 5 ml. of cyanmethemoglobin reagent in a 10 mm. cuvette.
3. Stopper the cuvette and allow it to stand for 15 minutes.
4. Half fill a second 10 mm. cuvette with cyanmethemoglobin reagent. This will be the Blank.
5. Using filter 550, set the meter of the LEITZ PHOTROMETER to 100 on the per cent transmittance scale with the Blank.
6. Immediately replace the Blank with the unknown and read the per cent transmittance on the meter.
7. Determine the concentration in grams per 100 ml. of blood from the calibration table.

Calculate the per cent hemoglobin as follows:

$$\frac{\text{grams hemoglobin}}{\text{considered normal}} \times 100 = \text{per cent}$$

BLOOD SUGAR DETERMINATION

(Somogyi-Nelson Method)

Reinhold (1953)

Semimicrotechnique:

1. Pipette 1.5 ml. of water into a centrifuge tube.
2. Draw cutaneous blood into a 0.1 ml. pipette and expel it into the water. Rinse the pipette two or three times by drawing up and expelling the solution into the centrifuge tube.
3. Add 0.2 ml. of Barium hydroxide solution and mix.
4. Add 0.2 ml. of Zinc sulfate solution and mix the contents of the tube thoroughly by tapping.
5. Allow the tubes to stand for three to five minutes and centrifuge for five minutes.
6. Pipette 0.5 ml. samples of the supernatant fluid, of each standard, and of water (for the blank) into separate tubes (test tubes or photometer cuvettes approximately 15x125 mm).
7. Add 1 ml. of Alkaline copper reagent and mix each tube by tapping.
8. Place a marble on top and heat in a vigorously boiling water bath for 20 minutes, or place in a pressure cooker for five minutes at 115°C.
9. Place tubes in water at room temperature for one minute.
10. Add 1 ml. of Arsenomolybdate reagent and mix.
11. Dilute to the 10 ml. mark or add 7.5 ml. of water. Mix by inverting.
12. Measure the absorbance at 540 mμ by means of a photo-colorimeter, using the blank for setting the zero.

Calculation: R_x is the reading of the unknown, and R_s is the reading of the standard. Using standard II (0.025 mg. of glucose):

$$\frac{R_x}{R_s} \times 100 = \text{mg. per cent glucose}$$

CHOLESTEROL IN SERUM

(Bloor Method)

Bloor (1916)

1. Into a dry 25 ml. glass stoppered volumetric flask, place: 17 ml. of alcohol-ether mixture.
2. Slowly pipette directly into the alcohol-ether mixture, and with constant shaking of the flask, 1 ml. of serum obtained from unhemolyzed blood.
3. Add alcohol-ether mixture to the graduation mark, stopper the flask, invert, and shake vigorously.
4. Let flask stand for 30 minutes—shake it occasionally.
5. Filter on a dry Whatman No. 40 paper into a dry flask.
6. Into a dry 50 ml. Erlenmeyer flask pipette: 10 ml. of the above filtrate.
7. Evaporate just to dryness on a water bath or an electric hot-plate.
8. Extract the cholesterol from the dried residue by boiling it with 3 ml. of chloroform and then decanting the solution into a dry 10 ml. glass stoppered graduated cylinder.
9. Repeat the extraction (Step 8) twice again, pouring each chloroform extract into the same graduated cylinder as before.
10. Add chloroform to the extracts in the cylinder until the volume is 10 ml.
11. Add 2 ml. of freshly prepared cholesterol reagent.
12. Stopper the cylinder and invert it several times.
CAUTION.
13. Place the cylinder in the dark for 20 minutes at 21-25°C.
14. Transfer some of the liquid to a 10 mm square cuvette.

15. Using filter 640, set the meter of the LEITZ PHOTROMETER to 100 on the per cent transmittance scale with a blank of 5 ml. of chloroform to which has been added 1 ml. of cholesterol reagent and contained in a 10 mm. square cuvette.
16. Immediately replace the blank with the unknown and read the per cent transmittance on the meter.
17. Determine the concentration in mg. per 100 ml. of serum from the calibration table.