

A STUDY OF HYPERVITAMINOSIS E

IN THE CHICK

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B.S.A., NATIONAL TAIWAN UNIVERSITY, 1961

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN THE DEPARTMENT

OF

POULTRY SCIENCE

WE ACCEPT THIS THESIS AS CONFORMING TO THE
REQUIRED STANDARD

THE UNIVERSITY OF BRITISH COLUMBIA

OCTOBER, 1970

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ACKNOWLEDGEMENT

I WISH TO THANK DR. W. D. KITTS, HEAD OF THE DEPARTMENT OF POULTRY SCIENCE, FOR PROVIDING THE FACILITY USED IN THIS STUDY.

I AM GRATEFUL TO MRS. B. E. MARCH, OF THE DEPARTMENT OF POULTRY SCIENCE, WHO SUPERVISED THE PLANNING AND EXECUTION OF THE EXPERIMENTS.

I ALSO THANK DRs. J. BIELY, R. FITZSIMMONS AND M. TAIT FOR THEIR ENCOURAGEMENT THROUGHOUT MY PROGRAM.

ABSTRACT

A STUDY WAS MADE OF THE EFFECTS OF FEEDING EXCESS VITAMIN E TO CHICKS. THREE GROUPS OF EXPERIMENTS WERE CONDUCTED TO INVESTIGATE (1) THYROIDAL RESPONSE TO EXCESS VITAMIN E, (2) RESPIRATION RATE OF MUSCLE MITOCHONDRIA FROM CHICKS FED EXCESS VITAMIN E, AND (3) SYMPTOMS OF HYPERVITAMINOSIS E.

IN TWO SEPARATE EXPERIMENTS, CHICKS WERE FED NORMAL AND EXCESS LEVELS (220 I.U./KG. OF DIET) OF VITAMIN E AND WERE SUBJECTED TO TEMPERATURES OF 14.5 AND 31.5°C. THE GOITROGENIC EFFECT OF THIOURACIL ON THE BIRDS IN THE DIFFERENT TREATMENTS WAS MEASURED. AT BOTH TEMPERATURES THE THIOURACIL-TREATED CHICKS FED EXCESS VITAMIN E EXHIBITED A LESSER ENLARGEMENT OF THE THYROID GLANDS THAN DID CHICKS RECEIVING A NORMAL LEVEL OF VITAMIN E. THIS FINDING INDICATES A REDUCTION IN THE SECRETION OF THYROID STIMULATING HORMONE (TSH) IN BIRDS FED EXCESS VITAMIN E. THE LEVEL OF VITAMIN E WHICH WAS FED DID NOT AFFECT THE GROWTH RATE OR FEED CONSUMPTION AT EITHER TEMPERATURE. IT, THEREFORE, APPEARS THAT THE METABOLIC RATES OF THE CHICKS FED THE LOW AND EXCESS LEVELS OF VITAMIN E WERE SIMILAR DESPITE DIFFERENCES IN THYROID ACTIVITY AND THAT TISSUE RESPIRATION IN BIRDS FED EXCESS VITAMIN E CAN BE MAINTAINED WITH A REDUCED SUPPLY OR TURNOVER OF THYROID HORMONE.

THE ACTIVITY OF THE THYROID GLAND ITSELF WAS STUDIED IN RESPONSE TO EXCESS VITAMIN E IN ANOTHER EXPERIMENT. USING RADIOIODINE (I^{131}) AS A

TRACER, IT WAS FOUND THAT THE RATES OF IODINE UPTAKE AND RELEASE BY THE THYROID GLAND WERE BOTH SLOWER IN BIRDS FED EXCESS VITAMIN E (220 I.U./KG. OF DIET) THAN IN CONTROL BIRDS. BECAUSE VITAMIN E ACTS AS A BIOLOGICAL ANTIOXIDANT, IT MAY, IN EXCESS AMOUNTS, DEPRESS THE RATE OF DEIODINATION OF THYROXINE IN THE PERIPHERAL TISSUE AT WHICH THYROID HORMONE IS REMOVED FROM THE CIRCULATION. AS A CONSEQUENCE, THE SECRETION OF TSH WOULD BE REDUCED.

IN ORDER TO OBTAIN SOME INDICATION OF CAUSE AND EFFECT IN THE MECHANISM BY WHICH THE EXCESS VITAMIN E AFFECTS THYROTROPIC HORMONE SECRETION RATE AND THYROID ACTIVITY, THE RESPIRATION RATE OF MITOCHONDRIA ISOLATED FROM THE PECTORAL MUSCLE OF CHICKS WHICH HAD BEEN FED EXCESS VITAMIN E (2200 I.U./KG. OF DIET) WAS COMPARED WITH THAT OF THE CONTROL BIRDS. THE RESULTS SHOWED A SIGNIFICANT REDUCTION IN OXYGEN UPTAKE BY THE MUSCLE MITOCHONDRIA OF CHICKS FED THE EXCESS AMOUNT OF VITAMIN E. THE GROWTH RATE OF THE CHICKS FED THE EXCESS LEVEL OF VITAMIN E IN THIS EXPERIMENT WAS MARKEDLY LOWER THAN THAT OF THE CONTROL CHICKS. IT IS SUGGESTED THAT THE CHICK MAY MAINTAIN A NORMAL RESPIRATION RATE WHEN FED EXCESS VITAMIN E UP TO A CERTAIN LEVEL THROUGH A REDUCTION IN THYROID ACTIVITY. WITH A LARGE EXCESS OF VITAMIN E, HOWEVER, NO FURTHER COMPENSATION IS POSSIBLE, AND RESPIRATION RATE IS DEPRESSED BELOW NORMAL.

EXCESS VITAMIN E CAUSED HYPOPROTHROMBINEMIA, INDICATIVE OF VITAMIN K-DEFICIENCY. OTHER SYMPTOMS NOTED WERE A REDUCTION IN HEMATOCRIT VALUES, RETICULOCYTOSIS AND AN ABNORMALLY FLUID APPEARANCE OF THE BONE MARROW. BASED UPON OBSERVATIONS OF BONE CALCIFICATION, THE CALCIUM REQUIREMENT APPEARED TO BE INCREASED IN THE PRESENCE OF EXCESS VITAMIN E.

TABLE OF CONTENTS

	PAGE
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION	1
REVIEW OF LITERATURE	3
EXPERIMENT I	12
THYROIDAL RESPONSE TO EXCESS DIETARY VITAMIN E	12
EXPERIMENT IA	12
MATERIALS AND METHODS	12
EXPERIMENT IB	14
RESULTS AND DISCUSSION	14
EXPERIMENT IC	20
MATERIALS AND METHODS	20
RESULTS AND DISCUSSION	22
EXPERIMENT II	26
RESPIRATION RATE OF MUSCLE MITOCHONDRIA FROM CHICKS FED EXCESS DIETARY VITAMIN E	26
MATERIALS AND METHODS	26
RESULTS AND DISCUSSION	29
EXPERIMENT III	35
SYMPTOMS OF HYPERVITAMINOSIS E IN CHICK	35
MATERIALS AND METHODS	35
RESULTS	38
DISCUSSION	44

	PAGE
SUMMARY	47
BIBLIOGRAPHY	49

LIST OF TABLES

TABLE	PAGE
1A. BASAL DIETS IN EXPERIMENT IA	61
1B. BASAL DIETS IN EXPERIMENT IB	61
2. AVERAGE THYROID WEIGHTS, BODY WEIGHTS AND FEED CONSUMPTION OF CHICKS IN EXPERIMENT IA	62
3. AVERAGE THYROID WEIGHTS, BODY WEIGHTS AND FEED CONSUMPTION OF CHICKS IN EXPERIMENT IB	63
4. ANALYSIS OF VARIANCE OF THYROIDAL RESPONSE TO THE EXCESS VITAMIN E AND/OR THIOURACIL AT DIFFERENT TEMPERATURES IN EXPERIMENT IA	64
5. ANALYSIS OF VARIANCE OF THYROIDAL RESPONSE TO EXCESS VITAMIN E AND/OR THIOURACIL AT DIFFERENT TEMPERATURES IN EXPERIMENT IB	65
6. AVERAGE THYROID WEIGHTS, BODY WEIGHTS AND FEED CONSUMPTION OF ETHOXYQUIN (EQ)-TREATED CHICKS AT 22°C IN EXPERIMENT IB	66
7. EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (¹³¹ I) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS	67
8. RESPIRATION RATES OF MUSCLE MITOCHONDRIA FROM CHICKS FED THE CONTROL DIET, EXCESS VITAMIN E AND/OR THIOURACIL . . .	68
9. BASAL DIET IN EXPERIMENT III	69
10. EFFECT OF EXCESS VITAMIN E ON HEMATOCRIT VALUE, PROTHROMBIN TIME AND RETICULOCYTE COUNT	70
11. PROTHROMBIN TIME OF CHICKS FED EXCESS VITAMIN E, 24 HOURS AFTER INJECTION OF MENADIONE	71
12. EFFECT OF EXCESS VITAMIN E ON BONE CALCIFICATION IN CHICKS FED DIETS ADEQUATE AND DEFICIENT IN CALCIUM	72

LIST OF FIGURES

FIGURE		PAGE
1.	THYROIDAL RESPONSE OF CHICKS RECEIVING TWO LEVELS OF VITAMIN E AND/OR ETHOXYQUIN TO THIOURACIL AT DIFFERENT AMBIENT TEMPERATURES IN EXPERIMENT 1A	16
2.	THYROIDAL RESPONSE OF CHICKS RECEIVING TWO LEVELS OF VITAMIN E AND/OR ETHOXYQUIN TO THIOURACIL AT DIFFERENT AMBIENT TEMPERATURES IN EXPERIMENT 1B	17
3.	EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (¹³¹ I) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS	23
4.	EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (¹³¹ I) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS	24
5.	ELECTRON MICROGRAPH OF BREAST MUSCLE MITOCHONDRIA FROM A CHICK FED A NORMAL LEVEL OF VITAMIN E	32
6.	ELECTRON MICROGRAPH OF BREAST MUSCLE MITOCHONDRIA FROM A CHICK FED EXCESS VITAMIN E	33
7.	ELECTRON MICROGRAPH OF BREAST MUSCLE MITOCHONDRIA FROM A CHICK FED A NORMAL LEVEL OF VITAMIN E SHOWING INTACT MITOCHONDRIA	34
8.	ERYTHROCYTES FROM A 40-DAY OLD CHICK FED A NORMAL LEVEL OF VITAMIN E	40
9.	RETICULOCYTES FROM A 40-DAY OLD CHICK FED EXCESS VITAMIN E	40
10.	ERYTHROBLAST OBSERVED ON THE BLOOD SMEAR FROM A 40-DAY OLD CHICK	41
11.	ABNORMAL BONE MARROW OF CHICKS FED EXCESS VITAMIN E	42

FIGURE	PAGE
12A. THE INFLUENCE OF EXCESS VITAMIN E ON PERCENT OF TIBIA ASH AT DIFFERENT AGES	45
12B. THE INFLUENCE OF EXCESS VITAMIN E ON PERCENT OF TIBIA ASH RELATIVE TO BODY WEIGHT	45

INTRODUCTION

A DEFICIENCY OF VITAMIN E CAUSES A VARIETY OF SYMPTOMS IN DIFFERENT SPECIES OF ANIMALS AND MAY AFFECT MANY TISSUES (SEE REVIEW BY MASON, 1949; MATTILL, 1952; DAM, 1957; ROELS, 1967; SCOTT ET AL., 1969). OTHER DIETARY COMPONENTS SUCH AS METHIONINE (CALVERT ET AL., 1962), SELENIUM (SCHWARZ, 1960; DESAI AND SCOTT, 1965) AND UNSATURATED FATTY ACIDS (CENTURY AND HORWITT, 1958; WITTING AND HORWITT, 1962) MAY AFFECT THE REQUIREMENT OF THE ANIMAL FOR VITAMIN E AND THE MANNER IN WHICH A DEFICIENCY OF THE VITAMIN MANIFESTS ITSELF. IT IS APPARENT THAT VITAMIN E HAS SEVERAL DIFFERENT METABOLIC FUNCTIONS BUT THE PRIMARY MECHANISM OF ACTION OF VITAMIN E IN THE LIVING CELL HAS NOT YET BEEN DETERMINED.

ONE FUNCTION OF VITAMIN E IS AS A BIOLOGICAL ANTIOXIDANT (TAPPEL, 1962). AS A RESULT OF ITS ANTIOXIDANT PROPERTIES VITAMIN E ACTS AS A NONSPECIFIC PROTECTOR OF MEMBRANE LIPIDS IN CELLS (HORWITT ET AL., 1968) OR ORGANELLES SUCH AS MITOCHONDRIA (TAPPEL AND ZALKIN, 1959A), LYSOSOMES (DESAI ET AL., 1964), AND MICROSOMES (TAPPEL AND ZALKIN, 1960). LACK OF SUFFICIENT TOCOPHEROL TO PREVENT UNCONTROLLED OXIDATION CAUSES THE EVENTUAL RUPTURE OF MEMBRANES AND THE RELEASE OF ENZYMES PECULIAR TO A SUBCELLULAR PARTICLE. VITAMIN E ALSO PROTECTS VITAMIN A AGAINST OXIDATION (DAM ET AL., 1952; PUDELKIEWICZ ET AL., 1964) AND HAS BEEN SHOWN TO REACTIVATE ASCORBIC ACID SYNTHESIS BY LIVER PREPARATIONS IN VITRO FROM VITAMIN E DEFICIENT ANIMALS (CAPUTTO ET AL., 1958). THE FACT THAT THE

TOCOPHEROLS READILY UNDERGO REVERSIBLE OXIDATION-REDUCTION, HAS LED TO THE SUGGESTION THAT α -TOCOPHEROL MAY FUNCTION IN TERMINAL ELECTRON-TRANSFER ENZYMES AS A COENZYME OR AS A STRUCTURAL AGENT (VASINGTON ET AL., 1960; DETWILER ET AL., 1966). BECAUSE VITAMIN E HAS ANTIOXIDANT PROPERTIES AND UNDERGOES REVERSIBLE OXIDATION-REDUCTION, IT IS POSSIBLE THAT AN EXCESS OF VITAMIN E MAY INTERFERE IN THE ACTION OF OTHER BIOLOGICAL REDOX SUBSTANCES.

ALTHOUGH THERE IS A LARGE VOLUME OF LITERATURE DEALING WITH VITAMIN E DEFICIENCY, THERE HAVE BEEN FEW STUDIES REGARDING THE EFFECTS OF EXCESS VITAMIN E. SYMPTOMS OF HYPERVITAMINOSIS E HAVE NOT BEEN DESCRIBED, NOR IS THERE EVIDENCE THAT TOCOPHEROLS PER SE EXERT ANY DELETERIOUS EFFECT IN ANIMALS OR MAN (DEMOLE, 1939; HILLMAN, 1957). THE PRESENT INVESTIGATION WAS CONDUCTED TO DETERMINE WHAT EFFECTS EXCESS DIETARY VITAMIN E MAY HAVE IN CHICKS.

THREE GROUPS OF EXPERIMENTS WERE CARRIED OUT TO STUDY (1) THYROIDAL RESPONSE TO EXCESS DIETARY VITAMIN E; (2) RESPIRATION RATE OF MUSCLE MITOCHONDRIA FROM CHICKS FED EXCESS VITAMIN E; (3) SYMPTOMS OF HYPERVITAMINOSIS E IN CHICKS.

REVIEW OF LITERATURE

GENERAL

VITAMIN E WAS DISCOVERED IN 1922 BY EVANS AND BISHOP AS A FACTOR PRESENT IN VEGETABLE OIL WHICH WAS NECESSARY FOR NORMAL REPRODUCTION IN THE RAT. THE DESIGNATION OF THE LETTER E, NEXT IN ORDER FOLLOWING VITAMIN D, WAS SUGGESTED BY SURE. PURE VITAMIN E WAS FIRST ISOLATED BY EVANS AND THE EMERSONS (1936) FROM THE UNSAPONIFICABLE FRACTION OF WHEAT GERM OIL. THE NAME TOCOPHEROL WAS PROPOSED FROM THE GREEK WORD, TOKOS (OFFSPRING), PEREIN (TO BEAR), AND OL (TO SIGNIFY AN ALCOHOL). THE PREFIXES α , β , γ , ETC. WERE USED TO INDICATE VARIOUS FORMS OF TOCOPHEROL. ALPHA-TOCOPHEROL, HAVING HIGH BIOLOGICAL ACTIVITY (CENTURY AND HORWITT, 1965) WAS IDENTIFIED BY FERNHOLZ (1937, 1938). DL- α -TOCOPHERYL ACETATE IS USED AS A REFERENCE STANDARD (HUME, 1941) AND FEED SUPPLEMENT. THIS FORM OF THE VITAMIN IS NOT READILY AUTOXIDIZABLE AND HAS A HIGH BIOPOTENCY.

VITAMIN E DEFICIENCY IN ANIMALS AND CHICKS HAS BEEN REPORTED BY A NUMBER OF INVESTIGATORS. VITAMIN E DEFICIENCY CAUSES A WIDE VARIETY OF SYMPTOMS IN DIFFERENT SPECIES OF ANIMALS AND MAY AFFECT MANY DIFFERENT TISSUES, INCLUDING THE EMBRYO (FETAL RESORPTION), GONADS (STERILITY), MUSCLE (MUSCULAR DYSTROPHY), BRAIN (ENCEPHALOMALACIA), BLOOD (HEMOLYSIS OF ERYTHROCYTES, AND ANEMIA), CAPILLARY WALLS (EXUDATIVE DIATHESIS), LIVER (LIVER NECROSIS), KIDNEY (DEGENERATION OF TUBULAR EPITHELIUM), DEPOT FAT

(STEATITIS) (SEE REVIEWS BY MASON, 1949; MATTIL, 1952; DAM, 1957; ROELS, 1967; SCOTT ET AL., 1969). ONE OF THE MOST WIDELY OCCURRING SYMPTOMS OF VITAMIN E DEFICIENCY IN MANY ANIMALS IS MUSCULAR DYSTROPHY. AS VITAMIN E DEFICIENCY AFFECTS SUCH A WIDE VARIETY OF ANIMAL TISSUES, AND SEVERAL FACTORS SUCH AS THE DIETARY LEVELS OF METHIONINE (CALVERT ET AL., 1962) SELENIUM (SCHWARZ, 1960), AND UNSATURATED FATTY ACIDS (CENTURY AND HORWITT, 1958; WITTING AND HORWITT, 1962) INFLUENCE VITAMIN E DEFICIENCY SYNDROMES IN A VARIETY OF ANIMAL SPECIES, IT IS APPARENT THAT VITAMIN E HAS SEVERAL DIFFERENT METABOLIC FUNCTIONS, BUT NO EXACT METABOLIC ROLE OF VITAMIN E HAS YET BEEN DEFINED.

VITAMIN E IS FOUND PRINCIPALLY IN THE ORGANELLA OF THE CELLS (WISS ET AL., 1962) SUCH AS MITOCHONDRIA, MICROSOMES, AND LYSOSOMES WHERE IT IS PRESENT MAINLY IN THE REDUCED STATE (MOORE, 1959). THERE SEEMS TO BE LITTLE DOUBT THEREFORE THAT IT FUNCTIONS AS A BIOLOGICAL ANTIOXIDANT WHICH PROTECTS THE STRUCTURAL AND METABOLIC INTEGRITY OF INTRACELLULAR UNITS (TAPPEL, 1962; GUHA AND ROELS, 1965; ROELS ET AL., 1965; LUCY AND DINGLE, 1964). PREVENTION OF PEROXIDATION APPEARS TO BE VERY IMPORTANT IN THE MAINTENANCE OF THE STRUCTURAL INTEGRITY OF THE CELLULAR MEMBRANES OR SUBCELLULAR MEMBRANES (ZALKIN AND TAPPEL, 1960) IN TISSUES. ONE INTERESTING FACT IS THAT TOCOPHEROLS ADMINISTERED EITHER IN VIVO OR IN VITRO INHIBIT THE DIALURIC ACID-INDUCED HEMOLYSIS OF ERYTHROCYTES IN RATS (ROSE AND GYORGY, 1950, 1952). TSEN AND COLLIER (1960) AND BUNYAN ET AL. (1960) HAVE PRESENTED EVIDENCE THAT DIALURIC ACID PROMOTES ERYTHROCYTE HEMOLYSIS BY CATALYZING LIPID PEROXIDATION AND THAT VITAMIN E ACTS AS AN ANTIOXIDANT. CHRISTENSEN ET AL. (1956) SHOWED THAT THE DIALURIC

ACID-INDUCED HEMOLYSIS MAY BE USED FOR ASSESSING VITAMIN E DEFICIENCY IN THE RAT BUT THAT IT IS NOT A RELIABLE CRITERION FOR DETERMINING A DEFICIENCY OF THIS VITAMIN IN THE CHICK.

VITAMIN E MAY PLAY A ROLE IN ENERGY METABOLISM. FOR EXAMPLE, IT MAY BE A COMPONENT OF THE CYTOCHROME C REDUCTASE SYSTEMS OF THE TERMINAL RESPIRATORY CHAIN FUNCTIONING DIRECTLY AS AN ELECTRON CARRIER (BIOLOGICAL OXIDATION AND REDUCTION) OR INDIRECTLY AS A BINDING AGENT (VASINGTON, REICHARD AND NASON, 1960). OLIVERIRA ET AL. (1969) DEMONSTRATED THAT α -TOCOPHEROL DID NOT HAVE A DIRECT QUANTITATIVE RELATIONSHIP WITH ENZYMATIC ACTIVITIES. IT HAS ALSO BEEN SUGGESTED THAT IT MAY FUNCTION DIRECTLY OR INDIRECTLY IN NORMAL OXIDATIVE PHOSPHORYLATION IN TISSUE RESPIRATION (VASINGTON, REICHARD AND NASON, 1960), OR IN THE METABOLISM OF COENZYME Q (GREEN, 1962).

THYROIDAL RESPONSE TO TEMPERATURE AND DIET

ALTHOUGH THE MECHANISM OF ACTION OF THYROID HORMONES STILL REMAINS UNKNOWN, THE THYROID GLAND IS CONSIDERED TO PLAY AN IMPORTANT ROLE IN THE ADAPTIVE PHENOMENA ASSOCIATED WITH CHANGES IN EXTERNAL ENVIRONMENT AND HAS BEEN THE SUBJECT OF NUMEROUS INVESTIGATIONS IN THIS REGARD. IN THESE STUDIES, THYROID ACTIVITY HAS BEEN EVALUATED BY DIFFERENT METHODS UTILIZING A VARIETY OF PARAMETERS SUCH AS BASAL METABOLIC RATE, THYROID WEIGHT, HISTOLOGICAL APPEARANCE OF THE TISSUE, PROTEIN-BOUND IODINE LEVELS, RADIOIODINE UPTAKE AND THYROIDAL IODINE TURNOVER RATE. MANY FACTORS SUCH AS GENETIC STRAIN, AGE, ENVIRONMENTAL TEMPERATURE, AND DIETARY INTAKE OF IODINE ARE KNOWN TO AFFECT THYROID ACTIVITY. HOWEVER, COMPARATIVELY LITTLE

IS KNOWN ABOUT THE EFFECTS OF NUTRITIONAL FACTORS OTHER THAN IODINE ON THYROID SECRETION RATE.

THERE IS MUCH DATA TO INDICATE THAT THE THYROID GLAND OF BIRDS RESPONDS TO ENVIRONMENTAL TEMPERATURE CHANGES (HOFFMANN AND SHAFFNER, 1950; JOINER AND HUSTON, 1957; PREMACHANDRA ET AL., 1958; HENINGER ET AL., 1960; STAHL AND TURNER, 1961; HUSTON ET AL., 1962). THE YOUNG BIRD HELD IN A COOL ENVIRONMENTAL TEMPERATURE WAS SHOWN TO HAVE HEAVIER THYROID GLANDS AND TO SECRETE MORE THYROXINE THAN THOSE EXPOSED TO HIGH TEMPERATURES (HOFFMANN AND SHAFFNER, 1950). LATER REPORTS (JOINER AND HUSTON, 1957; HUSTON ET AL., 1962) CONFIRMED THESE FINDINGS. HENDRICH AND TURNER (1963) ALSO DEMONSTRATED THAT SHORT TERM EXPOSURE TO COLD STIMULATED THE THYROID OF THE FOWL AS INDICATED BY THYROIDAL I^{131} UPTAKE AND RELEASE WAS SHOWN TO RETURN TO NORMAL BY LONGER TERM EXPOSURE TO COLD. A STUDY BY HENDRICH AND TURNER (1965) DEMONSTRATED THAT A CHANGE FROM SUMMER TEMPERATURE TO CONSTANT 4.4°C COLD HAD NO SIGNIFICANT EFFECT ON THE $T_{1/2}$ OF THYROXINE- I^{131} IN FOWLS AND SUGGESTED THAT FLUCTUATING ENVIRONMENTAL TEMPERATURES PRODUCE CHANGES IN THE THYROID HORMONE UTILIZATION.

KOBAYASHI AND GORBMAN (1960) AND ROSENBERG ET AL. (1964) REPORTED THAT THE TIME OF PEAK I^{131} -UPTAKE WAS INFLUENCED BY THE CONCENTRATION OF IODIDE IN THE DIET. KOBAYASHI AND GORBMAN (1960) DEMONSTRATED THAT WITH A LOW IODINE DIET, RADIOACTIVITY FELL RAPIDLY AFTER REACHING A PEAK AT 6 HOURS.

VERY LITTLE IS KNOWN OF THE RELATION OF THYROID FUNCTION AND VITAMIN E. THE RATE OF I^{131} UPTAKE BY THE THYROID IN VITAMIN E EXCESS HAS BEEN REPORTED TO BE REDUCED IN THE RAT (VALENTI AND BOTTARELLI, 1965) AND

A SIMILAR OBSERVATION IN HAMSTER WAS REPORTED (DURAND ET AL., 1968). IF A COMPOUND IS ADMINISTERED WHICH INHIBITS THE SYNTHESIS OF THYROID HORMONES, A COMPENSATORY HYPERTROPHY OF THE THYROID GLAND OCCURS IN RESPONSE TO AN ELEVATION IN THYROTROPIC HORMONE (TSH) SECRETION RATE. IN ANIMALS SUBJECTED TO DIFFERENT ENVIRONMENTAL TEMPERATURES, THE RESULTANT DIFFERENCES IN SECRETION RATES OF TSH (HENDRICH AND TURNER, 1964) AND THYROID HORMONE WILL BE REFLECTED IN DIFFERENCES IN THE GOITROGENIC RESPONSE TO ADMINISTRATION OF A GOITROGENIC COMPOUND SUCH AS THIOURACIL.

THIOURACIL EXERTS ITS EFFECT BY PREVENTING THE FORMATION OF ORGANIC IODINATED COMPOUNDS IN THE THYROID GLAND AND PREVENTING RECYCLING OF IODINE (FRANDLIN, LERNER AND CHAIKOFF, 1944; ASTWOOD, 1945; VANDERLAAN AND BISSEL, 1946). KOBAYASHI AND GORBMAN (1960) OBSERVED IN CHICKS THAT THE RATIO OF MONOIODOTYROSINE (MIT) TO DIIODOTYROSINE (DIT) WAS GREATER AT 48 HOURS AFTER ADMINISTRATION OF THIOURACIL THAN AT 6 HOURS, INDICATING ACCUMULATION OF MIT, AND SUGGESTED THAT THIOURACIL PRODUCED A BLOCK IN THYROXINE SYNTHESIS BETWEEN MIT AND DIT. IN RECENT YEARS, SEVERAL INVESTIGATORS (CUNNINGHAM, 1964; MALOOF AND SOODAF, 1963; JIROUSEK AND CUNNINGHAM, 1968), SUGGESTED THAT A PROTEIN SULFENYL IODIDE AS AN ACTIVE CARRIER FOR I^+ IS A KEY INTERMEDIATE IN THE ENZYMATIC IODINATION OF TYROSINE IN THE THYROID GLAND. THE SULFENYL IODIDE INTERMEDIATE SERVES TO STABILIZE I^+ WHICH IS INVOLVED IN THE IODINATION OF TYROSINE. A POSSIBLE MECHANISM IS THAT ANTITHYROID AGENTS SUCH AS THIOURACIL AND THIOUREA HAVE VERY HIGH RATES OF REACTION WITH SULFENYL IODIDE TO FORM AN INACTIVE MIXED DISULFIDE (CUNNINGHAM, 1964).

THIOURACIL NOT ONLY BLOCKS THE FORMATION OF THYROXINE IN THE THYROID

GLAND, BUT ALSO APPEARS TO INTERFERE WITH PERIPHERAL ACTIVITY OF THYROXINE. BARKER ET AL. (1949), ANDIK ET AL. (1949), AND STASILLI ET AL. (1960) SHOWED THAT THE CALORIGENIC EFFECTIVENESS OF THYROXINE WAS DECREASED BY FEEDING EITHER THIOURACIL OR METHYLTHIOURACIL. THE RESULTS OF SEVERAL REPORTS (VAN ARSDEL AND WILLIAMS, 1956; HOGNESS ET AL., 1954; STASILLI ET AL., 1960; JONES AND MIDDLESWORTH, 1960; ESCOBAR AND ESCOBAR, 1961, 1962; HERRERA ET AL., 1963) HAVE SHOWN THAT PROPYLTHIOURACIL DECREASED THE AMOUNT OF I^{131} EXCRETED IN THE URINE AND INCREASED THE FECAL EXCRETION OF I^{131} -THYROXINE AFTER THE ADMINISTRATION OF I^{131} LABELED THYROXINE, PRESUMABLY BY INHIBITING THEIR DEIODINATION. DEIODINATION OF BOTH PHENOLIC RING AND INNER RING IODINES IS AFFECTED (FLOCK AND BOLLMAN, 1962; HERRERA ET AL., 1963). JAGIELLOW AND MCKENZIE (1960) HAVE OBSERVED THAT THE PROTEIN-BOUND IODINE OF THIOURACIL-TREATED ANIMALS GIVEN GOITER-PREVENTING DOSES OF THYROXINE WAS HIGHER THAN THAT OF THYROXINE-TREATED, THYROIDECTOMIZED ANIMALS. HERSHMAN AND VAN MIDDLESWORTH (1962) SUPPORTED THESE FINDINGS. IT HAS BEEN SUGGESTED THAT THIOURACIL AND PROPYLTHIOURACIL ENHANCE THE BINDING OF THE HORMONE BY THE PLASMA PROTEIN, THEREBY DECREASING THE PERIPHERAL ACTIVITY OF THYROXINE (HERSHMAN AND VAN MIDDLESWORTH, 1962).

RADIOIODINE UPTAKE

THE DISCOVERY OF RADIOIODINE I^{131} BY ENRICO FERMI IN 1935 LED TO ITS USE BY HAMILTON AND SOLEY IN 1939 IN THE TEST OF THYROID FUNCTION. RADIOIODINE UPTAKE AS A PARAMETER OF THYROID ACTIVITY IS BASED UPON THE DYNAMICS OF IODINE METABOLISM WHICH MAY BE CONSIDERED FROM THREE ASPECTS:

(1) THE INORGANIC IODIDE IS TAKEN UP RAPIDLY FROM THE CIRCULATING BLOOD (VANDERLAAN AND VANDERLAAN, 1947; TAUROG ET AL., 1947; HALMI, 1961);

(2) IODINE MAY BE ORGANICALLY BOUND AS EARLY AS 15 MINUTES AFTER ADMINISTRATION (CHALKOFF AND TAUROG, 1949; WILLIAMS AND VICKERY, 1965);

(3) THE ORGANICALLY BOUND RADIOIODINE IS SOON RELEASED FROM THE THYROID GLAND (NADLER AND LEBLOND, 1955 AND 1958). A 24-HOUR-UP TAKE TEST IS WIDELY USED AND IS BASED ON THE ASSUMPTION THAT THE MAXIMUM LEVEL OF THYROIDAL I^{131} ACCUMULATION IS REPRESENTED BY THE LEVEL OF THYROIDAL I^{131} 24 HOURS AFTER ADMINISTRATION OF I^{131} (WAHLBERG, 1955; HAMILTON AND SOLEY, 1940; ROSENBERG ET AL., 1963). SOME INVESTIGATORS COMMENT THAT SINCE (1) THERE IS RELEASE OF INORGANIC IODINE FROM THE GLAND (HALMI AND PITT-RIVERS, 1962); (2) RADIOIODINE IN THE THYROID GLAND IS EASILY AFFECTED BY THE LEVEL OF IODINE IN THE DIET (ROSENBERG ET AL., 1964); (3) IODINE IS METABOLIZED IN THE THYROID GLAND IN A HETEROGENEOUS MANNER (ROSENBERG ET AL., 1966); THE VALIDITY OF UPTAKE OR RELEASE MEASUREMENTS AS AN INDEX OF THYROID ACTIVITY ACCORDINGLY BECOMES QUESTIONABLE. NEVERTHELESS, I^{131} UPTAKE AND RELEASE BY THE THYROID ARE EMPLOYED RATHER WIDELY AS COMPARATIVE INDICES OF THYROID ACTIVITY SINCE THE SPECIFIC RADIOACTIVITY OF THE IODINE DEMONSTRATED IN THE THYROID TISSUE IS EASILY MODIFIED BY CIRCUMSTANCES WITHIN THE NORMAL VARIATION OF THE PHYSIOLOGY OF THE ANIMALS. THE UPTAKE OF RADIOIODINE BY THE GLAND DOES NOT INDICATE THE ABSOLUTE AMOUNT OF IODINE PICKED UP, BUT MERELY REPRESENTS THE PERCENTAGE OF THE TOTAL STABLE IODIDE POOL TRAPPED (SILVER ET AL., 1955; ANDREWS, 1957).

VITAMIN E AND OXYGEN CONSUMPTION OF TISSUES

VITAMIN E DEFICIENCY HAS BEEN SHOWN TO INFLUENCE OXYGEN CONSUMPTION BUT THE EFFECT VARIES WITH THE TYPE OF TISSUE STUDIED. MARKED INCREASES IN OXYGEN CONSUMPTION FROM VARIOUS SPECIES OF VITAMIN E DEFICIENT ANIMALS HAVE BEEN REPORTED (VICTOR, 1934; MADSEN, 1936; FRIEDMAN AND MATTILL, 1941; HOUCHIN AND MATTILL, 1942A,B,C; HOUCHIN, 1942; KAUNITZ AND PAPPENHEIMER, 1943; RODERUCK ET AL., 1949; HIMMEL AND MELVILLE, 1951; ROSENKRANTZ, 1955; BIRD ET AL., 1963). RABBITS, HAMSTERS, RATS, AND GUINEA PIGS WERE USED AS THE EXPERIMENTAL ANIMALS IN THESE STUDIES. VICTOR (1934) OBSERVED NO EFFECT OF VITAMIN E DEFICIENCY ON THE OXYGEN CONSUMPTION OF DUCK MUSCLE. ROSENKRANTZ (1955) FOUND THAT RABBIT ADRENAL CORTEX AND LIVER SLICES SHOWED A DEFINITE INCREASE IN OXYGEN CONSUMPTION WHEREAS OXYGEN CONSUMPTION OF HEART AND KIDNEY TISSUES REMAINED UNCHANGED IN VITAMIN E DEFICIENCY. GRIGORYEVA AND SHCHUKINA (1967) OBSERVED THAT OXYGEN CONSUMPTION FOR THE OXIDATION OF α -KETO-GLUTARIC AND SUCCINIC ACIDS PER MG OF PROTEIN IN MITOCHONDRIA FROM DYSTROPHIC MUSCLE IN VITAMIN E DEFICIENCY WAS SIMILAR TO THAT FROM NORMAL TISSUE.

VITAMIN E AND MITOCHONDRIA

MITOCHONDRIA CONTAIN MUCH OF THE VITAMIN E PRESENT IN CELLS (WISS ET AL., 1962). OLIVEIRA ET AL. (1968) FOUND TOCOPHEROL TO BE PRESENT IN ALL THE ENZYMATICALLY ACTIVE LIPO-PROTEIN COMPLEXES OF THE ELECTRON TRANSPORT SYSTEM OF INNER MITOCHONDRIA. FURTHER, THEY DEMONSTRATED THAT THE TOCOPHEROL DID NOT APPEAR TO HAVE A DIRECT QUANTITATIVE RELATIONSHIP WITH THE ACTIVITY OF ENZYMES, SUCH AS CYTOCHROME OXIDASE, NADH- AND SUCCINATE-CYTOCHROME C REDUCTASES. MITOCHONDRIA CONTAIN ABOUT 25% LIPID

(SWANSON AND ARTOM, 1950), MUCH OF WHICH IS UNSATURATED AND GREATER THAN 90% IS PHOSPHOLIPID. MOST OF THE LIPID OCCURS IN THE MITOCHONDRIAL MEMBRANE (FLEISCHER AND ROUSER, 1965; FLEISCHER, 1967; CHAPMAN AND LESLIE, 1970). SALKIN AND TAPPEL (1960) REPORTED THAT IN VIVO PEROXIDATION OCCURS IN THE LIVER AND KIDNEY OF VITAMIN E DEFICIENT RABBITS. LIPID PEROXIDATION IS CATALYZED BY HEMATIN COMPOUNDS, SUCH AS THE CYTOCHROMES CONTAINED IN MITOCHONDRIA (TAPPEL AND ZALKIN, 1959B). AN ELECTRON MICROSCOPIC STUDY HAS SHOWN MITOCHONDRIAL ALTERATIONS IN SKELETAL MUSCLE OF YOUNG CHICKS FED A VITAMIN E DEFICIENT DIET (CHEVILLE, 1966). FURTHER, VAN VLEET ET AL. (1968) SUGGESTED THAT TISSUE IN WHICH VITAMIN E HAS BEEN DEPLETED, SKELETAL MUSCLE MITOCHONDRIA UNDERWENT LIPID PEROXIDATION WITH RESULTING DESTRUCTION OF THE INNER MEMBRANES. FRAGMENTATION OF CRISTAE, MITOCHONDRIAL SWELLING DUE TO ALTERED MEMBRANE PERMEABILITY, FORMATION OF INTRAMITOCHONDRIAL MYELIN-FIGURE-LIKE MEMBRANOUS PROFILES, AND ACCUMULATION OF INTRAMITOCHONDRIAL DENSE GRANULES RESULTED.

EXPERIMENT 1

THYROIDAL RESPONSE TO EXCESS DIETARY VITAMIN E

TWO EXPERIMENTS WERE CONDUCTED TO STUDY THE EFFECTS OF EXCESS VITAMIN E IN RELATION TO THYROID ACTIVITY. IN ONE EXPERIMENT, CHICKS WERE FED NORMAL AND EXCESS LEVELS OF VITAMIN E AND WERE SUBJECTED TO DIFFERENT TEMPERATURES. THE GOITROGENIC EFFECT OF THIOURACIL WAS MEASURED. IN A SECOND EXPERIMENT, THE RATES OF 131 UPTAKE AND RELEASE WERE COMPARED IN CHICKS WITH NORMAL AND EXCESS INTAKE OF VITAMIN E.

EXPERIMENT 1A

MATERIALS AND METHODS

THIS EXPERIMENT WAS STARTED IN NOVEMBER, 1969.

EIGHTY DAY-OLD MALE WHITE LEGHORN CHICKS WERE WING-BANDED AND DISTRIBUTED AT RANDOM INTO 3 GROUPS IN ELECTRICALLY HEATED BATTERY BROODERS. THE CONSTITUENTS OF BASAL EXPERIMENTAL DIET IS SHOWN IN TABLE 1A. GROUP A RECEIVED ONLY THE BASAL DIET (WITHOUT SUPPLEMENTARY VITAMIN E), GROUP B RECEIVED AN EXCESS VITAMIN E LEVEL OF 220 I.U. PER KG. OF DIET AND GROUP C RECEIVED THE BASAL DIET PLUS ETHOXYQUIN¹ (EQ) AT 0.25% OF THE DIET. ROOM TEMPERATURE WAS THERMOSTATICALLY CONTROLLED AT 22°C. THE EXPERIMENTAL DIETS AND WATER WERE FED AD LIBITUM.

¹1,2 DIHYDRO-6-ETHOXY-2, 2, 4-TRIMETHYLQUINOLINE.

AT 23 DAYS OF AGE, ALL THE BIRDS OF THE 3 GROUPS WERE WEIGHED AND 24 CHICKS FROM EACH GROUP WERE SELECTED BY A SYSTEMATIC PROCEDURE ACCORDING TO THE INDIVIDUAL BODY WEIGHTS IN ORDER TO REDUCE THE VARIATIONS OF THE INITIAL BODY WEIGHTS OF THE EXPERIMENTAL CHICKS AMONG THE 3 GROUPS.

EACH GROUP WAS DIVIDED INTO 2 SUBGROUPS FOR THE HOT AND COLD ENVIRONMENTAL CHAMBERS. THE COLD ENVIRONMENTAL CHAMBER WAS REGULATED AT 14.5°C AND THE HOT ENVIRONMENTAL CHAMBER AT 31.5°C . THE VARIOUS GROUPS OF CHICKS WERE PLACED IN THE ENVIRONMENTAL CHAMBERS FOR 2 DAYS ADAPTATION BEFORE THE EXPERIMENT WAS STARTED.

EACH SUBGROUP WAS DIVIDED INTO 2 LOTS OF 6 CHICKS EACH. ONE LOT WAS MAINTAINED ON THE ORIGINAL EXPERIMENTAL DIET AND THE OTHER LOT RECEIVED 0.1% THIOURACIL (TU) IN THE EXPERIMENTAL DIET. THE TREATMENTS ARE SUMMARIZED AS FOLLOWS:

EXPERIMENTAL DESIGN

	BASAL	TU	EXCESS VITAMIN E	EXCESS VITAMIN E +TU	EQ	EQ +TU
14.5°C	6*	6	6	6	6	6
31.5°C	6	6	6	6	6	6

*NUMBER OF CHICKS.

THE TEMPERATURES IN THE 2 ENVIRONMENTAL CHAMBERS WERE RECORDED BY A RECORDING THERMOGRAPH. FEED CONSUMPTION WAS ALSO RECORDED. THE EXPERIMENTAL PERIOD WAS 14 DAYS. AT THE END OF THE EXPERIMENT, ALL CHICKS WERE WEIGHED AND KILLED. THE THYROID GLANDS WERE REMOVED AND WEIGHED.

EXPERIMENT 1B

THIS EXPERIMENT WAS STARTED IN JULY, 1970. THE PROCEDURE AND THE TREATMENTS WERE THE SAME AS EXPERIMENT 1A WITH EXCEPTION OF LARGER POPULATION (15 INDIVIDUALS) IN EACH LOT AND TEMPERATURE (22°C) IN THE ETHOXYQUIN-TREATED BIRDS. THE BASAL DIET WAS ALSO MODIFIED AS SHOWN IN TABLE 1B, BECAUSE THE DIETS FED IN EXPERIMENT 1A WERE ACCIDENTLY FORMULATED WITHOUT ADEQUATE CALCIUM SUPPLEMENTATION.

RESULTS AND DISCUSSION

THE RESULTS FOR EXPERIMENTS 1A AND 1B ARE SUMMARIZED IN TABLES 2 AND 3. AVERAGE VALUES ARE EXPRESSED \pm SAMPLE STANDARD DEVIATION.

IN BOTH EXPERIMENTS, THE THYROID WEIGHTS OF THE CHICKS PER 100 GM. BODY WEIGHT WERE SIGNIFICANTLY GREATER IN THE 14.5°C ENVIRONMENTAL CHAMBER THAN THOSE IN THE 31.5°C ENVIRONMENTAL CHAMBER ($P \leq 0.05$). THIS RESULT IS IN AGREEMENT WITH PREVIOUSLY REPORTED FINDINGS (HOFFMANN AND SHAFFNER, 1950; JOINER AND HUSTON, 1957; HUSTON ET AL., 1962).

THIOURACIL CAUSES GOITER BY ENHANCING TSH OUTPUT IN RESPONSE TO A BLOCK IN THE SYNTHESIS OF THYROXINE BY THE THYROID GLAND (DEMPSEY AND ASTWOOD, 1943). THE EXTENT OF WHICH THYROID WEIGHT INCREASES IN RESPONSE TO THE FEEDING OF THIOURACIL VARIES WITH THE RATE OF SECRETION OF TSH (BARKER, 1955; BROWN-GRANT, 1957). THE RATE OF SECRETION OF TSH IS, IN TURN, DEPENDENT UPON THE NORMAL RATE AT WHICH THYROXINE IS SECRETED BY THE ANIMAL. ACCORDINGLY THE RESPONSE OF THE THYROID GLAND TO THIOURACIL IN THE PRESENT EXPERIMENTS IS CONSIDERED TO BE AN INDICATION OF THE RATE OF THYROXINE SECRETION UNDER THE EXPERIMENTAL TREATMENTS IMPOSED,

I.E. DIFFERENT ENVIRONMENTAL TEMPERATURES AND DIETARY LEVELS OF VITAMIN E.

EXCESS VITAMIN E DID NOT AFFECT THE THYROID SIZE OF THE CHICKS FED THE DIET WITHOUT THIOURACIL AT EITHER TEMPERATURE (TABLES 2 AND 3, FIGURES 1 AND 2). THYROID SIZE IS NOT A PARTICULARLY SENSITIVE PARAMETER OF THYROID ACTIVITY OR TSH SECRETION RATE. IT IS KNOWN THAT HIGHER LEVELS OF TSH ARE REQUIRED TO INCREASE THYROID SIZE THAN IS NEEDED TO INCREASE THYROXINE SECRETION RATE.

EXCESS VITAMIN E DID AFFECT THYROID WEIGHT OF THE THIOURACIL-TREATED CHICKS (FIGURES 1 AND 2). IN EXPERIMENT 1A, THE THIOURACIL-TREATED CHICKS FED EXCESS VITAMIN E EXHIBITED A LESSER THYROID RESPONSE THAN DID THE THIOURACIL-TREATED CHICKS FED THE LOW VITAMIN E. THE DIFFERENCE IN RESPONSE WAS NOT STATISTICALLY SIGNIFICANT (TABLE 4) AND MAY HAVE BEEN DUE TO SMALL NUMBERS OF CHICKS EMPLOYED IN THE EXPERIMENT. THE EXPERIMENT WAS, THEREFORE, REPEATED WITH A LARGER POPULATION. THE RESULTS OF THIS SECOND EXPERIMENT CONFIRMED THE EVIDENCE OF EXPERIMENT 1A. THE DIFFERENCE IN THYROIDAL RESPONSE OF CHICKS FED THE DIFFERENT LEVELS OF VITAMIN E WAS STATISTICALLY SIGNIFICANT ($P \leq 0.01$) AS SHOWN IN TABLE 5. IT WAS CONCLUDED THAT THE RATE OF TSH SECRETION IN THE CHICKS FED EXCESS VITAMIN E WAS LESS THAN THAT IN THE CHICKS FED THE DIETS WITHOUT VITAMIN E SUPPLEMENTATION AND AS A CONSEQUENCE, EXCESS VITAMIN E INDIRECTLY DEPRESSED THYROID ACTIVITY.

ONE HYPOTHESIS CONSIDERED FOR THYROID RESPONSE TO DIETARY VITAMIN E WAS BASED ON THE FACT THAT LOW VITAMIN E CAUSED A DECREASE IN CIRCULATING THYROXINE LEVEL DUE TO INCREASED PERIPHERAL DEIODINATION IN THE LIVER AND MUSCLE (GALTON AND HINGBAR, 1965). GALTON AND HINGBAR (1965) OBSERVED

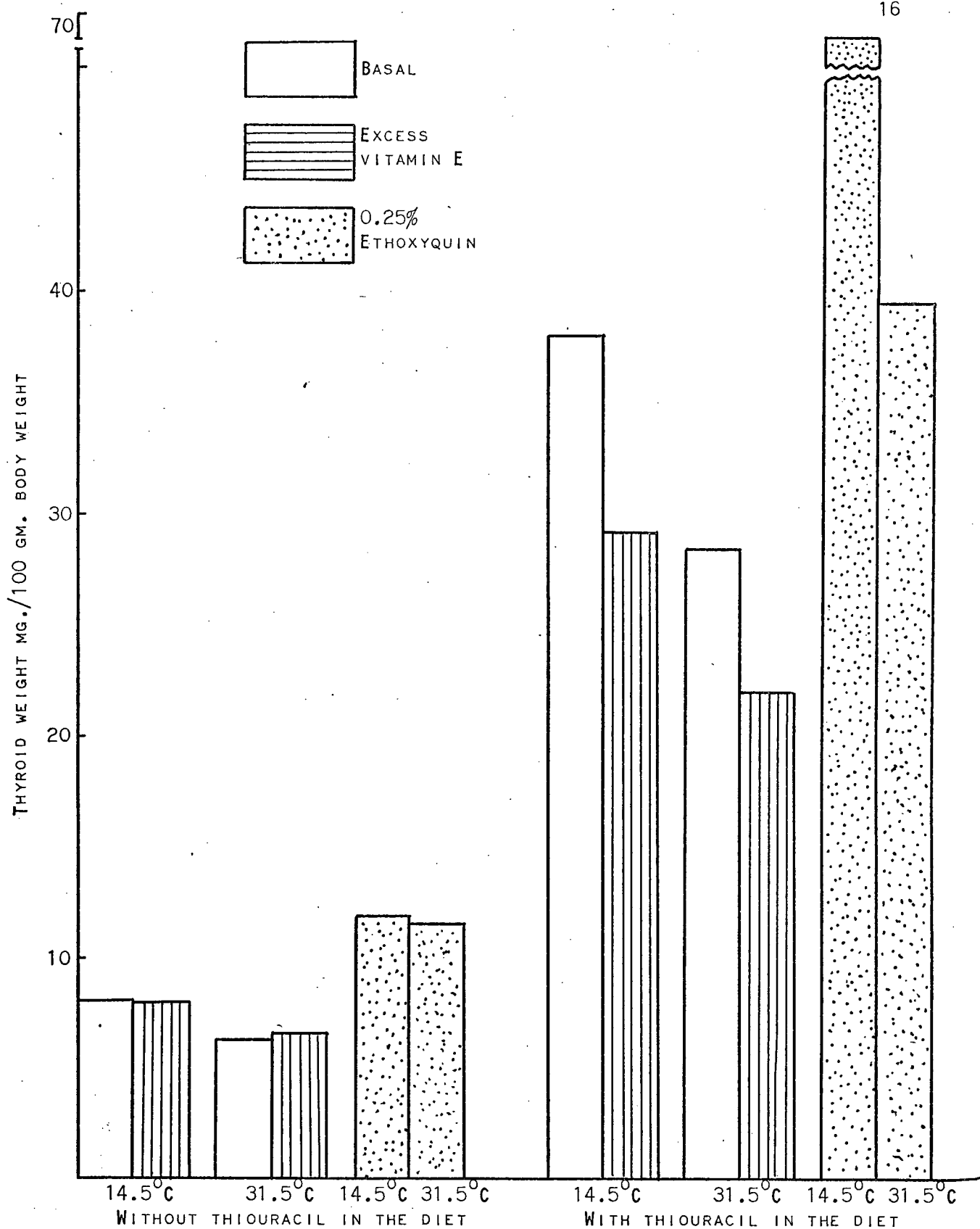


FIG. 1. THYROID RESPONSE OF CHICKS RECEIVING TWO LEVELS OF VITAMIN E AND/OR ETHOXYQUIN TO THIOURACIL AT DIFFERENT AMBIENT TEMPERATURES IN EXPERIMENT 1A.

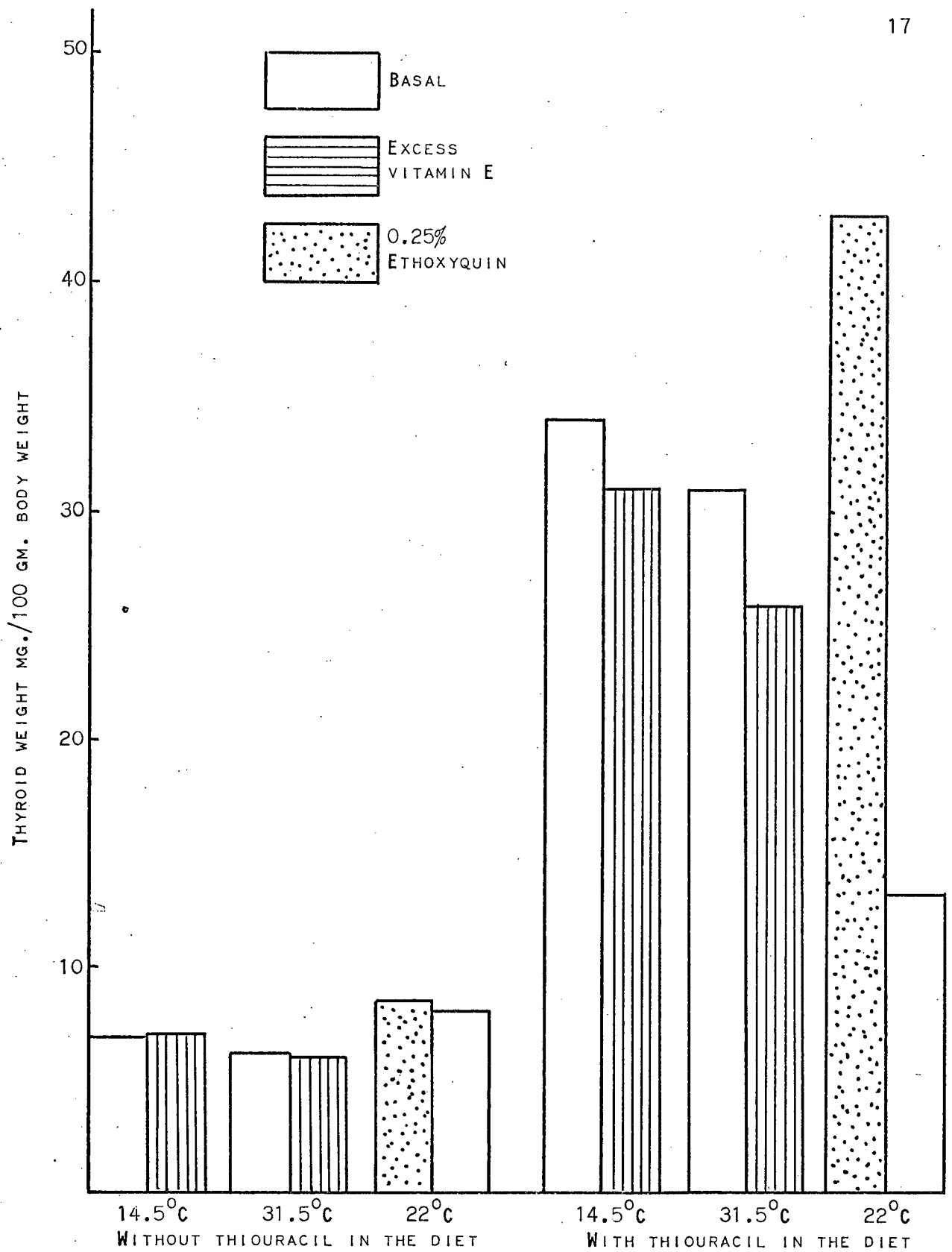


FIG. 2. THYROIDAL RESPONSE OF CHICKS RECEIVING TWO LEVELS OF VITAMIN E AND/OR ETHOXYQUIN TO THIOURACIL AT DIFFERENT AMBIENT TEMPERATURES IN EXPERIMENT 1B.

THAT DEIODINATION OF THYROXINE WAS INCREASED IN HOMOGENATES OF MUSCLE AND LIVERS FROM VITAMIN E DEFICIENT ANIMALS AND THAT LARGE DOSES OF TOCOPHEROL, EITHER ADMINISTERED IN VIVO OR ADDED TO TISSUE HOMOGENATES IN VITRO, GREATLY DECREASED HEPATIC OR MUSCULAR DEIODINATING ACTIVITY IN BOTH NORMAL AND VITAMIN E DEFICIENT RATS.

GRUENSTEIN (1970) HAS RECENTLY PROPOSED A THEORY FOR THE MECHANISM OF ACTION OF THYROXINE. HE SUGGESTS THAT THYROXINE ASSOCIATES WITH THE LIPIDS OF THE CELL MEMBRANE, WHEREUPON IT IS DEGRADED BY A FREE RADICAL MECHANISM, RESULTING IN THE RELEASE OF SOME OR ALL OF THE IODINE IN THE FORM OF I^+ OR I^0 . SEVERAL INVESTIGATORS (ROSENBERG AND JENDRASIAK, 1968; FINDELSTEIN AND CASS, 1968) HAD PREVIOUSLY SUGGESTED THAT A POSITIVE ION, I^+ OR A FREE RADICAL I^0 , RELEASED FROM THE THYROXINE MAY INTERACT WITH PHOSPHOLIPIDS AND CHOLESTEROL OF THE MEMBRANE, CHANGING THE PHYSICAL, STRUCTURAL AND/OR CHEMICAL PROPERTIES OF THESE LIPIDS SO THAT THE ELECTRICAL RESISTANCE OF THE MEMBRANE IS DECREASED. THE STUDIES OF WYNN (1968A,B) ON THYROXINE DEGRADATION IN RAT LIVER HAVE SHOWN THAT SOME OR ALL OF THE IODINE OF THYROXINE IS RELEASED AS PART OF THE INITIAL METABOLISM OF THE HORMONE AFTER IT REACHES TARGET TISSUES FROM THE PLASMA. HE FURTHER DEMONSTRATED THAT THYROXINE FUNCTIONS AS AN EXTREMELY POTENT ANTIOXIDANT, CAPABLE OF PREVENTING PEROXIDATION OF LECITHIN DERIVED FROM THE MICROSOMAL MEMBRANE AT EVEN LOWER CONCENTRATIONS THAN VITAMIN E (WYNN, 1968A). CASH ET AL. (1966) REPORTED THE ANTIOXIDANT PROPERTIES OF THYROXINE IN PREVENTING THE PEROXIDATION OF PHOSPHOLIPIDS IN MITOCHONDRIAL MEMBRANE. WYNN (1968A) HAS SHOWN THAT DURING THE ANTIOXIDATION REACTION IN THE PRESENCE OF METAL ION (Fe^{++}), OXYGEN AND PHOSPHOLIPID, THYROXINE IS ITSELF DEGRADED TO

RELEASE ITS IODINE. VITAMIN E IS GENERALLY CONSIDERED TO BE A BIOLOGICAL ANTIOXIDANT. THE RESULTS OF THE PRESENT EXPERIMENTS SUGGEST THAT EXCESS VITAMIN E MAY REPLACE THYROXINE IN ITS ANTIOXIDANT FUNCTION AND DEIODINATION OF THYROXINE MIGHT, THEREFORE, BE RETARDED. THE DEPRESSION OF DEIODINATION OF THYROXINE COULD THEN REDUCE THE RATE AT WHICH THYROID HORMONE IS REMOVED FROM THE CIRCULATION, THUS MAINTAINING THE LEVEL OF THE HORMONE IN THE CIRCULATION AND, THEREFORE, SUPPRESSING SECRETION OF TSH.

GROWTH RATE AND FEED CONSUMPTION WERE SIMILAR FOR LOW AND EXCESS VITAMIN E TREATED BIRDS KEPT AT TWO TEMPERATURES. THESE RESULTS INDICATE THAT METABOLIC RATES WERE SIMILAR IN BIRDS ON BOTH TREATMENTS DESPITE DIFFERENCES IN THYROID ACTIVITY. THIS SHOULD SUGGEST THAT TISSUE RESPIRATION IN BIRDS FED EXCESS VITAMIN E CAN BE MAINTAINED WITH A REDUCED SUPPLY OR TURNOVER OF THYROXINE.

ON THE OTHER HAND, ANBAR ET AL. (1965) REPORTED THE RESULTS OF EXPERIMENTS WHICH INDICATED DISSOCIATION OF THE CALORIGENIC EFFECT OF THYROID HORMONE FROM DEIODINATION. THEY FOUND THAT FOLLOWING THE INJECTION OF THYROXINE TO RATS, BASAL METABOLIC RATE WAS STIMULATED PRIOR TO DEIODINATION OF THYROXINE AND THAT BASAL METABOLIC RATE STIMULATION HAD LARGELY DIMINISHED BY THE TIME PEAK DEIODINATION ACTIVITY WAS OBSERVED. IF INCREASED DEIODINATION RATE IS A CONSEQUENCE OF ACCELERATED RESPIRATION RATHER THAN THE CAUSE, THEN SOME OTHER EXPLANATION FOR THE EFFECT OF VITAMIN E IN REDUCING THYROID ACTIVITY IS NECESSARY.

IT IS INTERESTING THAT 0.25% EQ EXERTED A GREAT EFFECT ON THE THYROIDAL RESPONSE (TABLES 2 AND 6, FIGURES 1 AND 2), BUT THE REVERSE

EFFECT OF THAT WAS OBTAINED WITH EXCESS VITAMIN E, ALTHOUGH BOTH VITAMIN E AND EQ ARE ANTIOXIDANTS. AT A LEVEL OF 0.25% EQ IS TOXIC (MARCH ET AL., 1968). THE MECHANISM BY WHICH EQ INCREASES THE GOITROGENICITY OF THIOURACIL IS OBSCURE.

EXPERIMENT 1c

THIS EXPERIMENT WAS DESIGNED TO STUDY THE RATES OF I^{131} UPTAKE AND RELEASE BY THE THYROID GLAND IN CHICKS IN RESPONSE TO FEEDING DIETS CONTAINING LOW AND EXCESSIVE LEVELS OF VITAMIN E.

MATERIALS AND METHODS

THE COMPOSITION OF THE BASAL DIET AND THE LEVEL OF VITAMIN E SUPPLEMENTATION WERE THE SAME AS IN EXPERIMENT 1B. TWO HUNDRED DAY-OLD WHITE LEGHORN MALE CHICKS WERE DISTRIBUTED AT RANDOM INTO TWO GROUPS OF ONE HUNDRED. ONE GROUP WAS FED THE BASAL DIET, AND THE OTHER GROUP THE VITAMIN E SUPPLEMENTED DIET. AT 23 DAYS OF AGE, ALL THE BIRDS OF THE TWO GROUPS WERE WEIGHED INDIVIDUALLY AND 40 CHICKS FROM EACH GROUP WERE SELECTED SYSTEMATICALLY AS IN EXPERIMENT 1A. ROOM TEMPERATURE WAS THERMOSTATICALLY CONTROLLED AT 22-23°C. THE 40 CHICKS OF EACH GROUP WERE DISTRIBUTED SYSTEMICALLY INTO 5 LOTS OF 8 CHICKS EACH. THE CHICKS WERE INJECTED INTRAVENOUSLY WITH 0.225 μ ci OF I^{131} IN 0.45 ML OF PHYSIOLOGICAL SALINE SOLUTION. FIVE, 10, 24, 48 AND 96 HOURS AFTER INJECTION, 8 CHICKS FROM EACH GROUP WERE WEIGHED. THE CHICKS WERE THEN KILLED AND THEIR THYROIDS REMOVED AND WEIGHED. THE I^{131} RADIOACTIVITY OF EACH PAIR OF GLANDS WAS MEASURED WITH A DEEP-WELL SCINTILLATION DETECTOR. MEASUREMENTS

OF RADIOACTIVITY WERE CORRECTED FOR COINCIDENCE LOSS, BACKGROUND RADIATION AND RADIOACTIVE DECAY.

THE CORRECTED COUNTS WERE EXPRESSED AS A PERCENTAGE OF THE INJECTED DOSE, THE RADIOACTIVITY OF WHICH WAS DETERMINED FROM A STANDARD. THE STANDARD WAS PREPARED BY DILUTING 5 ML. OF THE INJECTION SOLUTION INTO 500 ML. OF PHYSIOLOGICAL SALINE SOLUTION. RADIOACTIVITY WAS MEASURED IN A 1 ML. ALIQUOT OF THIS SOLUTION USING A DEEP-WELL SCINTILLATION DETECTOR. THE PERCENT I^{131} UPTAKE WAS EXPRESSED PER BIRD AND PER MG. OF THYROID GLAND.

RESULTS AND DISCUSSION

THYROIDAL RADIOACTIVITY AT DIFFERENT TIMES AFTER ADMINISTRATION OF I^{131} ARE SHOWN IN TABLE 7 AND IN FIGURES 3 AND 4. REGARDLESS OF THE METHOD OF EXPRESSING THYROIDAL RADIOACTIVITY (WHETHER PER BIRD OR PER MG. OF THYROID GLAND) THE DATA INDICATE THAT THE ACTIVITY OF THE THYROID GLAND IS DEPRESSED IN BIRDS FED EXCESS VITAMIN E. THE LEVELS OF I^{131} IN THE THYROID GLAND OF CHICKS FED SUPPLEMENTARY VITAMIN E WERE LOWER WHEN MEASURED 5 AND 10 HOURS AFTER I^{131} INJECTION THAN IN THE CONTROL CHICKS. THE DIFFERENCES BETWEEN THE CHICKS RECEIVING THE RESPECTIVE LEVELS OF VITAMIN E WERE STATISTICALLY SIGNIFICANT AT 5 AND 10 HOURS AFTER INJECTION WHEN CALCULATED ON THE BASIS OF RADIOIODINE UPTAKE PER BIRD AND WERE SIGNIFICANT AT 5, 10 AND 24 HOURS AFTER INJECTION WHEN CALCULATED ON THE BASIS OF RADIOIODINE UPTAKE PER MG. OF THYROID TISSUE. SUBSEQUENT TO 24 HOURS AFTER INJECTION TIME, THERE WAS A RAPID DECLINE IN THYROIDAL I^{131} IN THE CONTROL BIRDS WHEREAS THE LOSS OF RADIOACTIVITY FROM THE THYROID OF THE BIRDS FED EXCESS VITAMIN E WAS NOT SIGNIFICANT. THIS EXPERIMENT CONFIRMS THE PREVIOUS FINDINGS (VALENTI AND BOTTARELLI, 1965; DURAND ET AL., 1966) THAT I^{131} UPTAKE BY THE THYROID GLAND WAS REDUCED IN MAMMALS GIVEN A SUBCUTANEOUS INJECTION OF AN EXCESSIVE LEVEL OF VITAMIN E.

IN THIS EXPERIMENT, 0.5% IODIZED SALT WAS ADDED TO THE DIET TO ENABLE NORMAL IODINE METABOLISM IN THE THYROID GLAND. KOBAYASHI AND GORBMAN (1960) AND ROSENBERG ET AL. (1964) HAVE DEMONSTRATED IN RATS AND CHICKS THAT THE

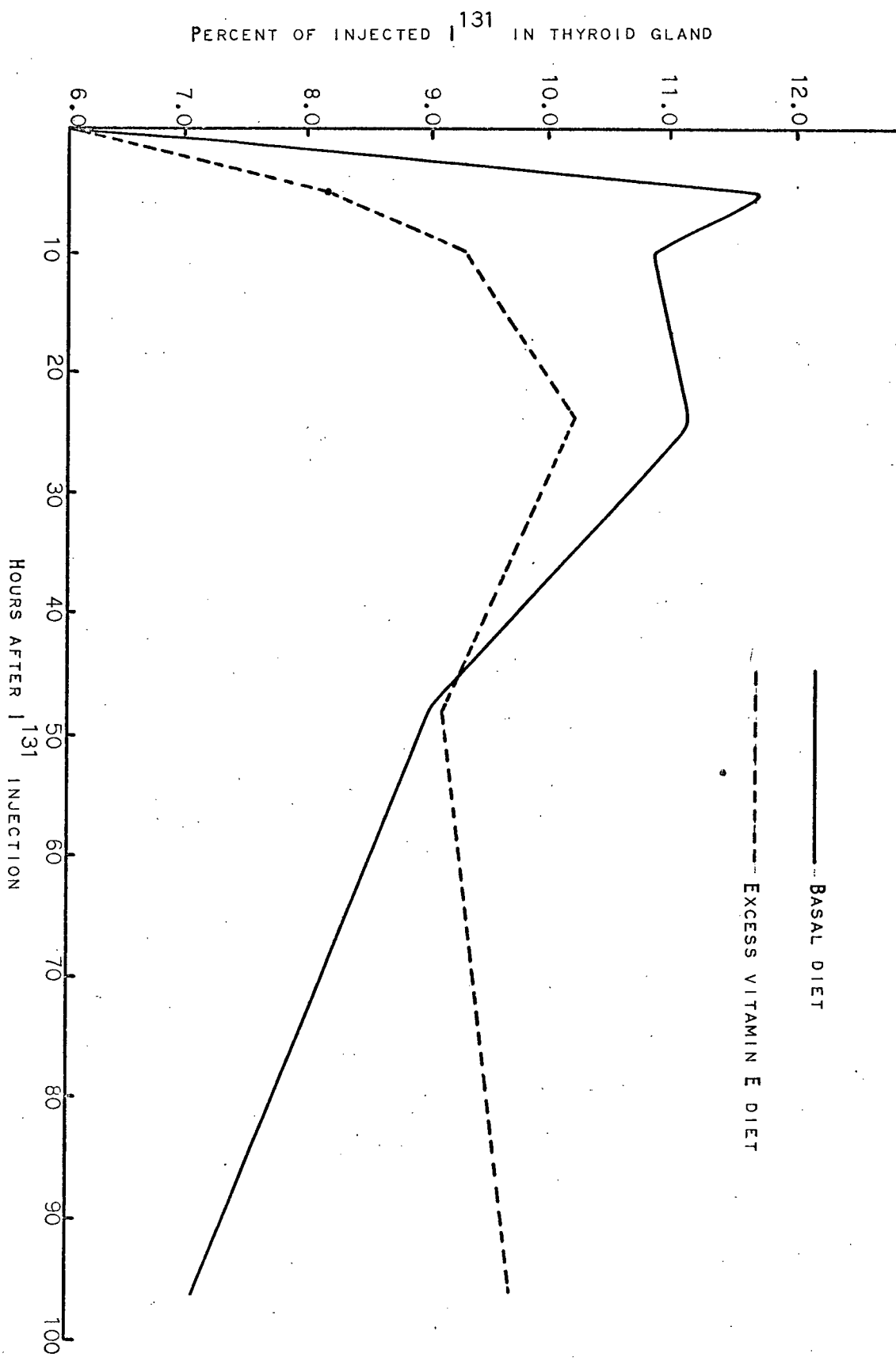


FIG. 3. EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (I^{131}) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS.

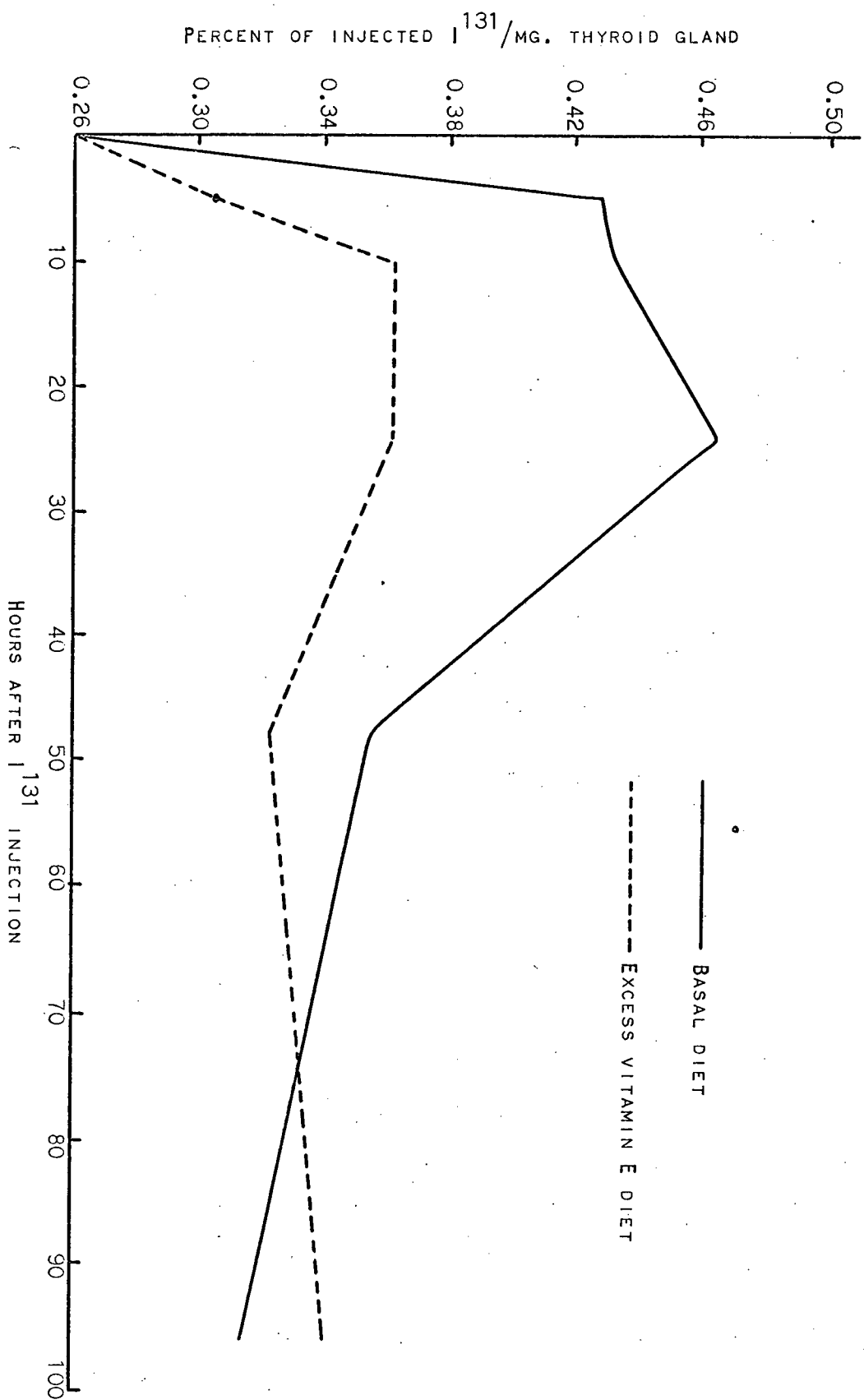


FIG. 4. EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (I^{131}) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS.

TIME OF PEAK I^{131} UPTAKE WAS INFLUENCED BY THE LEVEL OF DIETARY IODINE. KOBAYASHI AND GORBMAN (1960) HAVE SHOWN THAT IN CHICKS FED A LOW IODINE DIET THYROIDAL RADIOACTIVITY FELL RAPIDLY AFTER REACHING A PEAK AT 6 HOURS. ROSENBERG ET AL. (1964) FOUND THAT IN COCKERELS FED AN IODIDE SUPPLEMENTED DIET, AVERAGE MAXIMAL I^{131} UPTAKE OF 13% OF THE INJECTED DOSE WAS REACHED 12 HOURS AFTER INJECTION AND A VERY LONG RETENTION OF THYROIDAL RADIOIODIDE OCCURED.

THE RESULTS OF THIS EXPERIMENT, WHICH SHOWED THAT BOTH THE RATES OF IODINE UPTAKE AND OF IODINE RELEASE BY THE THYROID GLAND ARE DEPRESSED IN CHICKS FED EXCESS VITAMIN E, SUBSTANTIATE THE CONCLUSIONS OF EXPERIMENTS 1A, AND B, WHEREAS IN EXPERIMENT 1, HOWEVER, THE DATA INDICATED THAT TSH SECRETION WAS REDUCED IN HYPERVITAMINOSIS E, EXPERIMENT 1C PROVIDES DIRECT EVIDENCE THAT THYROID SECRETION RATE IS LESS IN EXCESS VITAMIN E FED CHICKS.

EXPERIMENT II

RESPIRATION RATE OF MUSCLE MITOCHONDRIA FROM CHICKS

FED EXCESS DIETARY VITAMIN E

THE EXPERIMENT WAS DESIGNED TO STUDY THE OXYGEN CONSUMPTION OF MUSCLE MITOCHONDRIA FROM NORMAL AND THIOURACIL-TREATED CHICKS FED NORMAL AND EXCESS LEVELS OF VITAMIN E.

MATERIALS AND METHODS

SIXTY DAY-OLD MALE WHITE LEGHORN CHICKS WERE DISTRIBUTED INTO TWO GROUPS IN ELECTRICALLY HEATED BATTERY BROODERS. THE CONTROLS RECEIVED THE BASAL DIET WHILE THE OTHER GROUP WAS FED THE BASAL DIET SUPPLEMENTED WITH 2200 I.U. OF DL- α -TOCOPHERYL ACETATE PER KG. AFTER 40 DAYS, 15 CHICKS OF EACH GROUP WERE FED THE RESPECTIVE DIET WITH THE ADDITION OF 0.1% OF THIOURACIL. FIFTEEN DAYS LATER, THE CHICKS (55 DAYS OLD) WERE KILLED AND THE PECTORAL MUSCLE EXCISED IMMEDIATELY FOR THE ISOLATION OF MITOCHONDRIA.

PREPARATION OF MITOCHONDRIA

APPROXIMATELY 15 GM OF MUSCLE FROM EACH CHICK WAS IMMERSSED IN ICE-CHILLED 0.15M KCl SOLUTION. THE TISSUE WAS THEN HOMOGENIZED WITH 100 ML OF THE ICE-CHILLED CHAPPELL-PERRY TRIS-KCl MEDIUM (1954), USING A VIRTIS HOMOGENIZER AT A SETTING OF 70 FOR 45 SECONDS. MYOFIBRILS, NUCLEI AND UNBROKEN CELLS WERE REMOVED BY TWO SUCCESSIVE CENTRIFUGATIONS AT 600 X G FOR 10 MINUTES AT 0-4°C (AZZONE ET AL., 1961). THE FINAL

SUPERNATANT FLUID WAS THEN CENTRIFUGED AT 8500 X G FOR 10 MINUTES AT 0-4°C. THE MITOCHONDRIA WERE WASHED TWICE BY RESUSPENSION IN THE ISOLATION MEDIUM AND RECENTRIFUGED AT 8500 X G. THE MITOCHONDRIAL PELLET WAS RINSED WITH 0.15M KCl AT A CONCENTRATION OF APPROXIMATELY 3 MG. OF MITOCHONDRIA PER ML. THE PROTEIN CONTENT OF THE MITOCHONDRIAL SUSPENSION WAS DETERMINED BY THE BIURET METHOD (GORNAL ET AL., 1949) AFTER CLEARING THE SUSPENSION WITH 0.5% SODIUM DEOXYCHOLATE. CRYSTALLINE BOVINE SERUM ALBUMIN WAS USED AS A STANDARD TO PLOT A CALIBRATION CURVE FOR PROTEIN ESTIMATIONS.

OXYGEN UPTAKE BY MITOCHONDRIA

RESPIRATION OF MITOCHONDRIA WAS MEASURED BY THE WARBURG MANOMETRIC TECHNIQUE (UMBRIET ET AL., 1959). THE MEDIUM USED WAS THAT OF AZZONE (1961) WITH SOME MODIFICATION. EACH VESSEL CONTAINED 25 MM TRIS BUFFER (PH 7.5), 50 MM KCl, 6 MM MgCl₂, 20 MM Pi (PH 7.5), 0.01 MM α -KETO-GLUTARATE, 0.02 MM CYTOCHROME C, 1 MM ATP (DI-NA-SALT), 30 MM GLUCOSE AND 0.33 MG. YEAST HEXOKINASE (SIGMA TYPE V). THE ADDITION OF GLUCOSE, HEXOKINASE AND CYTOCHROME C CAN STIMULATE THE RESPIRATION IN THE MEDIUM DURING INCUBATION PERIOD (HEDMAN, 1965). ATP WAS USED FOR PREVENTION OF MITOCHONDRIAL SWELLING (CHAPPEL AND PERRY, 1954). THE TOTAL LIQUID VOLUME WAS 3 ML PER WARBURG VESSEL. ALL FLASKS WERE EQUILIBRATED AT 30°C FOR 5 MINUTES IMMEDIATELY AFTER ADDITION OF 1 ML OF THE MITOCHONDRIA TO THE REACTION MIXTURE. INCUBATION WAS AT 30°C WITH OXYGEN AS THE GAS PHASE FOR A PERIOD OF 2 HOURS. ALL DETERMINATIONS WERE PERFORMED IN DUPLICATE OR TRIPLICATE. THE OXYGEN UPTAKE OF THE MITOCHONDRIA WAS MEASURED AT 30 MINUTE INTERVALS

FOR TWO HOURS. OXYGEN UPTAKE IS EXPRESSED AS $\mu\text{L}/\text{MG}$ OF MITOCHONDRIAL PROTEIN/HOUR.

ELECTRON MICROSCOPIC STUDY OF MUSCLE MITOCHONDRIA

A SMALL SAMPLE OF A PELLET OF THE FRESH ISOLATED MITOCHONDRIA WAS TRANSFERRED INTO A MICROCENTRIFUGE TUBE ACCORDING TO THE METHOD OF MALAMED (1963) AND THEN FIXED IN 1% OsO_4 WITH A VERONAL-ACETATE ISOTONIC BUFFER AT PH 7.4 FOR 2 HOURS AT 4°C (KAY, 1966). THE SLURRY OF MITOCHONDRIA WAS REAGGREGATED INTO A MANAGEABLE PELLET IN 5 MINUTES OF CENTRIFUGATION. AFTER DEHYDRATION IN A GRADED ALCOHOL SERIES AND FINALLY IN PROPYLENE OXIDE, THE SAMPLES WERE EMBEDDED IN EPOXY 812 (LUFT, 1961). ULTRATHIN SECTIONS WERE MADE WITH MT-2 MICROTOME AND MOUNTED ON CARBONED-COLLODION-COATED 200 MESH COPPER GRIDS. DOUBLE STAINING WAS USED WITH URANYL ACETATE FOR 20 MINUTES (STEMPAK AND WARD, 1964) FOLLOWED BY LEAD CITRATE FOR 15 MINUTES (REYNOLDS, 1963). THE STAINED SECTIONS WERE EXAMINED WITH A HITACHI-78 ELECTRON MICROSCOPE.

RESULTS AND DISCUSSION

THE RESULTS SHOW A SIGNIFICANT DECREASE IN THE OXYGEN CONSUMPTION OF THE MUSCLE MITOCHONDRIA OF THE CHICKS FED EXCESSIVE LEVEL OF VITAMIN E, COMPARED WITH THE CHICKS FED THE CONTROL DIET (TABLE 8). THE DEPRESSION IN RESPIRATION DUE TO EXCESS VITAMIN E WAS SIGNIFICANT EVEN WHEN METABOLIC RATE HAD BEEN REDUCED BY THE FEEDING OF THIOURACIL. IT SHOULD BE NOTED THAT THE LEVEL OF VITAMIN E SUPPLEMENTATION IN THIS EXPERIMENT WAS 10 TIMES THAT USED IN EXPERIMENT 1. BECAUSE TISSUE RESPIRATION RATE IS KNOWN TO BE ACCELERATED IN VITAMIN E DEFICIENCY, IT MAY BE DEDUCED THAT THE RESPONSE TO EXCESS VITAMIN E WILL BE RELATIVE TO THE LEVEL ADMINISTERED AND THAT A DEPRESSION IN RESPIRATION RATE WAS LIKEWISE ASSOCIATED WITH THE REDUCTION IN THYROID ACTIVITY WITH THE LEVEL OF VITAMIN E FED IN EXPERIMENT 1. A POSITIVE ION, I^+ OR I^0 RELEASED DURING THYROXINE DEGRADATION INTERACTS WITH THE PHOSPHOLIPIDS OF CELLULAR MEMBRANE, RESULTING IN A LOWERING OF THE ELECTRICAL RESISTANCE OF THE MEMBRANE (FINKELSTEIN AND CASS, 1968; ROSENBERG AND JENDRASIAK, 1968). THIS LOWERED ELECTRICAL RESISTANCE, WHICH IS POSTULATED TO BE THE PRIMARY ACTION OF THYROXINE, MAY BE THE RESULT OF EITHER INCREASED IONIC CONDUCTIVITY, OR INCREASED ELECTRONIC CONDUCTIVITY, AND MAY LOWER THE RESISTANCE EITHER TRANSVERSE OR PARALLEL TO THE PLANE OF THE MEMBRANE. MITOCHONDRIA TRANSFER ELECTRONS FROM SUBSTRATES SUCH AS NADH OR SUCCINATE TO MOLECULAR OXYGEN ALONG THE CYTOCHROME CHAIN ASSOCIATED WITH THE MITOCHONDRIAL MEMBRANE (GREEN ET AL., 1964). IT HAS BEEN SHOWN

THAT PHOSPHOLIPID FOR THE ACTIVITY OF THE ELECTRON TRANSPORTING SUBMITOCHONDRIAL FRACTIONS I, II, III AND IV WERE REQUIRED IN THE MEMBRANE OF MITOCHONDRIA (BRIERLY ET AL., 1962; BRIERLY AND MEROLA, 1962; FLEISCHER AND FLEISCHER, 1964). GRUENSTEIN (1970) SUGGESTED THAT, BY INCREASING THE CONDUCTIVITY OF THE PHOSPHOLIPIDS, THYROXINE MAY EITHER FACILITATE THE TRANSFER OF ELECTRONS FROM CYTOCHROME TO CYTOCHROME, OR EVEN TO SOME EXTENT "SHORT CIRCUIT" PARTS OF THE ELECTRON TRANSPORT CHAIN, THUS CAUSING MORE RAPID REDUCTION OF OXYGEN AND PARTIAL UNCOUPLING OF PHOSPHORYLATION. THIS ACTION IS IN AGREEMENT WITH THE OBSERVED INCREASED OXYGEN CONSUMPTION EFFECT OF THYROXINE (TATA ET AL., 1963). EXCESS VITAMIN E MAY DEPRESS THE DEIODINATION OF THYROXINE (GALTON AND HINGBAR, 1965). WITH A LESSER AMOUNT OF I^+ OR I^0 RELEASED IN MEMBRANE OF MUSCLE MITOCHONDRIA, CONDUCTIVITY OF THE PHOSPHOLIPID WILL BE LOWER, PHOSPHORYLATION RATE WILL BE REDUCED AND OXYGEN UPTAKE IS DECREASED.

IT IS CONCLUDED THAT EXCESS VITAMIN E NOT ONLY DEPRESSED THE ACTIVITY OF THYROID GLAND BUT ALSO REDUCE THE RESPIRATION RATE OF MUSCLE MITOCHONDRIA.

THE GROWTH RATE OF THE CHICKS FED THE EXCESS LEVEL OF VITAMIN E IN THIS EXPERIMENT WAS MARKEDLY LOWER THAN THAT OF THE CONTROL CHICKS. IT IS SUGGESTED THAT THE CHICK MAY MAINTAIN NORMAL RESPIRATION RATE WHEN FED EXCESS VITAMIN E UP TO A CERTAIN LEVEL THROUGH A REDUCTION IN THYROID ACTIVITY (SEE DISCUSSION OF EXPERIMENT 1). WITH A LARGE EXCESS OF VITAMIN E, HOWEVER, NO FURTHER COMPENSATION IS POSSIBLE AND RESPIRATION RATE IS DEPRESSED BELOW NORMAL.

FROM THE ELECTRON MICROGRAPHS, SWOLLEN MITOCHONDRIA WERE CLEARLY

SEEN IN PREPARATIONS FROM BOTH CONTROL CHICKS AND CHICKS FED AN EXCESS OF VITAMIN E (FIGURES 5 AND 6). SOME SPECIMENS, HOWEVER, SHOWED NORMAL INTACT MITOCHONDRIA (FIGURE 7). THE SWOLLEN APPEARANCE OF THE MITOCHONDRIA MAY BE DUE TO THE SEVERAL WASHINGS WITH CHAPPEL AND PERRY TRIS-KCL MEDIUM. TABLE 8 INDICATES THAT THE RESPIRATION RATE OF THE MITOCHONDRIA IN THE PERIOD OF 0-30 MINUTES AND OF 30-60 MINUTES ARE NEARLY EQUAL. ORDINARILY IT IS REPORTED THAT RESPIRATION RATE DECLINES IN SUCCESSIVE 30 MINUTE PERIODS. IT APPEARS THAT THE MITOCHONDRIA MAY BE SWELLING CONTINUOUSLY DURING THE INCUBATION PERIOD OF 0-30 MINUTES UNTIL A CERTAIN AMOUNT OF ATP IS FORMED. THE SWELLING OF MUSCLE MITOCHONDRIA CAN BE REVERSED BY THE PRESENCE OF LOW CONCENTRATIONS OF ATP (CHAPPEL AND PERRY, 1954) AND THUS THE MITOCHONDRIA UNDERGO A NORMAL RESPIRATION IN THE PERIOD OF 30-60 MINUTES. THE ELECTRON MICROGRAPH ALSO SHOWS THAT THE MITOCHONDRIA PREPARATION WAS CONTAMINATED WITH SMALL PARTICLES WHICH MIGHT BE SUBMITOCHONDRIA. IT APPEARS THAT THE METHOD OF PREPARATION OF MITOCHONDRIA USING THE FRACTION OBTAINED AT 8500 X G IS NOT A GOOD METHOD FOR OBTAINING INTACT MITOCHONDRIA FOR RESPIRATION STUDIES ALTHOUGH THE MITOCHONDRIAL YIELD IS GOOD. HEDMAN (1965) REPORTED THAT THE 3500 X G MITOCHONDRIAL FRACTION REPRESENTED RELATIVELY INTACT MITOCHONDRIA BUT THE MITOCHONDRIAL YIELD WAS REDUCED BY ABOUT ONE THIRD.

IN THE PRESENT EXPERIMENT, TWO ADVERSE EFFECTS OF HYPERVITAMINOSIS E WERE OBSERVED VIZ. BONE FRAGILITY AND AN INCREASE IN PROTHROMBIN TIME.

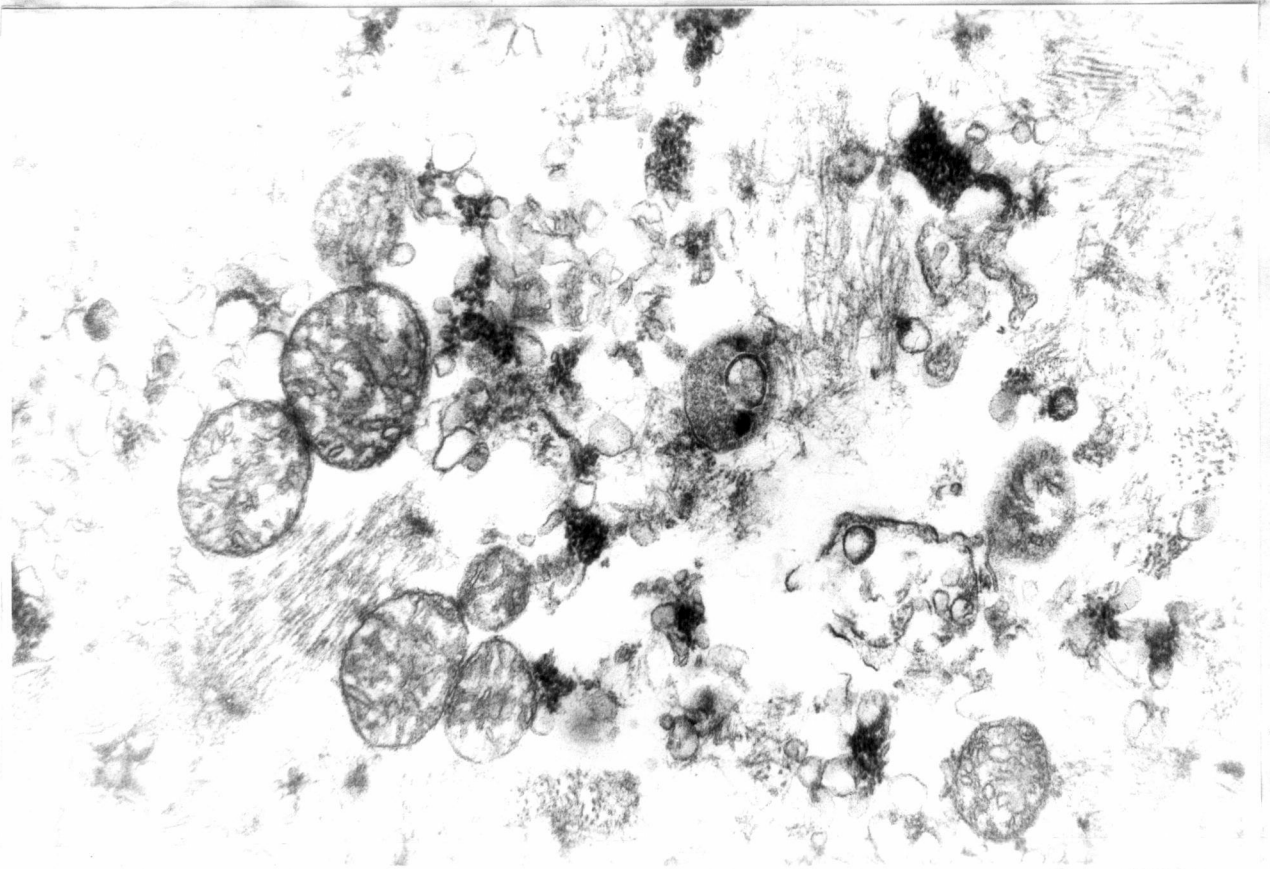


FIG. 5. ELECTRON MICROGRAPH OF BREAST MUSCLE MITOCHONDRIA FROM A CHICK FED A NORMAL LEVEL OF VITAMIN E. THE CONCENTRATION OF MITOCHONDRIA WAS LOW AND THE SUSPENSION CONTAMINATED WITH SUBMITOCHONDRIA. THE SWOLLEN MITOCHONDRIA WERE CLEARLY SEEN. X 30,000.

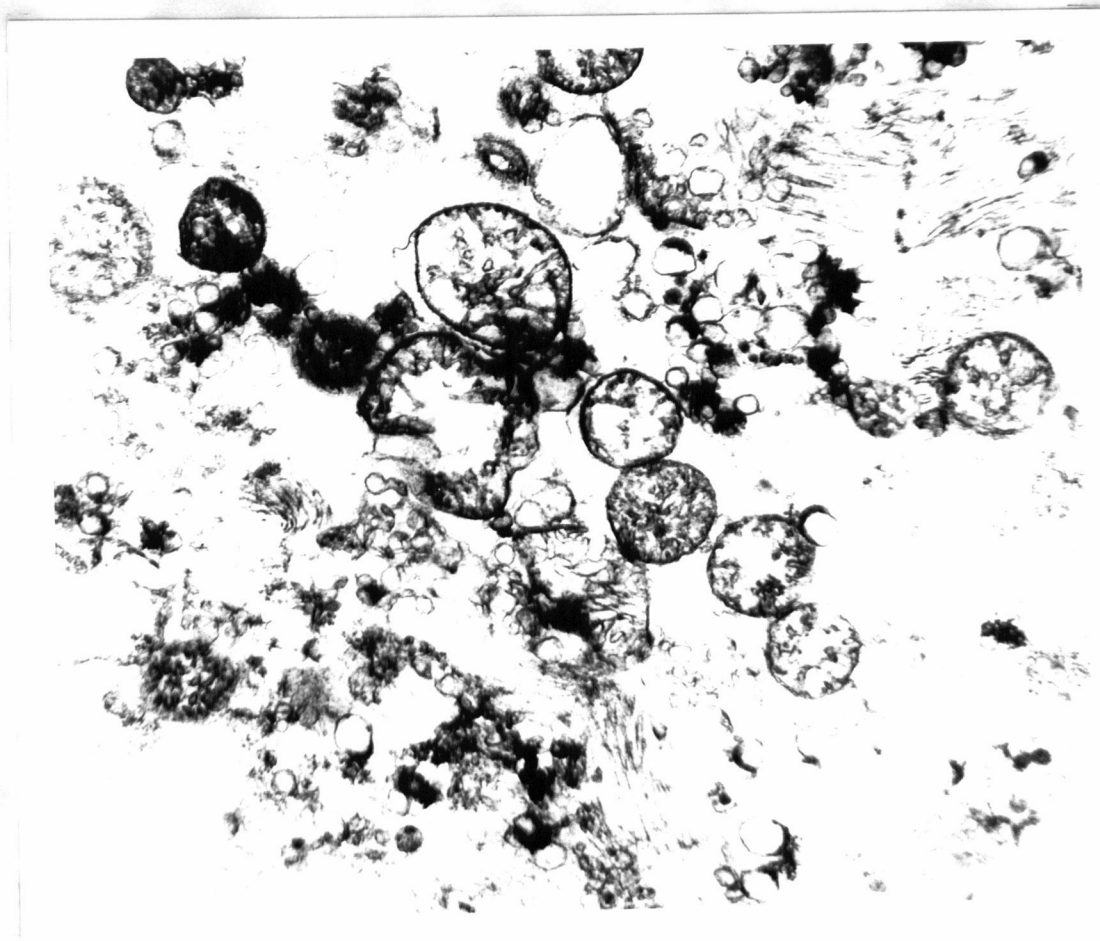


FIG. 6. ELECTRON MICROGRAPH OF A SECTION OF MUSCLE MITOCHONDRIA FROM A CHICK FED EXCESS VITAMIN E. THE SWOLLEN MITOCHONDRIA WERE CLEARLY SEEN AND THE SUSPENSION CONTAMINATED WITH SUBMITOCHONDRIA. x 30,000.

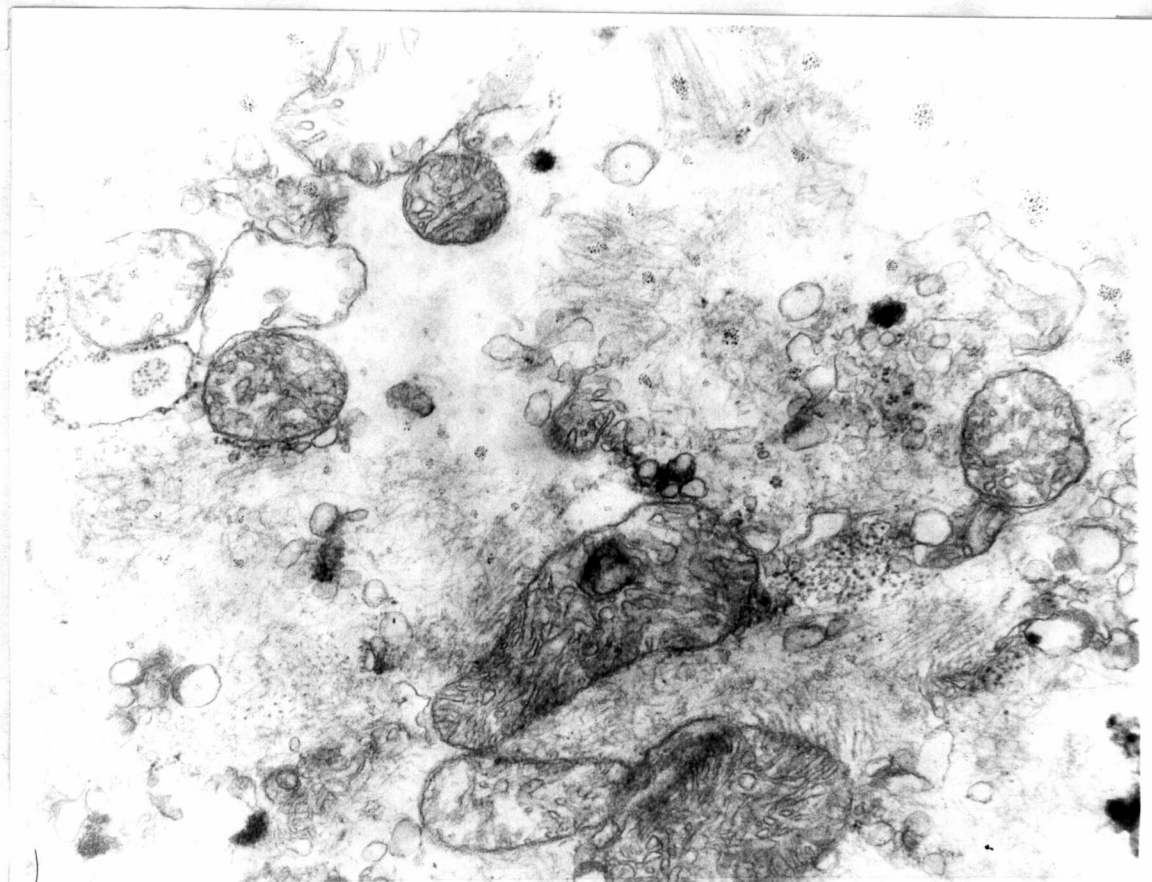


FIG. 7. ELECTRON MICROGRAPH OF A SECTION OF BREAST MUSCLE MITOCHONDRIA FROM A CHICK FED A NORMAL LEVEL OF VITAMIN E SHOWING INTACT MITOCHONDRIA. NO SWELLING PHENOMENON WAS SEEN BUT THE MITOCHONDRIAL SUSPENSION WAS CONTAMINATED WITH SUBMITOCHONDRIA X 36,000.

EXPERIMENT III

SYMPTOMS OF HYPERVITAMINOSIS E IN CHICKS

THIS EXPERIMENT WAS DESIGNED TO INVESTIGATE THE EFFECTS OF EXCESS VITAMIN E IN CHICKS FED DIETS DEFICIENT AND ADEQUATE IN CALCIUM. SINCE THERE IS NO INFORMATION AVAILABLE REGARDING THE EFFECTS OF LONG TERM ADMINISTRATION OF EXCESS VITAMIN E TO CHICKS, THE EXPERIMENT WAS CONDUCTED WITH CHICKS FED THE DIFFERENT DIETS FROM HATCH TO 50 DAYS OF AGE.

IN EXPERIMENT II, TWO ADVERSE EFFECTS OF HYPERVITAMINOSIS E WERE OBSERVED VIZ. BONE FRAGILITY AND AN INCREASE IN PROTHROMBIN TIME. THE DIETS FED IN THIS EXPERIMENT WERE ACCIDENTLY FORMULATED WITHOUT ADEQUATE CALCIUM SUPPLEMENTATION. THERE WAS, THEREFORE, THE POSSIBILITY OF AN INTERRELATIONSHIP BETWEEN THE CALCIUM DEFICIENCY AND EXCESS VITAMIN E IN THE RESPONSE OF THE CHICKS IN THIS EXPERIMENT.

A SEARCH OF THE LITERATURE FOR RELATED OBSERVATIONS REVEALED ONE REPORT THAT HIGH DIETARY LEVELS OF EITHER VITAMIN A OR VITAMIN E DEPRESSED PROTHROMBIN LEVEL OF THE BLOOD IN THE RAT (MELLETE AND LEONE, 1960). NO REPORT OF ANY EFFECT OF EXCESS VITAMIN E ON BONE CALCIFICATION WAS FOUND.

MATERIALS AND METHODS

DAY-OLD WHITE LEGHORN MALE CHICKS WERE WING-BANDED AND DISTRIBUTED AT RANDOM INTO 8 LOTS OF 25 CHICKS EACH IN COMPARTMENTS OF ELECTRICALLY HEATED BATTERY BROODERS. THE FOUR DIFFERENT DIETS USED IN THE EXPERIMENT

ARE SHOWN IN TABLE 9. EACH DIET WAS FED TO DUPLICATE LOTS OF CHICKS.

ROOM TEMPERATURE WAS THERMOSTATICALLY CONTROLLED AT 22-23°C. THE FEED AND WATER WERE SUPPLIED AD LIBITUM.

AFTER A PERIOD OF 30 DAYS, BLOOD SAMPLES (1 ML PER CHICK) OF 3 CHICKS FROM EACH LOT WERE DRAWN AT RANDOM FROM THE WING VEIN BY USING TUBERCULIN SYRINGE. BLOOD SAMPLES CONTAINED SODIUM OXALATE OR HEPARIN FOR THE DIFFERENT HEMATOLOGICAL DETERMINATIONS. PROTHROMBIN TIMES, HEMATOCRIT VALUES AND RETICULOCYTE COUNTS WERE DETERMINED ON THE BLOOD SAMPLES.

PROTHROMBIN TIME

THE MEASUREMENT OF PROTHROMBIN TIME IS USED AS AN IMPORTANT SCREENING TEST FOR THE DEFICIENCY OF THE VARIOUS FACTORS OF THE PROTHROMBIN COMPLEX SUCH AS PROTHROMBIN, FACTORS V, VII AND X (QUICK, 1961).

NINE-TENTH ML OF BLOOD WAS DRAWN IN A SYRINGE CONTAINING 0.1 ML OF SODIUM OXALATE AND MIXED WELL. PROTHROMBIN TIME WAS ESTIMATED BY THE QUICK ONE STAGE METHOD (QUICK, 1936) WITH MODIFICATION BY ALMQUIST (1941).

HEMATOCRIT VALUE

THE DETERMINATION OF HEMATOCRIT VALUE (OR PACKED CELL VOLUME) IS USED AS A SIMPLE SCREENING TEST FOR ANEMIA. THE MICROMETHOD WAS APPLIED. MICRO-HEMATOCRIT TUBES (1.1-1.2 MM. IN I.D., 75 MM IN 1 LENGTH) WERE USED FOR THE HEMATOCRIT DETERMINATIONS. THE HEPARINIZED BLOOD SAMPLE WAS ALLOWED TO ENTER THE TUBE BY CAPILLARITY. THE TUBE WAS THEN SEALED WITH PLASTICINE. AFTER CENTRIFUGATION FOR 3 MINUTES, USING A INTERNATIONAL MICRO-CAPILLARY CENTRIFUGE, MODEL M.B. THE HEMATOCRIT VALUE WAS MEASURED USING A READING DEVICE.

RETICULOCYTE COUNTS

THE RETICULOCYTE STAIN TECHNIQUE (COATES AND MARCH, 1966) WAS USED FOR RETICULOCYTE COUNTS.

AT 30 DAYS OF AGE, 4 CHICKS FROM EACH LOT WERE WEIGHED AND KILLED. THE LEFT TIBIA OF EACH CHICK WAS TAKEN FOR ASH ANALYSIS. BONE ASH, EXPRESSED ON A DRY FAT-FREE BASIS, WAS USED TO SHOW THE DEGREE OF BONE CALCIFICATION IN THE LEFT TIBIA ACCORDING TO THE A.O.A.C. PROCEDURE (1965), USED IN THE CHICK ASSAY FOR VITAMIN D. THE RIGHT TIBIAS WERE CUT AND THE APPEARANCE OF THE MARROW NOTED.

THESE OBSERVATIONS WERE REPEATED¹ ON THE REMAINING CHICKS OF 40 AND 50 DAYS OF AGE.

INJECTION OF VITAMIN K

AT THE CONCLUSION OF THE EXPERIMENT, THREE BIRDS FED EXCESS VITAMIN E WERE INJECTED INTRAMUSCULARLY WITH MENADIONE (5 MG./KG. OF BODY WEIGHT) IN OIL SOLUTION. TWENTY-FOUR HOURS AFTER INJECTION, PROTHROMBIN TIME WAS DETERMINED.

¹ PROTHROMBIN TIMES WERE NOT MEASURED AT 50 DAYS OF AGE.

RESULTS

PROTHROMBIN TIME

THE MEAN PROTHROMBIN TIME OF THE CHICKS FROM EACH DIETARY TREATMENT AND THE STATISTICAL ANALYSIS OF THE RESULTS ARE SHOWN IN TABLE 10. THE ADDITION OF EXCESS VITAMIN E TO EITHER THE BASAL DIET OR TO THE BASAL DIET SUPPLEMENTED WITH ADDITIONAL CALCIUM SIGNIFICANTLY ($P \leq 0.01$) PROLONGED PROTHROMBIN TIME OF BLOOD FROM THE CHICKS. AT 30 DAYS OF AGE, THERE WAS AN EFFECT OF DIETARY CALCIUM LEVEL ON PROTHROMBIN TIME. AT 40 DAYS OF AGE EXCESS VITAMIN E LENGTHENED PROTHROMBIN TIME TO A GREATER DEGREE WHEN THE DIET WAS DEFICIENT IN CALCIUM. COMPARISON SHOULD NOT BE MADE BETWEEN THE ABSOLUTE PROTHROMBIN TIMES MEASURED AT 30 AND 40 DAYS OF AGE BECAUSE OF A POSSIBLE DIFFERENCE IN THE ACTIVITY OF THE THROMBOPLASTIN PREPARATION USED IN THE TWO SETS OF MEASUREMENTS.

HEMATOCRIT VALUES

THE HEMATOCRIT VALUES ARE ALSO SHOWN IN TABLE 10. EXCESS VITAMIN E RESULTED IN DECREASED HEMATOCRIT VALUES IN THE PRESENT EXPERIMENT. SUPPLEMENTATION OF THE BASAL DIET TO SUPPLY ADEQUATE CALCIUM INCREASED SIGNIFICANTLY THE HEMATOCRIT VALUE OF BLOOD SAMPLES AT 30 DAYS OF AGE ($P \leq 0.01$). BY 40 DAYS OF AGE THE DIFFERENCES IN HEMATOCRIT VALUES BETWEEN THE CHICKS FED THE CALCIUM DEFICIENT AND CALCIUM-ADEQUATE DIETS WERE NO LONGER SIGNIFICANT. AT 50 DAYS OF AGE, THE DIFFERENCES IN HEMATOCRIT VALUES OF BLOOD SAMPLES FROM THE CHICKS FED NORMAL AND EXCESS LEVELS OF

VITAMIN E, ALTHOUGH STATISTICALLY SIGNIFICANT ($P \leq 0.01$) WERE SMALLER THAN THOSE OBSERVED WITH YOUNGER BIRDS.

RETICULOCYTE COUNTS

RETICULOCYTE COUNTS OF THE CHICKS FED THE BASAL DIET AND THE BASAL DIET SUPPLEMENTED WITH CALCIUM ARE SIMILAR (TABLE 10). SUPPLEMENTATION OF THE DIETS WITH EXCESS VITAMIN E INCREASED RETICULOCYTE COUNTS SIGNIFICANTLY ($P \leq 0.01$) IN THE BLOOD FROM CHICKS FED DIETS OF EITHER CALCIUM LEVEL. IN THE BLOOD SAMPLE FROM CHICKS FED EXCESS VITAMIN E, MANY EARLY RETICULOCYTES WITH A HEAVILY STAINING RETICULUM WERE SEEN. THE CELLS OBSERVED IN MANY OF THE BLOOD SMEARS FROM THE CHICKS FED EXCESS VITAMIN E APPEARED TO BE ERYTHROBLASTS (COMPARABLE TO FIGURES 9-13 OR 121-124, LUCAS AND JAMROZ, 1961). PHOTOGRAPHS OF REPRESENTATIVE CELLS ARE SHOWN IN FIGURES 8, 9 AND 10.

BONE MARROW

THE MARROW CAVITY OF THE TIBIA FROM BIRDS FED EXCESS VITAMIN E SHOWED RESORPTION OF ALL CALCIFIED TISSUE AND CONTAINED ONLY BLOODY MATERIAL. THE TYPICAL APPEARANCE IS SHOWN IN FIGURE 11 FROM A BIRD 50 DAYS OF AGE.

BONE CALCIFICATION

DATA RELATING TO THE CALCIFICATION OF BONE ARE SHOWN IN TABLE 12. IN GROUPS 3 AND 4 RECEIVING ON ADEQUATE LEVEL OF CALCIUM (1% CALCIUM) NO SIGNIFICANT DIFFERENCE IN BONE ASH PERCENTAGE WAS DETECTED IN HYPERVITAMINOSIS E. IN COMPARING GROUPS, 1 (NORMAL LEVEL OF VITAMIN E) AND 2 (HYPERVITAMINOSIS E), BOTH RECEIVING A LOW LEVEL OF CALCIUM

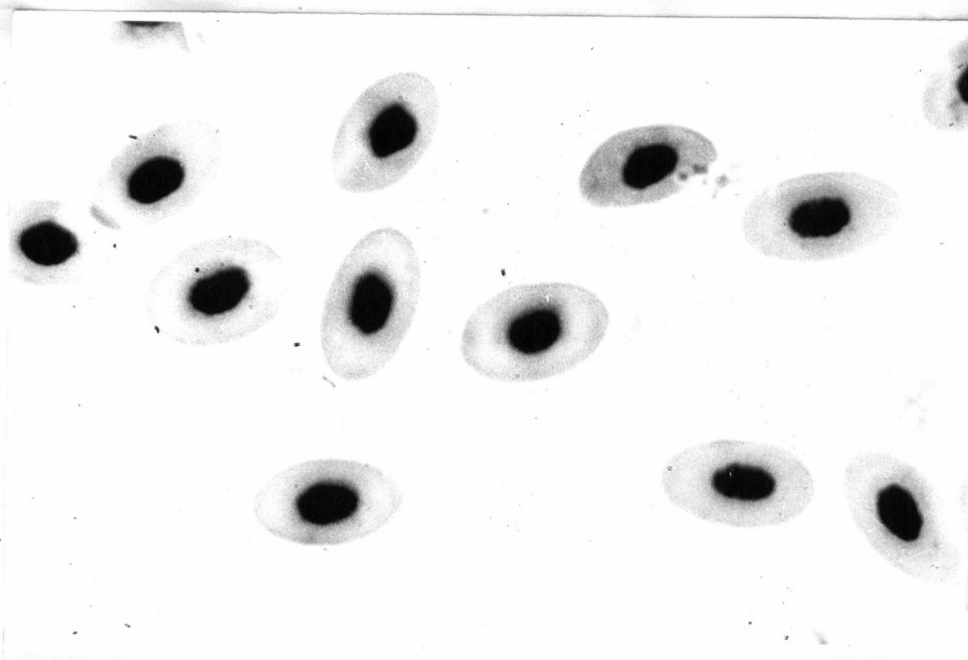


FIG. 8. ERYTHROCYTES FROM A 40-DAY OLD CHICK FED A NORMAL LEVEL OF VITAMIN E $\times 1000$.

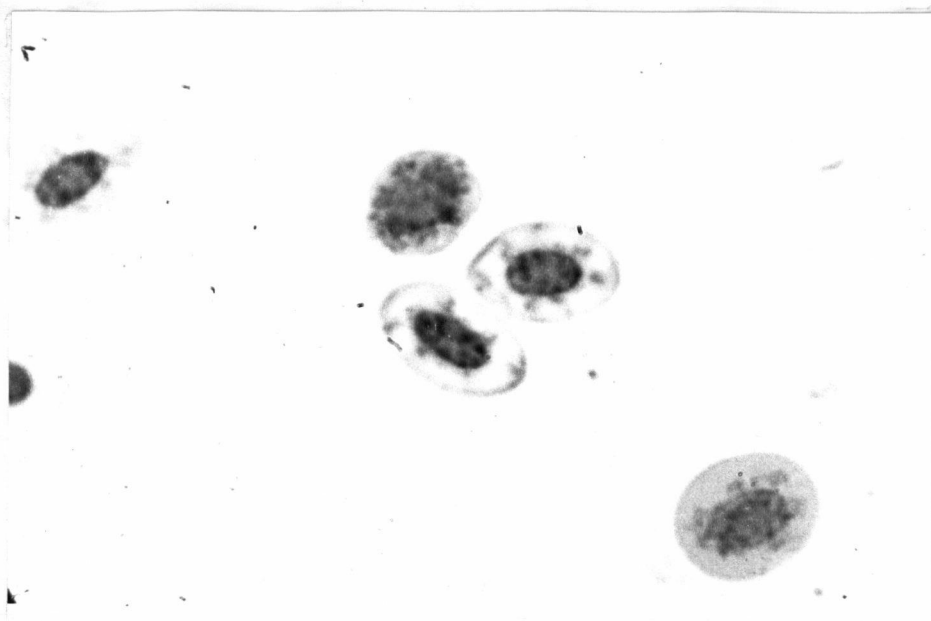


FIG. 9. RETICULOCYTES FROM A 40-DAY OLD CHICK FED EXCESS VITAMIN E $\times 1000$.

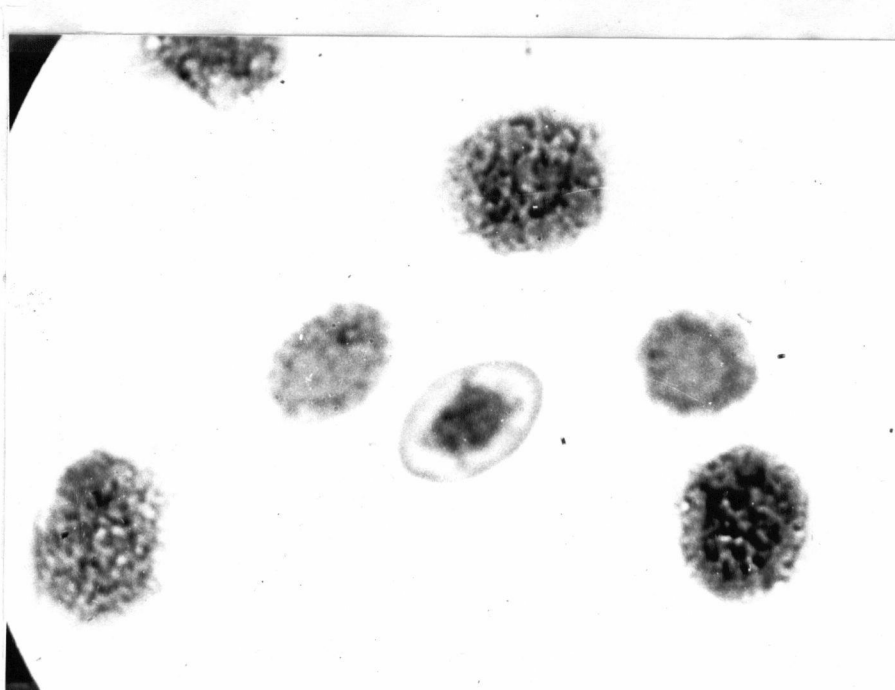


FIG. 10. ERYTHROBLASTS OBSERVED ON THE BLOOD SMEAR FROM A 40-DAY OLD CHICK X 1000.

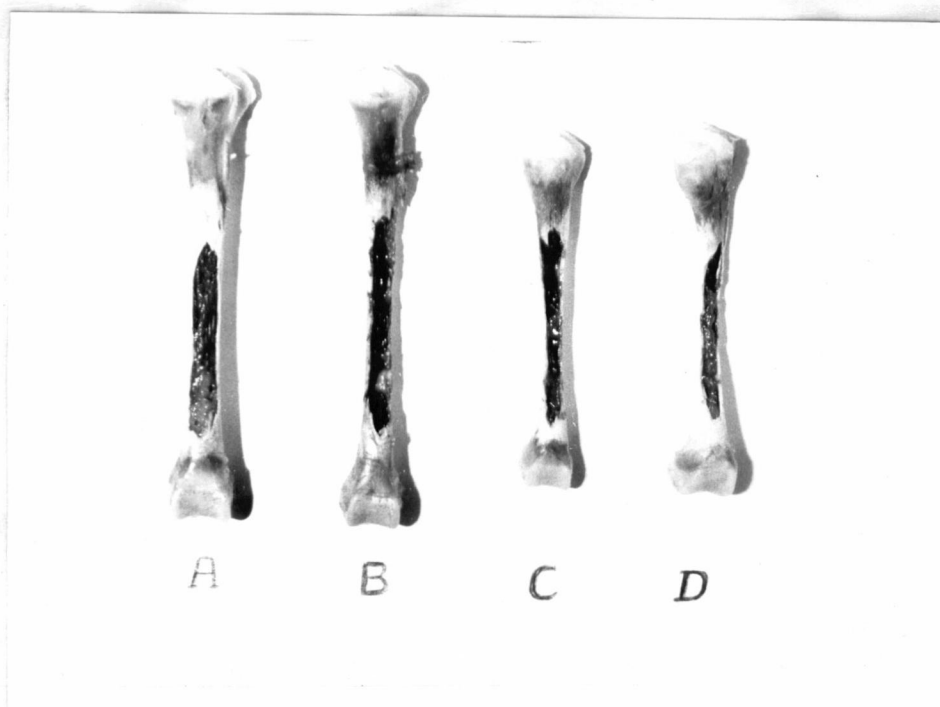


FIG. 11. ABNORMAL BONE MARROW OF CHICKS FED EXCESS VITAMIN E.

- A. NORMAL BONE MARROW FROM THE 50-DAY OLD CONTROL CHICK.
- B. SEVERE HYPOPLASTIC BONE MARROW FROM THE 50-DAY OLD CHICK FED EXCESS VITAMIN E.
- C. MEDIATE HYPOPLASTIC BONE MARROW FROM A 40-DAY OLD CHICK FED EXCESS VITAMIN E DIET.
- D. SLIGHTLY HYPOPLASTIC BONE MARROW FROM A 40-DAY OLD CHICK FED EXCESS VITAMIN E.

(0.59% CALCIUM), THE ASH PERCENTAGE WAS SIGNIFICANTLY LOWER IN GROUP 2 IN PERIODS OF 30, 40 AND 50 DAYS. BECAUSE BONE MINERALIZATION INCREASED AS THE CHICK BECAME OLDER AND HEAVIER AND BECAUSE EXCESS VITAMIN E DEPRESSED GROWTH, AT LEAST PART OF THE DIFFERENCE IN BONE ASH BETWEEN THE CHICKS FED THE NORMAL AND EXCESS LEVELS OF VITAMIN E MAY HAVE BEEN DUE TO THE DIFFERENCE IN BODY WEIGHT OF THE BIRDS OF THE TWO LEVELS OF VITAMIN E. PERCENT TIBIA ASH WAS ACCORDINGLY GRAPHED AGAINST BODY WEIGHT IN FIGURE 12B. FOR COMPARISON, A GRAPH WAS ALSO MADE OF PERCENT TIBIA ASH RELATIVE TO AGE OF THE BIRDS. USING THE VALUES FOR PERCENT ASH AT A BODY WEIGHT OF 300 GM., IT WILL BE SEEN THAT, WHEN THE DIET WAS DEFICIENT IN CALCIUM, AN EXCESS OF VITAMIN E DEPRESSED CALCIFICATION.

INJECTION OF VITAMIN K

TWENTY-FOUR HOURS AFTER INJECTION OF MENADIONE, PROTHROMBIN TIME OF THE CHICKS FED EXCESS VITAMIN E WAS SHORTENED TO THAT OF CHICKS FED THE NORMAL LEVEL OF VITAMIN E (TABLE 11).

DISCUSSION

THE PROLONGED PROTHROMBIN TIME IN THE CHICKS FED EXCESS VITAMIN E AND THE SHORTENING OF PROTHROMBIN TIME BY ADMINISTRATION OF MENADIONE WERE INDICATIVE OF VITAMIN K-DEFICIENCY (ALMQUIST AND STOKSTAD, 1936). THE PROLONGED PROTHROMBIN TIME IN CHICKS FED EXCESS VITAMIN E CONFIRMED THE OBSERVATION BY MELLETE AND LEONE (1960) IN RATS. THE MECHANISM BY WHICH HYPOPROTHROMBINEMIA IS CAUSED MAY BE INTERPRETED IN ONE OF THREE WAYS. ONE POSSIBLE MECHANISM IS THAT AN EXCESSIVE AMOUNT OF VITAMIN E OR ITS METABOLITES MAY ANTAGONIZE THE ACTION OF VITAMIN K. IN THE COURSE OF EARLIER STUDIES ON THE METABOLISM OF α -TOCOPHERYL-5-METHYL-C¹⁴ IN ANIMALS, THREE LABELED METABOLITES, α -TOCOPHERYL-P-QUINONE (CSALLANY ET AL., 1962), A DIMER (CSALLANY AND DRAPER, 1963) AND A TRIMER (DRAPER ET AL., 1967) WERE ISOLATED FROM THE LIVER TISSUE. IT IS SUGGESTED THAT THE STRUCTURE OF THESE END METABOLITES, HOWEVER, ARE SIMILAR TO THAT OF VITAMIN K AND PROBABLY COMPETE WITH VITAMIN K FOR CERTAIN ENZYME SITES. THE SECOND POSSIBLE MECHANISM IS THAT AN EXCESSIVE AMOUNT OF VITAMIN E MAY INTERFERE WITH THE ABSORPTION OF FAT SOLUBLE VITAMINS, SUCH AS VITAMINS K AND D. THE THIRD IS THAT EXCESSIVE AMOUNTS OF VITAMIN E MAY RETARD THE SYNTHESIS OF VITAMIN K.

EXCESS VITAMIN E DID NOT EXERT AN EFFECT ON THE BONE FORMATION IN CHICKS FED ADEQUATE CALCIUM DIET. WHEN FED A LOW CALCIUM DIET, PERCENT ASH IS SIGNIFICANTLY LOWER IN CHICKS FED EXCESS VITAMIN E THAN THAT IN THE CHICKS FED THE BASAL DIET, SHOWN IN TABLE 12 AND FIGURES 12A AND B. IT

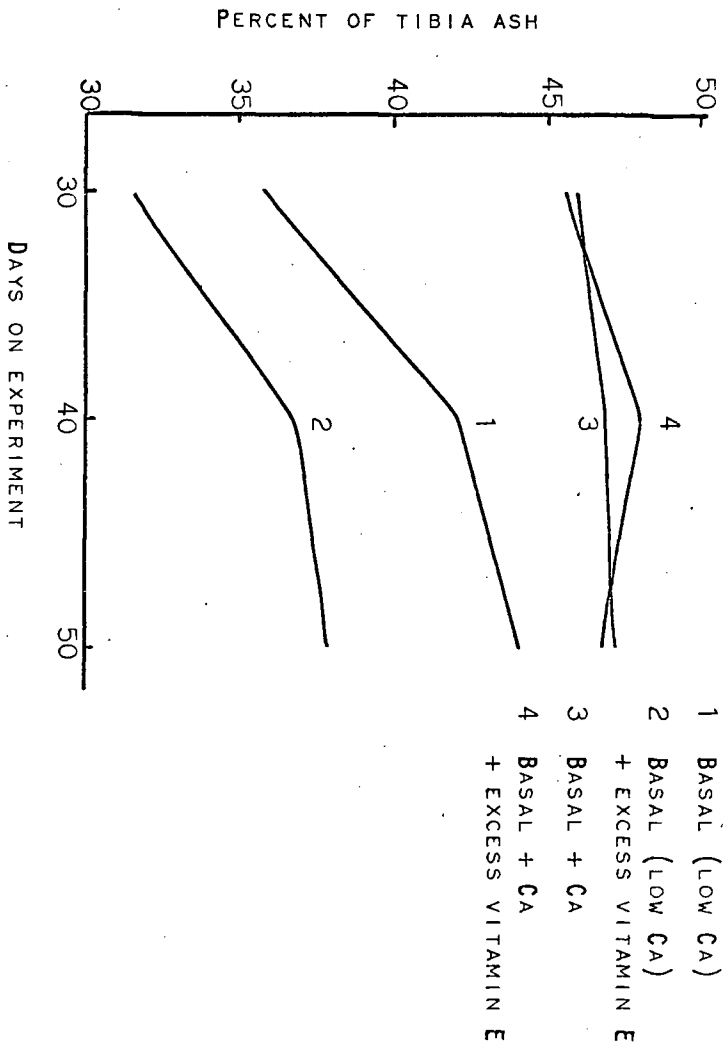


FIG. 12A. THE INFLUENCE OF EXCESS VITAMIN E ON PERCENT OF TIBIA ASH AT DIFFERENT AGES.

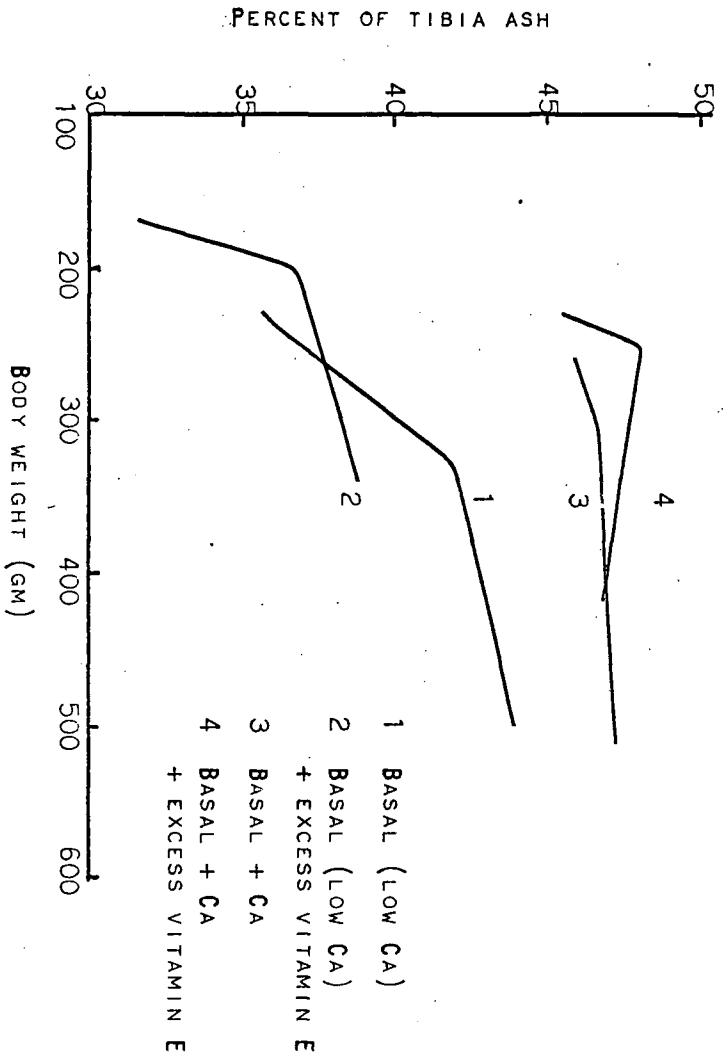


FIG. 12B. THE INFLUENCE OF EXCESS VITAMIN E ON PERCENT OF TIBIA ASH RELATIVE TO BODY WEIGHT.

APPEARS THAT EXCESS VITAMIN E MAY ALSO INTERFERE WITH THE ABSORPTION OF VITAMIN D OR SLOW DOWN THE BONE CALCIFICATION PROCESS.

RETICULOCYTOSIS HAS BEEN REPORTED TO OCCUR IN RESPONSE TO TOCOPHEROL TREATMENT OF VITAMIN E-DEFICIENT MONKEYS, HUMAN INFANTS AND CHILDREN (FITCH, 1958; HASSAN ET AL., 1966). MARCH ET AL. (1969) OBSERVED THAT RECICULOCYTOSIS OCCURED IN CHICKS FED EXCESS VITAMIN E (220 I.U. PER KG OF DIET) OR OTHER ANTIOXIDANTS AND ATTRIBUTED THE CONDITION TO RETARDATION OF THE MATURATION PROCESS OF THE RED BLOOD CELLS. MARCH ET AL. ALSO REPORTED THAT ERYTHROCYTE TURNOVER WAS SLOWER IN SOME OF THE BIRDS FED EXCESS VITAMIN E AND THAT HEMATOCRIT VALUES WERE NOT REDUCED. IT IS INTERESTING* IN THE PRESENT EXPERIMENT THAT THE LEVEL OF VITAMIN E (2200 I.U. PER KG OF DIET OR 100 TIMES THE NORMAL LEVEL) RESULTED IN THE DECREASED HEMATOCRIT VALUE, RETICULOCYTOSIS AND ALTERATION OF BONE MARROW. ONE POSSIBLE MECHANISM IS THAT AN EXCESSIVE AMOUNT OF VITAMIN E NOT ONLY RETARDS THE MATURATION OF THE RETICULOCYTES IN THE BLOOD BUT ALSO SLOWS DOWN OR EVEN PREVENTS THE ERYTHROPOIESIS IN THE BONE MARROW, THEREFORE, RESULTING IN THE DECREASED HEMATOCRIT VALUE (ANEMIA). IN SOME SEVERE CASES, JELLY-LIKE BLOODY MATERIAL WAS OBSERVED IN THE MARROW CAVITY AND ERYTHROBLASTS WERE PRESENT IN THE SMEARS OF THE CIRCULATING BLOOD INDICATING A RETARDATION OF ERYTHROPOIESIS. UNFORTUNATELY, MICROSCOPIC STUDIES HAVE NOT BEEN CARRIED OUT FOR BONE MARROW SMEAR. FURTHER STUDIES ARE REQUIRED.

SUMMARY

A STUDY WAS MADE OF THE EFFECTS OF FEEDING EXCESS VITAMIN E TO CHICKS. THREE GROUPS OF EXPERIMENTS WERE CONDUCTED TO INVESTIGATE (1) THYROIDAL RESPONSE TO EXCESS VITAMIN E, (2) RESPIRATION RATE OF MUSCLE MITOCHONDRIA FROM CHICKS FED EXCESS VITAMIN E, AND (3) SYMPTOMS OF HYPERVITAMINOSIS E.

IN TWO SEPARATE EXPERIMENTS, CHICKS WERE FED NORMAL AND EXCESS LEVELS (220 I.U./KG. OF DIET) OF VITAMIN E AND WERE SUBJECTED TO TEMPERATURES OF 14.5 AND 31.5°C. THE GOITROGENIC EFFECT OF THIOURACIL ON THE BIRDS IN THE DIFFERENT TREATMENTS WAS MEASURED. AT BOTH TEMPERATURES THE THIOURACIL-TREATED CHICKS FED EXCESS VITAMIN E EXHIBITED A LESSER ENLARGEMENT OF THE THYROID GLANDS THAN DID CHICKS RECEIVING A NORMAL LEVEL OF VITAMIN E. THIS FINDING INDICATES A REDUCTION IN THE SECRETION OF THYROID STIMULATING HORMONE (TSH) IN BIRDS FED EXCESS VITAMIN E. THE LEVEL OF VITAMIN E WHICH WAS FED DID NOT AFFECT THE GROWTH RATE OR FEED CONSUMPTION AT EITHER TEMPERATURE. IT, THEREFORE, APPEARS THAT THE METABOLIC RATES OF THE CHICKS FED THE LOW AND EXCESS LEVELS OF VITAMIN E WERE SIMILAR DESPITE DIFFERENCES IN THYROID ACTIVITY AND THAT TISSUE RESPIRATION IN BIRDS FED EXCESS VITAMIN E CAN BE MAINTAINED WITH A REDUCED SUPPLY OR TURNOVER OF THYROID HORMONE.

THE ACTIVITY OF THE THYROID GLAND ITSELF WAS STUDIED IN RESPONSE TO EXCESS VITAMIN E IN ANOTHER EXPERIMENT. USING RADIOIODINE (I^{131}) AS A

TRACER, IT WAS FOUND THAT THE RATES OF IODINE UPTAKE AND RELEASE BY THE THYROID GLAND WERE BOTH SLOWER IN BIRDS FED EXCESS VITAMIN E (220 I.U./KG. OF DIET) THAN IN CONTROL BIRDS. BECAUSE VITAMIN E ACTS AS A BIOLOGICAL ANTIOXIDANT, IT MAY, IN EXCESS AMOUNTS, DEPRESS THE RATE OF DEIODINATION OF THYROXINE IN THE PERIPHERAL TISSUE AT WHICH THYROID HORMONE IS REMOVED FROM THE CIRCULATION. AS A CONSEQUENCE, THE SECRETION OF TSH WOULD BE REDUCED.

IN ORDER TO OBTAIN SOME INDICATION OF CAUSE AND EFFECT IN THE MECHANISM BY WHICH THE EXCESS VITAMIN E AFFECTS THYROTROPIC HORMONE SECRETION RATE AND THYROID ACTIVITY, THE RESPIRATION RATE OF MITOCHONDRIA ISOLATED FROM THE PECTORAL MUSCLE OF CHICKS WHICH HAD BEEN FED EXCESS VITAMIN E (2200 I.U./KG. OF DIET) WAS COMPARED WITH THAT OF THE CONTROL BIRDS. THE RESULTS SHOWED A SIGNIFICANT REDUCTION IN OXYGEN UPTAKE BY THE MUSCLE MITOCHONDRIA OF CHICKS FED THE EXCESS AMOUNT OF VITAMIN E. THE GROWTH RATE OF THE CHICKS FED THE EXCESS LEVEL OF VITAMIN E IN THIS EXPERIMENT WAS MARKEDLY LOWER THAN THAT OF THE CONTROL CHICKS. IT IS SUGGESTED THAT THE CHICK MAY MAINTAIN A NORMAL RESPIRATION RATE WHEN FED EXCESS VITAMIN E UP TO A CERTAIN LEVEL THROUGH A REDUCTION IN THYROID ACTIVITY. WITH A LARGE EXCESS OF VITAMIN E, HOWEVER, NO FURTHER COMPENSATION IS POSSIBLE AND RESPIRATION RATE IS DEPRESSED BELOW NORMAL.

EXCESS VITAMIN E CAUSED HYPOPROTHROMBINEMIA, INDICATIVE OF VITAMIN K-DEFICIENCY. OTHER SYMPTOMS NOTED WERE A REDUCTION IN HEMATOCRIT VALUES, RECTICULOCYTOSIS AND AN ABNORMALLY FLUID APPEARANCE OF THE BONE MARROW. BASED UPON OBSERVATIONS OF BONE CALCIFICATION THE CALCIUM REQUIREMENT APPEARED TO BE INCREASED IN THE PRESENCE OF EXCESS VITAMIN E.

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TABLE 1.--BASAL DIET¹ IN EXPERIMENT 1A.

INGREDIENTS	%
GROUND WHEAT	83.0
HERRING MEAL	14.5
DISTILLERS' DRIED SOLUBLES	2.0
IODIZED SALT	0.5
TOTAL	100.0

TABLE 1B.--BASAL DIET¹ IN EXPERIMENT 1B.

INGREDIENTS	%
GROUND WHEAT	81.5
HERRING MEAL	14.5
BONE MEAL	0.5
LIME STONE	1.0
DISTILLERS' DRIED SOLUBLES	2.0
IODIZED SALT	0.5
TOTAL	100.0

¹ EACH KG. OF BASAL DIET CONTAINED THE FOLLOWING INGREDIENTS: MANGANESE SULPHATE, 12.54 MG.; VITAMIN A, 5505 I.U.; VITAMIN D₃, 440 I.U., MENADIONE, 0.60 MG.; RIBOFLAVIN, 3.61 MG.; CALCIUM PANTOTHENATE, 10.21 MG.; NIACIN, 10.12 MG.; FOLACIN, 1.21 MG.; CHOLINE CHLORIDE, 128.7 MG.; AMPROLIUM, 0.125 MG.

TABLE 2.--AVERAGE THYROID WEIGHTS, BODY WEIGHTS AND FEED CONSUMPTION OF CHICKS IN EXPERIMENT 1A.

	TEMPERATURE C°	CONTROL	EXCESS VITAMIN E	EQ
THYROID WEIGHT MG.	14.5 C° 31.5 C°	28.13±5.53 21.67±4.27	28.70±2.86 22.14±5.40	19.29±5.07 18.50±4.08
BODY WEIGHT GM.	14.5 C° 31.5 C°	340±21.8 341±30.5	359±16.5 335±25.9	161±12.4 187±16.5
THYROID WT. MG./100GM.B.W.	14.5 C° 31.5 C°	8.25±1.31 6.35±1.09	8.09±0.67 6.55±1.20	11.92±2.80 11.51±2.57
FEED CONSUMED PER CHICK IN 13 DAYS GM.	14.5 C° 31.5 C°	401 341	454 341	242 168

	TEMPERATURE	THIOURACIL	EXCESS VITAMIN E +THIOURACIL	EQ +THIOURACIL
THYROID WEIGHT MG.	14.5 C° 31.5 C°	123.26±56.20 95.96±16.49	85.02±52.74 72.43±17.51	123.30±42.50 71.57±23.37
BODY WEIGHT GM.	14.5 C° 31.5 C°	316±27.7 335±28.8	280±28.5 321±29.2	176±14.9 183±13.8
THYROID WT. MG./100GM.B.W.	14.5 C° 31.5 C°	38.16±14.27 28.65±6.20	29.37±15.66 22.70±5.20	68.86±16.01 39.53±14.73
FEED CONSUMED PER CHICK IN 13 DAYS GM.	14.5 C° 31.5 C°	378 325	310 250	272 227

TABLE 3.--AVERAGE THYROID WEIGHTS, BODY WEIGHTS, FEED CONSUMPTION OF CHICKS IN EXPERIMENT 1B.

	TEMPERATURE	CONTROL	EXCESS VITAMIN E
THYROID WEIGHT MG.	14.5 C ⁰ 31.5 C ⁰	25.02±3.46 23.26±3.16	25.98±6.00 22.68±4.66
BODY WEIGHT GM.	14.5 C ⁰ 31.5 C ⁰	364±22 376±41	369±38 373±33
THYROID WT. MG./100GM.B.W.	14.5 C ⁰ 31.5 C ⁰	6.67±0.84 6.20±0.64	7.02±1.24 6.05±0.89
FEED CONSUMED PER CHICK IN 13 DAYS GM.	14.5 C ⁰ 31.5 C ⁰	549 362	561 357

	TEMPERATURE	THIOURACIL	EXCESS VITAMIN E +THIOURACIL
THYROID WEIGHT MG.	14.5 C ⁰ 31.5 C ⁰	123.10±25.30 109.31±25.03	105.63±16.44 94.06±16.69
BODY WEIGHT GM.	14.5 C ⁰ 31.5 C ⁰	335±35 350±31	342±34 353±32
THYROID WT. MG./100GM.B.W.	14.5 C ⁰ 31.5 C ⁰	34.03±5.11 31.09±5.27	30.98±4.07 26.63±4.36
FEED CONSUMED PER CHICK IN 13 DAYS GM.	14.5 C ⁰ 31.5 C ⁰	423 328	416 333

TABLE 4.--ANALYSIS OF VARIANCE OF THYROIDAL RESPONSE TO THE EXCESS VITAMIN E AND/OR THIOURACIL AT DIFFERENT TEMPERATURES IN EXPERIMENT 1A.

SOURCE	DF	MEAN SQUARE	F
TEMPERATURE (A)	1	292.842	4.611*
VITAMIN E (B)	1	165.169	2.601
THIOURACIL (C)	1	6005.450	94.560**
AB	1	2.799	0.044
BC	1	167.179	2.632
AC	1	124.614	1.962
ERROR	41	63.509	
TOTAL	47		

* SIGNIFICANT ($P < 0.05$).

** HIGHLY SIGNIFICANT ($P < 0.01$).

TABLE 5.--ANALYSIS OF VARIANCE OF THYROIDAL RESPONSE TO THE EXCESS VITAMIN E AND/OR THIOURACIL AT DIFFERENT TEMPERATURES IN EXPERIMENT 1B.

SOURCE	DF	MEAN SQUARE	F
TEMPERATURE (A)	1	103.937	5.982*
VITAMIN E (B)	1	166.519	9.584**
THIOURACIL (C)	1	17523.512	1008.606**
AB	1	0.164	0.0094
BC	1	178.202	10.257**
AC	1	238.754	13.742**
ERROR	109	17.374	
TOTAL	115		

* SIGNIFICANT ($P < 0.05$).

** HIGHLY SIGNIFICANT ($P < 0.01$).

TABLE 6.--AVERAGE THYROID WEIGHTS, BODY WEIGHTS, FEED CONSUMPTION OF
EQ-TREATED CHICKS AT 22 C° IN EXPERIMENT 1B.

	EQ	EQ +THIOURACIL	CONTROL	THIOURACIL
THYROID WEIGHT MG.	25.90±6.09	138.28±34.14	28.73±7.11	49.82±11.83
BODY WEIGHT GM.	307±47	313±36	353±41.4	380±30
THYROID WT. MG./100GM.B.W.	8.50±1.67	43.67±7.70	8.09±1.58	13.22±3.45
FEED CONSUMED PER CHICK IN 13 DAYS GM.	292	302	333	318

TABLE 7.--EFFECT OF EXCESS DIETARY VITAMIN E ON THYROIDAL IODINE (I^{131}) UPTAKE AND RELEASE IN WHITE LEGHORN MALE CHICKS.

A. MEAN VALUES, THYROIDAL LEVEL OF I^{131} PER BIRD:% OF INJECTED DOSE \pm S.D.					
	5 HR	10 HR	24 HR	48 HR	96 HR
BASAL	11.75 \pm 1.52	10.88 \pm 2.03	11.17 \pm 3.25	9.60 \pm 2.57	7.17 \pm 2.19
EXCESS VITAMIN E	8.11 \pm 1.69	9.35 \pm 2.19	10.26 \pm 2.24	9.14 \pm 2.16	9.86 \pm 2.68
B. MEAN VALUES, THYROIDAL LEVEL OF I^{131} :% OF INJECTED DOSE/MG. THYROID \pm S.D.					
	5 HR	10 HR	24 HR	48 HR	96 HR
BASAL	0.427 \pm 0.061	0.433 \pm 0.037	0.466 \pm 0.063	0.355 \pm 0.059	0.317 \pm 0.045
EXCESS VITAMIN E	0.306 \pm 0.047	0.363 \pm 0.081	0.362 \pm 0.048	0.323 \pm 0.053	0.342 \pm 0.057

TABLE 8.--RESPIRATION RATES¹ OF MUSCLE MITOCHONDRIA FROM THE CHICKS FED THE CONTROL DIET, EXCESS VITAMIN E AND/OR THIOURACIL. INCUBATION WAS AT 30°C.

INCUBATION PERIOD	DIETARY TREATMENT ²			
	CONTROL	EXCESS VITAMIN E	THIOURACIL	EXCESS VITAMIN E +THIOURACIL
	(7) ³	(7)	(6)	(7)
0-30 MIN.	11.80±2.78	7.05±3.28	8.50±3.85	5.37±1.94
30-60 MIN.	12.03±1.18	7.70±3.36	8.84±2.89	4.84±2.21
60-90 MIN.	10.71±3.19	8.03±4.65	8.51±2.62	4.44±1.91
90-120 MIN.	7.01±2.62	4.64±2.20	6.35±2.47	3.97±2.06
TOTAL	41.55±8.22 ^A	27.42±12.81 ^B	32.20±11.67 ^A	18.63±7.64 ^B
UL/HR/MG. PROTEIN	20.78±4.11 ^A	13.71±6.41 ^B	16.10±5.84 ^A	9.32±3.82 ^B
BODY WEIGHT (GM.)	597±91	363±60	482±66	332±40

¹ EXPRESSED AS UL O₂ UPTAKE PER MG. MITOCHONDRIAL PROTEIN±S.D. DURING SUCCESSIVE 30 MINUTE PERIODS.

² VALUES WITHIN A LINE HAVING THE SAME SUPERScript ARE NOT STATISTICALLY DIFFERENT AT THE 5% LEVEL OF PROBABILITY BY THE DUNCAN'S MULTIPLE RANGE TEST.

³ NUMBER OF CHICKS TESTED.

TABLE 9.--COMPOSITION OF THE EXPERIMENTAL DIETS.

	<u>DIET 1</u> BASAL ¹	<u>DIET 2</u> +CA	<u>DIET 3</u> +EXCESS +VITAMIN E	<u>DIET 4</u> +EXCESS +VITAMIN E +CA
	%	%	%	%
BASAL DIET	98.0	98.0	98.0	98.0
CELLULOSE	2.0		2.0	
BONE MEAL		1.5		1.5
LIME STONE		0.5		0.5
TOTAL	100.0	100.0	100.0	100.0
DL- α -TOCOPHERYL ACETATE (I.U./KG)	10.	10.	2200	2200

¹ BASAL DIET WAS THE SAME AS SHOWN IN TABLE 1A.

TABLE 10. --EFFECT OF EXCESS VITAMIN E ON HEMATOCRIT VALUE, PROTHROMBIN TIME AND RETICULOCYTE COUNT.

	AGE OF CHICKS (DAYS)	DIET ¹			
		BASAL	BASAL +CALCIUM	EXCESS VITAMIN E	EXCESS VITAMIN E +CALCIUM
HEMATOCRIT (%) ± S.D.	30	24.94±1.57 ^A	28.06±0.81 ^B	14.51±2.57 ^C	18.13±0.78 ^D
	40	25.08±1.50 ^A	27.69±0.72 ^A	13.81±3.54 ^B	15.89±2.37 ^B
	50	27.63±1.78 ^A	27.00±2.18 ^A	21.67±2.18 ^B	23.58±2.06 ^B
PROTHROMBIN TIME (SECONDS)	30	56.94±4.90 ^A	51.90±6.72 ^A	96.36±12.44 ^B	92.34±9.75 ^B
	40	43.44±5.19 ^A	38.28±5.58 ^A	141.60±18.78 ^C	105.00±18.33 ^B
RETICULOCYTE (%) ± S.D.	30	11.73±0.95 ^A	10.68±1.02 ^A	47.45±3.99 ^B	44.50±9.17 ^B
	40	12.98±2.70 ^A	9.65±0.73 ^A	42.08±9.37 ^B	40.60±19.07 ^B
	50	11.92±3.25 ^A	11.64±1.20 ^A	52.44±5.02 ^C	24.38±3.83 ^B

¹ VALUES WITHIN A LINE HAVING THE SAME SUPERScript ARE NOT STATISTICALLY DIFFERENT AT THE 1% LEVEL OF PROBABILITY BY THE DUNCAN'S NEW MULTIPLE RANGE TEST.

TABLE 11 -- PROTHROMBIN TIME OF CHICKS FED EXCESS VITAMIN E, 24 HOURS AFTER INJECTION OF MENADIONE.

	CONTROL	EXCESS VITAMIN E	EXCESS VITAMIN E (24 HR AFTER INJ. OF MENADIONE)
PROTHROMBIN TIME (SECONDS)	34.7±3.8	77.9±15.9	27.1±3.2

TABLE 12.--EFFECT OF EXCESS VITAMIN E ON BONE CALCIFICATION IN CHICKS FED DIETS ADEQUATE AND DEFICIENT IN CALCIUM.

		AGE OF CHICKS (DAYS)	BODY WEIGHT	ASH%
GROUP 1	BASAL	30	229±81.7	35.74±4.04
		40	333±75	42.14±0.52
		50	497±110	43.97±3.22
GROUP 2	BASAL EXCESS +VITAMIN E	30	169±43	31.61±3.43
		40	202±37	36.76±2.42
		50	338.9±38	37.83±2.13
GROUP 3	BASAL + CALCIUM	30	257±23	45.94±0.87
		40	307±42	46.71±0.90
		50	513±48	47.20±2.39
GROUP 4	BASAL +EXCESS VITAMIN E +CALCIUM	30	231±28	45.54±0.56
		40	250±32	48.20±1.36
		50	416±55	46.92±0.66