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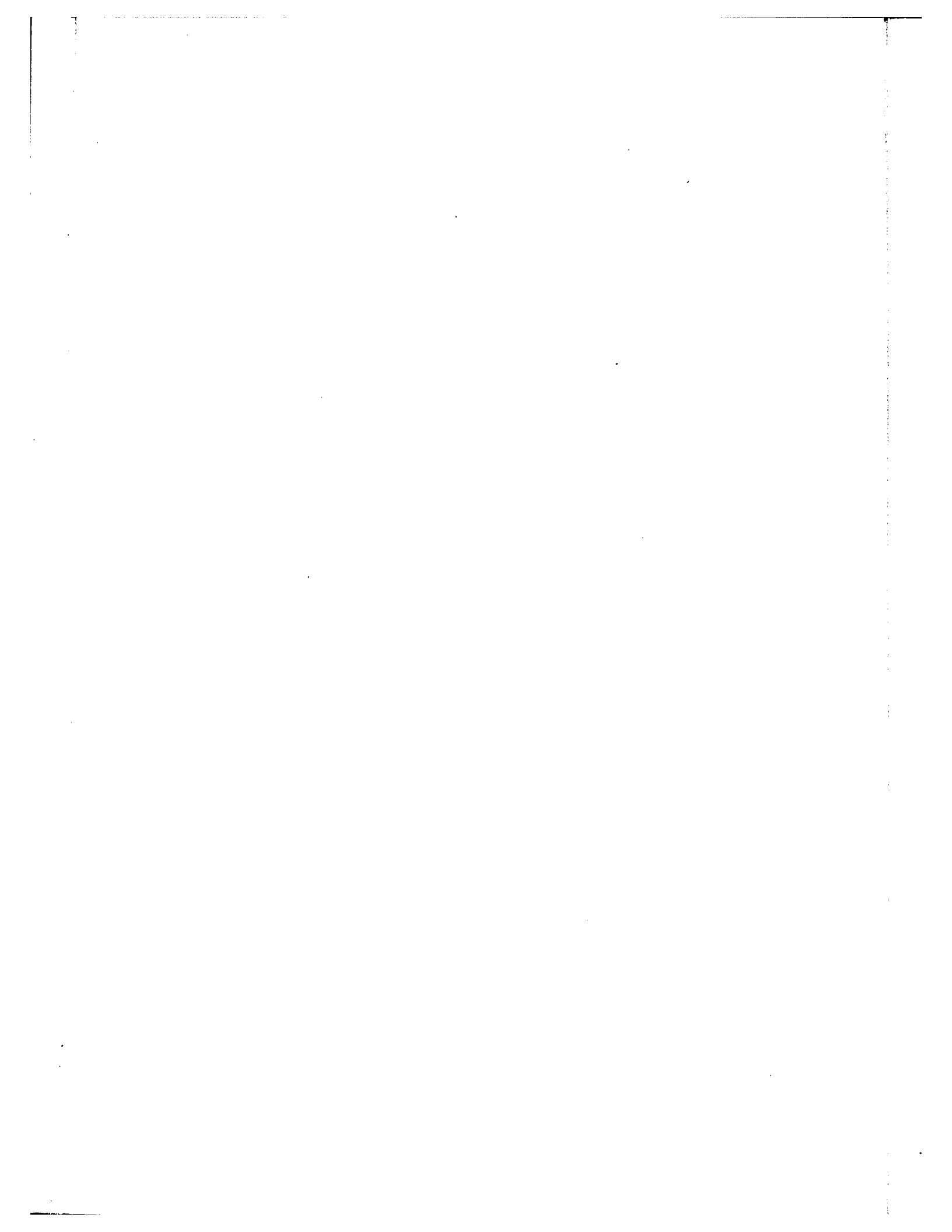
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THYROXINE AND CATECHOLAMINE BINDING AND METABOLISM IN THE RAT

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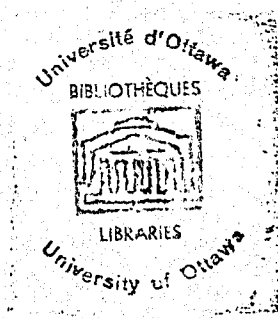
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TO PROFESSOR ANTOINE D' IORIO, WHO HAS DIRECTED
THIS WORK WITH CONSIDERATION AND GREAT UNDERSTANDING,
I EXPRESS MY GRATITUDE.

I WISH TO THANK DR H. L. BENOITON FOR HIS
PATIENT HELP, AND MISSES A. AND C. BARRIEUX FOR THEIR
VALUABLE ASSISTANCE IN THE TYPING OF THIS MANUSCRIPT.

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INTRODUCTION

THE POTENTIATION OF SEVERAL PHYSIOLOGICAL EFFECTS OF CATECHOLAMINES BY THYROID HORMONES HAS BEEN OBSERVED FOR MANY YEARS. NUMEROUS INVESTIGATORS HAVE NOTICED AN INCREASE IN THE EFFECT OF ADRENALINE ON OXYGEN CONSUMPTION IN HYPERTHYROIDISM. THE TACHYCARDIA INDUCED BY ADMINISTRATION OF ADRENALINE HAS BEEN FOUND TO BE AUGMENTED IN HYPERTHYROID SUBJECTS OR THYROID-FED ANIMALS. A SIMILAR INCREMENT IN THE PRESSOR EFFECT OF NORADRENALINE HAS BEEN REPORTED AFTER THYROID FEEDING IN VARIOUS SPECIES. THE THYROID STATE OF THE ORGANISM HAS ALSO BEEN SHOWN TO AFFECT THE GLYCEMIC AND GLYCOGENOLYTIC RESPONSES TO ADMINISTERED CATECHOLAMINES. FURTHERMORE HYPERGLYCEMIA PROVOKED BY ADRENALINE INJECTION IS INCREASED IN HYPERTHYROIDISM AND DECREASED IN HYPOTHYROIDISM (1).

THE AUGMENTATION OF THE PHYSIOLOGICAL EFFECTS OF EXOGENOUS CATECHOLAMINES AFFECTS THE MAGNITUDE RATHER THAN THE TIME-COURSE OF THE RESPONSE. THYROID HORMONES COULD ACT BY INCREASING THE CONCENTRATION OF " AVAILABLE " CATECHOLAMINES IN THE TISSUE, AT THE EFFECTOR SITE. THIS MAY BE BROUGHT ABOUT EITHER BY A GREATER UPTAKE OF CIRCULATING CATECHOLAMINES BY TISSUE OR BY A SLOWER RATE OF INACTIVATION OF THE BIOGENIC AMINES ALREADY PRESENT IN THE CELL. THYROID HORMONES COULD AFFECT EITHER PROCESS OR BOTH OF THEM.

ALTHOUGH THE BIOLOGICAL EFFECTS OF EXOGENOUS CATECHOLAMINES ARE WELL ESTABLISHED, THE MECHANISM LEADING TO THE

DISAPPEARANCE OF THESE EFFECTS IS STILL UNKNOWN. THE RAPID DISAPPEARANCE OF CATECHOLAMINE ACTIONS HAVE LED SOME INVESTIGATORS TO POSTULATE THAT THE ENZYMES RESPONSIBLE FOR CATECHOLAMINE DEGRADATION: MONOAMINE OXIDASE AND CATECHOL-O-METHYL TRANSFERASE, ARE NOT PRIMARILY INVOLVED IN THE BIOLOGICAL DISPOSAL OF SYMPATHETIC AMINES. LATELY, GREAT ATTENTION HAS BEEN GIVEN TO A SECOND MODE OF INACTIVATION OF ADRENALINE AND NORADRENALINE : BINDING TO SITES (2). IT IS POSTULATED THAT THYROID HORMONES WOULD ACT BY OCCUPYING THESE SITES AND THUS WOULD PREVENT THE BINDING OF INTRACELLULAR CATECHOLAMINES. THE AMINES WOULD THEN BE FREE TO ATTACH TO THE EFFECTOR SITES.

WHEN THIS WORK WAS STARTED, THE " BINDING THEORY " WAS JUST BEING DEVELOPPED AND AN ATTEMPT WAS MADE TO TEST IT IN VIVO, IN THE WHOLE RAT. IT WAS FOUND THAT TOO MANY UNCONTROLLABLE VARIABLES WERE INVOLVED AND THAT A SIMPLER SYSTEM WAS REQUIRED FOR A BETTER UNDERSTANDING OF THE MECHANISM. THEREFORE, IN VITRO EXPERIMENTS ON ISOLATED SMALL INTESTINE STRIPS WERE UNDERTAKEN. THE EFFECTS OF ENDOGENOUS AND EXOGENOUS THYROXINE ON THE UPTAKE AND BINDING OF ADRENALINE WERE TESTED.

IT WAS DURING THIS PERIOD THAT C. MAVRIDES, FROM THIS LABORATORY, SHOWED THAT VARIOUS THYROXINE ANALOGUES HAVE AN INHIBITORY EFFECT ON CATECHOL-O-METHYL TRANSFERASE ACTIVITY (3). OUR WORK QUITE NATURALLY WAS ORIENTATED TOWARDS A CONFIRMATION OF MAVRIDES' RESULTS. THE EFFECTS OF THYROXINE ON INTESTINE

CATECHOL-O-METHYL TRANSFERASE WERE ALSO STUDIED.

WE HOPED THAT WHATEVER CONTRIBUTION THIS WORK WILL MAKE
IN THE FUTURE, IT WILL NOT ADD TO THE " .. CURRENT CONFUSION IN
ADRENAL MEDULLARY-THYROID RELATIONSHIPS ... ONE OF THE MOST
CHARACTERISTIC FEATURES OF RESEARCH IN THIS FIELD. " (1).

REVIEW OF LITERATURE

PART I. MODIFICATION OF THE PHYSIOLOGICAL EFFECTS OF EXOGENOUS CATECHOLAMINES BY THYROID HORMONES

GREAT PROGRESS HAS BEEN MADE SINCE 1908 WHEN KRAUS AND FRIEDENTHAL (4) OBSERVED THAT ADRENALINE INJECTION COULD MIMIC THE THYROTOXIC STORM. NUMEROUS EXPERIMENTAL RESULTS HAVE SHOWN THAT THE POTENTIATION OF CATECHOLAMINE EFFECTS BY THYROID HORMONES OCCURS IN THE WHOLE ANIMAL AS WELL AS IN THE ISOLATED ORGAN (7). IN THE FOLLOWING DISCUSSION, THE PHYSIOLOGICAL EFFECTS OF ADMINISTERED CATECHOLAMINES WILL FIRST BE EXAMINED IN THE CASE OF ANIMALS IN VARIOUS THYROID STATES. THE SECOND SECTION WILL BE DEVOTED TO THE ACTION OF CATECHOLAMINES ON ISOLATED TISSUES AS A FUNCTION OF ENDOGENOUS OR EXOGENOUS THYROID HORMONE CONCENTRATION.

A) THE PHYSIOLOGICAL EFFECTS OF ADMINISTERED CATECHOLAMINES IN ANIMALS IN VARIOUS THYROID STATES : 1) EUTHYROID ANIMALS

IT WAS IN 1895 THAT OLIVER AND SCHAPER (5) OBSERVED FOR THE FIRST TIME THAT AN INJECTION OF ADRENAL EXTRACT INTO DOGS RESULTED IN A RISE OF BLOOD PRESSURE. THE EFFECT WAS ATTRIBUTED TO THE PRESENCE IN THE EXTRACT OF SYMPATHIN. AFTER ADRENALINE AND NORADRENALINE HAD BEEN ISOLATED AND SYNTHESIZED, THEIR RESPECTIVE EFFECTS COULD BE STUDIED INDEPENDENTLY. THE D-ISOMERS OF BOTH AMINES WERE FOUND TO HAVE THE SAME PHYSIOLOGICAL EFFECTS AS THOSE OF THE L-FORM, BUT HIGHER DOSES OF THE FORMER WERE REQUIRED.

IN ALL SPECIES WHICH HAVE BEEN STUDIED, IT HAS BEEN OBSERVED THAT THE INTRAVENOUS INJECTION OF ADRENALINE OR NORADRENALINE IS FOLLOWED BY AN INCREASE OF THE OXYGEN CONSUMPTION. SUBCUTANEOUS INJECTION HAS THE SAME EFFECT BUT A DELAY IN THE RESPONSE IS OBSERVED. THE SLOW INTRAVENOUS INFUSION OF EITHER AMINE INCREASES OXYGEN CONSUMPTION ALSO (6, 7). IF THE DOSE OF INJECTED ADRENALINE OR THE RATE OF INFUSION IS AUGMENTED, THE OXYGEN CONSUMPTION IS DECREASED (8).

INTRAVENOUS OR INTRAARTERIAL INJECTION OF NORADRENALINE CAUSES A RAPID DECREASE IN BLOOD PRESSURE DUE TO PERIPHERAL VASOCONSTRICTION (9). INJECTION OF SMALL DOSES OF ADRENALINE, EITHER BY INTRAVENOUS OR BY INTRAARTERIAL ROUTE DOES NOT AFFECT BLOOD PRESSURE, BUT WITH LARGE DOSES A DECREASE IN BLOOD PRESSURE HAS BEEN OBSERVED (10).

AT SMALL DOSES, ADRENALINE HAS A POSITIVE CHRONOTROPIC AND INOTROPIC EFFECT ON THE HEART (11, 12). NORADRENALINE DOES NOT APPEAR TO AFFECT THE HEART AT SMALL DOSES (13). WHILE NORADRENALINE HAS MAINLY A CONSTRICTOR EFFECT ON PERIPHERAL BLOOD VESSELS (14), ADRENALINE ACTION VARIES WITH THE ORGAN STUDIED. THERE IS A MARKED CONSTRICTION OF THE SKIN BLOOD VESSELS FOLLOWING ADRENALINE INJECTION (15). BLOOD VESSELS OF SKELETAL MUSCLE SEEM TO BE DILATED (16). PORTAL VEIN APPEARS CONSTRICTED, THE EFFECT OF ADRENALINE ON HEPATIC VEIN IS NOT ELUCIDATED (17, 18).

THE VARIOUS EFFECTS OF CATECHOLAMINES ON PERIPHERAL ORGANS

ARE NOT CLEAR. ADRENALINE INJECTION CONTRACTS THE SPLENIC CAPSULE (19) AND INCREASES LIVER VOLUME (20). THE EFFECTS ON THE UTERUS ARE DIFFERENT DEPENDING UPON THE STATE OF THE ORGAN (21). THE TONUS OF THE STOMACH IS DECREASED BY ADRENALINE (22) AND THAT OF THE SMALL INTESTINE INHIBITED (23). BOTH ADRENALINE AND NORADRENALINE HAVE A DILATOR ACTION ON THE BRONCHI, THE FORMER BEING MORE POTENT (24). THERE IS STILL CONTROVERSY CONCERNING THE EFFECT OF ADRENALINE ON THE THYROID GLAND. RESULTS OBTAINED USING 131 IODINE-RELEASE AS A MEASURE OF THYROID GLAND ACTIVITY ARE NOT CONCLUSIVE (25). SOME AUTHORS CONSIDER THAT ADRENALINE ACTION ON THYROID GLAND MIGHT BE INDIRECT. BLOOD VESSELS TO AND FROM THE GLAND ARE KNOWN TO BE EXTREMELY SENSITIVE TO ADRENALINE DILATOR EFFECT. AN INCREASE IN BLOOD FLOW RATHER THAN A DIRECT EFFECT ON THE GLAND ITSELF, HAS BEEN ASSUMED TO BE RESPONSIBLE FOR THE INCREASE IN THE RELEASE OF 131 IODINE IN THE BLOOD LEAVING THE GLAND (26).

THE HYPERGLYCEMIC RESPONSE TO ADMINISTERED CATECHOLAMINES HAS BEEN A PHENOMENON OBSERVED FROM THE EARLIEST REPORTS (27, 28). THIS HYPERGLYCEMIA IS ACCOMPANIED BY AN INCREASED GLYCOGENOLYSIS IN THE LIVER (29) AND IN STRIATED MUSCLE (30). IN THE CASE OF ADRENALINE INJECTION, A SIMULTANEOUS INCREASE IN BLOOD LACTATE IS NOTICED (31). SOME AUTHORS HAVE ATTEMPTED TO RELATE THIS LACTATE INCREASE TO THE CALORIGENIC EFFECT OF ADRENALINE (32). THE MECHANISM OF ACTION OF ADRENALINE IN GLYCOGENOLYSIS HAS BEEN STUDIED EXTENSIVELY (33). ADRENALINE, BY INCREASING THE

CONCENTRATION OF CYCLIC 3',5'-AMP, ACTIVATES THE INACTIVE PHOSPHORYLASE. THIS ENZYME IS THE FIRST STEP IN THE TRANSFORMATION OF GLYCOGEN INTO GLUCOSE, AND, MOREOVER, IS THE LIMITING STEP IN THE RATE OF THE REACTION. IN ALL THE TISSUES STUDIED, ADRENALINE INJECTION CAUSED AN INCREASE IN CYCLIC AMP CONCENTRATION. A CONCOMITANT INCREASE IN ACTIVE PHOSPHORYLASE ACTIVITY WAS FOUND IN HEART, SKELETAL MUSCLE AND LIVER. BECAUSE THE INCREASE IN CYCLIC AMP AND IN ACTIVE PHOSPHORYLASE WAS TAKING PLACE AT THE SAME TIME THAT TACHYCARDIA WAS OCCURRING, SEVERAL ATTEMPTS WERE MADE TO EXPLAIN THE CARDIAC ACTION OF ADRENALINE AS A FUNCTION OF THESE FACTORS (34, 35). AT THE PRESENT TIME, THERE IS NO AGREEMENT ON THIS THEORY (36).

2) HYPERTHYROID ANIMALS

LONG BEFORE ADRENALINE AND NORADRENALINE SPECIFIC EFFECTS HAD BEEN ELUCIDATED, THE RELATIONSHIP BETWEEN SYMPATHETIC SYSTEM AND THYROID GLAND HAD BEEN RECOGNIZED. THE OBSERVATIONS MADE ON HYPERTHYROID PATIENTS STIMULATED MANY INVESTIGATORS: NUMEROUS CONTRADICTIONARY REPORTS ON THE SYNERGISM BETWEEN CATECHOLAMINES AND THYROID HORMONES APPEARED. AS PREVIOUSLY STATED, THE DISCREPANCIES IN EXPERIMENTAL RESULTS HAVE NOT YET BEEN EXPLAINED.

THERE IS GENERAL AGREEMENT, HOWEVER, THAT IN HYPERTHYROIDISM, THE RISE IN OXYGEN CONSUMPTION INDUCED BY CATECHOLAMINE ADMINISTRATION IS LARGER THAN THAT OBSERVED IN EUTHYROIDISM.

(1). SUB-CUTANEOUS INJECTION OF ADRENALINE INTO NORMAL OR THYROID-FED RATS HAS BEEN SHOWN TO ELEVATE OXYGEN CONSUMPTION. THIS

INCREASE, SIGNIFICANTLY HIGHER IN THYROID-FED RATS, COULD BE OBSERVED UP TILL 74 HOURS FOLLOWING ADRENALINE INJECTION (37). SIMILAR OBSERVATIONS WERE MADE ON THYROTOXIC PATIENTS OR ON TRIIODOTHYRONINE-TREATED SUBJECTS. DURING ADRENALINE INFUSION THE INCREASE IN OXYGEN CONSUMPTION WAS HIGHER IN THESE TWO GROUPS WHEN COMPARED TO EUTHYROID PATIENTS. MOREOVER, THE RISE IN OXYGEN CONSUMPTION IN THYROTOXIC PATIENTS, WAS BROUGHT ABOUT AT A RATE OF ADRENALINE INFUSION LOWER THAN THAT AT WHICH THE NORMAL SUBJECTS REACTED (38). RING (39) STUDIED THE RESPONSE OF NORMAL AND THYROXINE-TREATED RATS TO A SINGLE INJECTION OF ADRENALINE. AN INCREASED RESPONSE TO ADRENALINE, AS SHOWN BY A GREATER INCREASE IN OXYGEN CONSUMPTION, WAS OBSERVED IN THYROXINE-TREATED RATS. ANIMALS KEPT EIGHT WEEKS ON THYROXINE INJECTIONS EXHIBITED 94.9% INCREASE AFTER ADRENALINE INJECTION. PRETREATMENT WITH THYROXINE FOR A PERIOD OF FOUR WEEKS RESULTED IN ONLY 54.3% INCREASE. WHEN THYROXINE WAS GIVEN ONLY FOR ONE WEEK, NO SENSITIZATION WAS SHOWN ; THE SAME INCREASE WAS OBSERVED IN BOTH TREATED AND NORMAL ANIMALS. SWANSON CONFIRMED RING'S OBSERVATIONS THAT THE POTENTIATION OF ADRENALINE ACTION DEPENDED UPON THE THYROID STATE OF THE ANIMALS. IN ORDER TO CONTROL MORE ACCURATELY THE LEVEL OF CIRCULATING THYROID HORMONES, SHE ELIMINATED ENDOGENOUS THYROID HORMONES BY SURGICAL REMOVAL OF THE THYROID GLAND. THE RATS WERE THEN INJECTED WITH VARIOUS AMOUNTS OF THYROXINE, AFTER A CERTAIN PERIOD OF TIME HAD ELAPSED. THE RANGE OF THYROXINE CONCENTRATIONS INJECTED INTO THE RAT, SUBCUTANEOUSLY DAILY FOR A PERIOD OF FOUR

WEEKS WAS FROM 0 TO 48 UG. (0,3,12 AND 48 UG.). THE RESPONSE OF THESE RATS TO A SINGLE INJECTION OF ADRENALINE WAS THEN COMPARED TO THAT OF NORMAL RATS. IN THYROXINE-TREATED ANIMALS, THE RESPONSE TO ADRENALINE WAS INCREASED. THE INCREASE IN THE RESPONSE WAS PROPORTIONAL TO THE LOGARITHM OF THE DOSE OF THYROXINE RECEIVED (40).

IN EXPERIMENTS WHERE THE EFFECTS OF ENDOGENOUS CATECHOLAMINES WERE BLOCKED BY EPIDURAL COCAINE INJECTION (41), OR BY DIBENZYLAMINE (42), A POTENTIATION OF THE CALORIGENIC EFFECT OF EXOGENOUS ADRENALINE OR NORADRENALINE WAS FOUND IN BOTH THYROID-FED DOGS (41), AND THYROXINE-TREATED RATS (42).

THE CARDIOVASCULAR SYSTEM HAS PROVIDED A CONVENIENT TOOL FOR THE INVESTIGATIONS ON ADRENAL-THYROID INTERRELATIONS. CLINICIANS HAVE OFTEN OBSERVED THE SENSITIZATION OF THE HEART OF THYROTOXIC PATIENTS TO EXOGENOUS ADRENALINE. A MORE PROMINENT EFFECT OF ADRENALINE ON TACHYCARDIA HAS BEEN REPORTED IN HYPERTHYROID DOGS (43). IN THIS SERIES OF EXPERIMENTS, ADRENALINE INJECTION HAD A DEPRESSOR EFFECT WHICH WAS STILL ENHANCED IN HYPERTHYROID ANIMALS. FURTHERMORE, THE THRESHOLD DOSE OF ADRENALINE WAS LOWER IN THE HYPERTHYROID THAN IN THE NORMAL DOGS. IN ANOTHER SERIES OF EXPERIMENTS (44), THYROID FUNCTION IN THE DOG WAS DESTROYED BY TREATMENT OF THE ANIMAL WITH LARGE DOSES OF ¹³¹IODINE. THE DOGS WERE THEN FED DESICCATED THYROID. NORMAL DOGS RESPONDED TO ADRENALINE INTRAVENOUS INJECTION BY A RISE IN BLOOD PRESSURE. THYROID-FED ¹³¹IODINE-PRETREATED DOGS EXHIBITED A LOWER

INCREASE IN BLOOD PRESSURE THAN THAT OBSERVED WITH NORMAL ONES. INTRAVENOUS INJECTION OF NORADRENALINE ALSO RESULTED IN A SMALLER RISE IN BLOOD PRESSURE AMONG THE THYROID-FED ¹³¹IODINE-PRETREATED DOGS WHEN COMPARED TO THE NORMALS. THE CONCENTRATION OF ADRENALINE OR NORADRENALINE USED IN THESE STUDIES WAS NOT GIVEN. INTACT DOGS PRETREATED WITH THYROXINE FOR TWO WEEKS DID NOT SHOW ANY SENSITIZATION OF THE HEART TO EITHER ADRENALINE OR NORADRENALINE INJECTIONS (45). SEVERAL AUTHORS, HOWEVER, HAVE REPORTED A LOWERING OF ADRENALINE LETHAL DOSE IN HYPERTHYROID ANIMALS (48, 49, 46, 47). IN MAN AN INCREASE IN THE PRESSOR EFFECTS OF NORADRENALINE HAS BEEN REPORTED IN A TYROTOXIC SUBJECT; BUT NO CHANGE IN HEART RATE WAS OBSERVED. THERE WAS ALSO A MARKED DIFFERENCE IN THE TOLERABLE DOSE OF NORADRENALINE IN THE SAME THYROTOXIC PATIENT (50).

EXTENSIVE INVESTIGATIONS HAVE BEEN CONDUCTED ON THE EFFECT OF THYROID HORMONES ON ADRENALINE-INDUCED HYPERGLYCEMIA. IN THYROID-FED RABBITS, PREVIOUSLY THYROIDECTOMIZED, ADRENALINE CAUSED A GREATER RISE IN BLOOD GLUCOSE THAN IT DID IN NORMAL RABBITS (51). THESE RESULTS HAVE BEEN CONFIRMED IN THYROID-FED RABBITS WITH INTACT THYROID GLANDS (52). ABBOT AND VANSURSKIRK COULD NOT FIND ANY DIFFERENCE BETWEEN THYROID-FED AND NORMAL CATS OR DOGS IN THEIR BLOOD SUGAR RESPONSE TO ADRENALINE (53). THE LIVER GLYCOGEN WAS UNCHANGED BY THYROID-FEEDING AND THUS DEPLETION OF LIVER GLYCOGEN COULD NOT BE INVOLVED IN THIS LACK OF INCREASED RESPONSE. IN THYROIDECTOMIZED

GUINEA-PIGS GIVEN A SINGLE DOSE OF THYROXINE TWENTY-FOUR HOURS BEFORE THE INJECTION OF ADRENALINE, THE THRESHOLD DOSE OF ADRENALINE FOR HYPERGLYCEMIA WAS LOWER THAN THAT FOR THE NORMAL ANIMALS. MOREOVER, THE DECREASE IN THE THRESHOLD DOSE WAS PROPORTIONAL TO THE AMOUNT OF THYROXINE INJECTED (54). IN HYPOPHYSECTOMIZED FEMALE RATS, PRETREATED WITH THYROXINE, THE GLYCOGEN LEVEL IN STRIATED MUSCLE WAS DECREASED BY THE INJECTION OF ADRENALINE. THE GLYCOGEN LEVEL WAS MEASURED IN RECTUS PECTORIS, BEFORE A SINGLE ADRENALINE INJECTION. AFTER ADRENALINE INJECTION, THE PERCENT DECREASE OF THE GLYCOGEN CONCENTRATION IN RECTUS PECTORIS, ABDOMINAL MUSCLE AND DIAPHRAGM, COMPARED TO THE LEVEL IN RECTUS PECTORIS BEFORE THE INJECTION, WAS RESPECTIVELY : - 36%, - 62%, - 62% (55). IN RABBITS FED WITH DESIGNATED THYROID FOR FOURTEEN DAYS, THE THRESHOLD DOSES OF ADRENALINE AND NORADRENALINE TO HYPERGLYCEMIA WERE MEASURED BY TENDENBURG (52). DOSES OF CATECHOLAMINES RANGING FROM 30 TO 480 UG PER KILOGRAM OF BODY WEIGHT WERE INJECTED SUBCUTANEOUSLY INTO NORMAL RABBITS. IN THYROID-FED RABBITS, A SINGLE DOSE OF ADRENALINE OF 60 UG WAS ASSAYED AND GAVE A RISE IDENTICAL TO THAT OBTAINED WITH A DOSE OF 85 UG IN THE NORMAL CONTROLS. NORADRENALINE WAS LESS EFFICIENT IN BOTH NORMAL AND THYROID-FED RABBITS IN THE INDUCTION OF HYPERGLYCEMIA. IN THE NORMAL RABBITS, 225 UG/KG WAS NECESSARY TO ELICIT A RESPONSE IDENTICAL TO THAT OBTAINED WITH ADRENALINE. MOREOVER, THYROID-FEEDING FURTHER INCREASED THE DOSE OF ADRENALINE NECESSARY, SINCE 250 UG/KG WAS NOW NECESSARY TO ELICIT HYPERGLYCEMIA.

3) HYPOTHYROID ANIMALS

IN HYPOTHYROIDISM OR IN THYROIDECTOMIZED ANIMALS, THE RESPONSES TO CATECHOLAMINE INJECTION ARE EITHER UNCHANGED OR DECREASED.

(*)

OXYGEN CONSUMPTION FOLLOWING "ADRENALIN" INJECTION WAS FOUND TO BE INCREASED IN THYROIDECTOMIZED RATS (37). THE INCREASE IN OXYGEN CONSUMPTION, HOWEVER, WAS LESS PRONOUNCED THAN THAT OBSERVED IN NORMAL CONTROLS. THESE RESULTS WERE CONFIRMED BY SEVERAL WORKERS IN DIFFERENT LABORATORIES (39, 56). THE INJECTION OF ADRENALINE TO THYROIDECTOMIZED ANIMALS RESULTED IN A DECREASE IN THE RESPONSE AS MEASURED BY OXYGEN CONSUMPTION. ALTHOUGH THE OXYGEN CONSUMPTION WAS LESS ELEVATED, THERE WAS STILL A RESPONSE TO ADRENALINE INJECTION IN THE THYROIDECTOMIZED ANIMALS. SWANSON DID NOT FIND A RISE IN OXYGEN CONSUMPTION AFTER ADRENALINE HAD BEEN INJECTED INTO RATS THYROIDECTOMIZED FROM FIFTEEN TO SIXTY DAYS PRIOR ADRENALINE INJECTION. THE OXYGEN CONSUMPTION WAS MEASURED INTERMITTENTLY FOR FOUR HOURS AFTER A SINGLE INTRAMUSCULAR INJECTION OF ADRENALINE (40). THIS WORKER CLAIMED THAT THE DISCREPANCIES BETWEEN HER RESULTS AND THOSE PREVIOUSLY REPORTED WERE PROBABLY DUE TO THE FACT THAT THE

(*) ADRENALIN WAS A PURIFIED EXTRACT OF OX ADRENAL MEDULLA AND CONTAINED THE THREE AMINES FOUND NORMALLY IN THIS GLAND : DOPAMINE, NORADRENALINE AND ADRENALINE.

ANIMALS USED IN THE PREVIOUS EXPERIMENTS WERE NOT COMPLETELY DEPLETED OF THYROID HORMONES. THE DIET OF THE ANIMALS USED IN THE EARLIER EXPERIMENTS WAS NOT CONTROLLED AND THE POSSIBILITY OF AN EXTERNAL SOURCE OF THYROID HORMONES IN THE DIET WAS NOT ELIMINATED. THE LEVEL OF CIRCULATING THYROID HORMONES IN THESE ANIMALS MIGHT THEREFORE HAVE BEEN CLOSE TO NORMAL, DUE TO INGESTION OF THYROID HORMONES FROM THE DIET.

AFTER A SINGLE INJECTION OF ADRENALINE, THE RESPONSE OF THE HEART OF THYROIDECTOMIZED RABBITS WAS IDENTICAL TO THAT OBSERVED WITH THE HEART OF NORMAL RABBITS (46). IN MYXEDEMATOUS PATIENTS, THE HEART RATE WAS UNAFFECTED BY ADRENALINE OR NORADRENALINE INFUSION (50). TREATMENT OF HYPOTHYROID PATIENTS WITH EITHER D- OR L-THYROXINE DID NOT AFFECT THE CARDIOVASCULAR RESPONSE TO ADRENALINE. IT WAS SUGGESTED BY THE AUTHORS THAT ONLY A HIGH LEVEL OF CIRCULATING THYROID HORMONES AFFECTS THE RESPONSE OF THE CARDIOVASCULAR SYSTEM TO CATECHOLAMINES (57). IN ¹³¹IODINE-TREATED DOGS, A DECREASE IN THE BLOOD PRESSURE RESPONSE WAS FOUND WITH EITHER ADRENALINE OR NORADRENALINE INJECTION (44). SURPRISINGLY, FEEDING DESSICATED THYROID TO THESE DOGS PROVOKED A FURTHER DECREASE IN THE RESPONSE.

A GRADUAL FALL IN THE SENSITIVITY OF THE SMOOTH MUSCLE OF THE HIND LEGS OF THE CAT OCCURRED AFTER THYROIDECTOMY. SENSITIVITY TO ADRENALINE WAS INDUCED BY DENERVATION OF THE HIND LEGS (58).

CONCERNING ADRENALINE-INDUCED HYPERGLYCEMIA, THYROIDECTOMY WAS FOUND TO HAVE EITHER NO EFFECT OR A DECREASED EFFECT. IN RABBITS,

A. DECREASE IN THE HYPERGLYCEMIA PROVOKED BY ADRENALINE WAS OBSERVED AFTER THYROIDECTOMY (51). THE DECREASE WAS PROPORTIONAL TO THE GLUCOSE BLOOD LEVEL BEFORE ADRENALINE INJECTION. A SMALLER DECREASE WAS OBSERVED AMONG THE ANIMALS WHICH EXHIBITED THE LOWER GLYCEMIA BEFORE THE INJECTION. IN GUINEA-PIGS, THE THRESHOLD FOR ADRENALINE WAS ELEVATED, IN THE THYROIDECTOMIZED ANIMALS COMPARED TO THE NORMALS. WHILE 3 MICROGRAMS WERE THE MINIMAL DOSE OF ADRENALINE REQUIRED TO PRODUCE HYPERGLYCEMIA IN NORMAL ANIMALS, IN THE THYROIDECTOMIZED ANIMALS, 15 MICROGRAMS PER KILOGRAM OF BODY WEIGHT WAS THE MINIMAL DOSE REQUIRED (53). IN RATS, THYROIDECTOMY WAS FOUND TO INCREASE THE RESPONSE TO ADRENALINE, WHERE GLYCEMIA WAS CONCERNED. SKELETAL MUSCLE GLYCOGEN WAS SLIGHTLY DECREASED, AFTER ADRENALINE INJECTION (59).

B. THE ACTION OF CATECHOLAMINES ON ISOLATED TISSUES IN RELATION TO ENDOGENOUS OR EXOGENOUS THYROID HORMONE LEVELS.

CATECHOLAMINES HAVE BEEN SHOWN TO EXERT THEIR ACTION ON ALL TISSUES INNERVATED BY SYMPATHETIC FIBERS. BOTH ADRENALINE AND NORADRENALINE HAVE BEEN FOUND TO HAVE EITHER AN INHIBITORY OR AN EXCITATORY EFFECT, DEPENDING UPON THE TISSUE UNDER INVESTIGATION. IN ORDER TO EXPLAIN SUCH A DIFFERENCE OF ACTION CAUSED BY A SINGLE MOLECULE, ALQUIST POSTULATED THE EXISTENCE OF RECEPTOR (OR EFFECTOR) SITES (61). THE STRUCTURE OF THE RECEPTOR SITES WOULD BE DIFFERENT AND THIS WOULD EXPLAIN HOW A SINGLE SUBSTRATE COULD ELICIT SUCH OPPOSITE RESPONSES. THE DIFFERENTIATION BETWEEN TWO RECEPTOR SITES

WAS BASED UPON THE OBSERVATION THAT COMPLETE BLOCKAGE OF THE SYMPATOMIMETIC SYSTEM WAS BROUGHT ABOUT BY THE USE OF VARIOUS SYMPATHOLYTIC DRUGS. DEPENDING UPON THE ACTIONS BLOCKED BY ONE OF TWO DRUGS, THE RECEPTOR IN THE TISSUES WAS IDENTIFIED AS ALPHA OR BETA. FURGNOTT POSTULATED THE EXISTENCE OF A THIRD RECEPTOR SITE, SPECIFIC FOR THE INHIBITORY ACTION OF CATECHOLAMINES ON THE SMOOTH MUSCLE OF THE SMALL INTESTINE (60). HOWEVER, IN LATER WORK THIS AUTHOR AGREED ON THE EXISTENCE OF ONLY TWO RECEPTOR SITES, ON THE BASIS THAT SYMPATHOLYTIC DRUGS FOR BOTH ALPHA AND BETA RECEPTORS WERE NECESSARY TO INDUCE COMPLETE BLOCKAGE OF THE CATECHOLAMINE ACTION IN THE SMALL INTESTINE.

ALTHOUGH NO DIRECT PROOF OF THE EXISTENCE OF RECEPTOR SITES ARE AVAILABLE PRESENTLY, AND IN SPITE OF THE FACT THAT ALMOST NOTHING IS KNOWN ABOUT THESE SITES, IT WAS STIMULATING TO SEARCH FOR AN ACTION OF THYROID HORMONES AT THIS LEVEL. THYROID HORMONES COULD ACT AT THIS SITE AS ACTIVATORS, IN A FASHION SIMILAR TO THAT EXHIBITED BY THE ACTIVATORS OF ENZYMES. BECAUSE THIS THEORY PRESENTLY IS NOT BASED ON CONCLUSIVE EXPERIMENTAL FACTS, IT WILL NOT BE DEVELOPPED FURTHER. EXPERIMENTAL OBSERVATIONS WILL BE DISCUSSED, AND IT WILL BE ATTEMPTED TO CORRELATE THE FINDINGS TO A POSSIBLE ACTION OF THYROID HORMONES AT THE RECEPTOR SITES FOR CATECHOLAMINES.

IN THE FIRST SECTION, THE EFFECT OF ENDOGENOUS THYROID HORMONE LEVELS ON THE PHYSIOLOGICAL ACTIONS OF CATECHOLAMINES ON ISOLATED TISSUES WILL BE DISCUSSED.

THE OBSERVATIONS RELATED TO THE EFFECT OF EXOGENOUS THYROID HORMONES ON CATECHOLAMINE ACTIONS ON ISOLATED TISSUES WILL BE PRESENTED IN THE SECOND SECTION.

1) EFFECTS OF ADDED CATECHOLAMINES ON ISOLATED TISSUES FROM ANIMALS IN VARIOUS THYROID STATES

BEFORE ENTERING THE MAIN SUBJECT MATTER, IT IS NECESSARY TO EXPLAIN THAT ONLY THE OBSERVATIONS OBTAINED WITH PURE HORMONES WILL BE PRESENTED HERE. NUMEROUS EXPERIMENTS HAVE BEEN MADE WITH CATECHOLAMINES AND THYROID HORMONES, USING HORMONES WHICH WERE NOT PURE. IT SEEMED THAT THE CONFUSION EXISTING ALREADY, WHEN STUDIES WERE MADE WITH PURE HORMONES, WAS GREAT ENOUGH ; IT WAS THOUGHT THAT IT SHOULD NOT BE INCREASED BY CONSIDERING THOSE EXPERIMENTS WERE "ADRENIN" (CRUDE EXTRACT FROM THE ADRENAL MEDULLA) OR "ADRENALIN" (PURIFIED EXTRACT OF ADRENAL MEDULLA) WERE USED. THE RESTRICTION MADE HERE, DOES NOT IMPLY THAT THESE EXPERIMENTS ARE NOT VALID, BUT THAT THEY WOULD NOT CONTRIBUTE MUCH TO OUR DISCUSSION.

THE EFFECT OF ADRENALINE ON THE WHOLE HEART WAS STUDIED BY Mc DONALD (62). THE WHOLE HEART WAS ISOLATED FROM NORMAL OR THYROID-FED TERRAPINS. THE INCREASE IN OXYGEN CONSUMPTION, FOLLOWING THE ADDITION OF ADRENALINE TO THE PERFUSION MEDIUM, WAS MEASURED. A SIGNIFICANTLY HIGHER INCREASE WAS FOUND WITH HYPERTHYROID HEARTS COMPARED TO THAT OBSERVED WITH THE NORMAL ONES. HOFFMANN ET AL. STUDIED, IN THE WHOLE HEART OF CATS AND GUINEA-PIGS, THE RESPONSE TO

ADRENALINE AS A FUNCTION OF THE AMINE CONCENTRATION IN THE MEDIUM (63). THE ENDOGENOUS THYROXINE WAS ELIMINATED BY SURGICAL THYROIDECTOMY OF THE ANIMALS. AFTER EIGHT TO FIFTEEN DAYS HAD ELAPSED, THE ANIMALS WERE INJECTED WITH THYROXINE FOR A PERIOD OF TWO TO EIGHT WEEKS. IN HEARTS FROM ATHYROID CONTROLS, INCREASE IN AMPLITUDE AND IN RATE WAS BROUGHT ABOUT BY A MINIMAL DOSE OF 0.05 MICROGRAM OF ADRENALINE, IN HEARTS FROM THYROXINE-TREATED ANIMALS THE THRESHOLD DOSE AVERAGED 0.01 MICROGRAM. THE RESPONSE TO ADRENALINE BOTH IN RATE AND IN AMPLITUDE WAS LARGER WITH THE THYROXINE-TREATED HEARTS THAN THAT WITH THE ATHYROID CONTROLS. NO DATA WERE PRESENTED CONCERNING THE RESPONSES OF THE HEART AS A FUNCTION OF THE DURATION OF THYROXINE TREATMENT.

THIER ET AL. STUDIED THE RESPONSE OF ISOLATED ATRIA FROM HYPERTHYROID OR EUTHYROID RATS, TO BOTH ADRENALINE AND NORADRENALINE (64). THE DOSE-RESPONSE CURVE WAS HIGHER IN THE CASE OF THE HYPERTHYROID ANIMALS THAN IN THE CASE OF THE CONTROLS. AT EQUIVALENT DOSES OF EITHER CATECHOLAMINE IT SHOWED THAT A GREATER INCREASE IN ATRIAL RATE WAS OBTAINED IN THIS CASE. THE SHAPES OF THE CURVE WERE IDENTICAL, AND NO DISPLACEMENT TO THE LEFT OBSERVED WITH THE DOSE-RESPONSE CURVE FROM HYPERTHYROID ATRIA. THIS SHOWED THAT THE THRESHOLD DOSE OF EITHER ADRENALINE OR NORADRENALINE WAS NOT LOWERED IN ATRIA FROM HYPERTHYROID RATS. THE AUTHORS CONCLUDED THAT ATRIA FROM HYPERTHYROID ANIMALS, WERE NOT SENSITIZED TO THE ACTION OF BIOGENIC AMINES. THE CHANGE IN ATRIAL RATE FOLLOWING ADDITION OF EITHER

CATECHOLAMINE TO THE MEDIUM WAS, NEVERTHELESS, SIGNIFICANTLY HIGHER IN HYPERTHYROID HEARTS THAN IN NORMAL ONES.

THE RESPONSE OF VENTRICULAR STRIPS TO NORADRENALINE WAS STUDIED IN THE CAT BY BENFEY AND VARMA (65). THE TISSUE USED FOR THE STRIPS WAS THE RIGHT VENTRICLE ISOLATED FROM SPINAL CATS UNTREATED, OR TREATED WITH TRIIODOTHYRONINE. TRIIODOTHYRONINE WAS INJECTED SUBCUTANEOUSLY, DAILY, FOR FIVE TO SIX DAYS AT A DOSE OF 0.200 MG-0.400 MG. FOR THE TWO DOSES OF NORADRENALINE ASSAYED (2 MICROGRAMS & 8 MICROGRAMS PER MILLILITER OF INCUBATION MEDIUM) THE RESPONSE OF THE HYPERTHYROID TISSUE WAS UNCHANGED COMPARED TO THAT OF THE NORMAL TISSUE.

IN 1962, MAC MILLAN AND RAND INVESTIGATED THE EFFECT OF TRIIODOTHYRONINE ON THE RESPONSE OF THORACIC AORTA AND SPLEEN CAPSULE TO EXCESSIVE NORADRENALINE, IN THE RABBIT (66). IN ORDER TO ELIMINATE ANY ENDOGENOUS THYROID HORMONE, THE ANIMALS WERE THYROIDECTOMIZED PRIOR TO TREATMENT WITH TRIIODOTHYRONINE. FOR A PERIOD OF EIGHT TO TWENTY DAYS, 0.100 MILLIGRAMS OF TRIIODOTHYRONINE WAS INJECTED SUBCUTANEOUSLY INTO THE RABBIT. THORACIC AORTA WAS THEN REMOVED AND THE CHANGE IN CONTRACTION FOLLOWING THE ADDITION OF NORADRENALINE TO THE MEDIUM NOTED. THE MINIMAL DOSE NECESSARY TO ELICIT A RESPONSE WAS HIGHER IN THE HYPERTHYROID AORTA THAN THE MINIMAL EFFECTIVE DOSE FOR THE NORMAL CONTROL. MOREOVER, THE MAXIMAL RESPONSE TO NORADRENALINE WAS ALSO LOWER IN THE AORTA FROM HYPERTHYROID RABBIT THAN THAT IN THE NORMAL CONTROL. ON THE OTHER HAND,

WITH THE HYPERTHYROID SPLEEN, CONTRACTION OF THE CAPSULE WAS OBSERVED AT A DOSE OF NORADRENALINE LOWER THAN THAT USED FOR THE NORMAL SPLEEN ; THE MAXIMAL RESPONSE ELICITED, HOWEVER, WAS LESS IN THE HYPERTHYROID SPLEEN THAN IN THE NORMAL. THE AUTHORS CONCLUDED THAT NOT ALL THE TISSUES FROM HYPERTHYROID ANIMALS WERE SENSITIZED TO CATECHOLAMINES.

AS EARLY AS 1928, REYNOLDS REPORTED THAT FEEDING THYROID EXTRACT TO RABBITS DID NOT AFFECT THE RESPONSE OF THE SMALL INTESTINE TO ADRENALINE (67). THESE RESULTS WERE CONFIRMED BY AUMANN IN 1940 (68). NO CHANGE IN THE REACTIVITY OF SMALL INTESTINE TO ADRENALINE WAS OBSERVED IN THYROID-FED RABBITS (69). THIBAUT, HOWEVER, FOUND THAT IN THE SMALL INTESTINE FROM THYROXINE-TREATED RABBITS, PREVIOUSLY THYROIDECTOMIZED, THE INHIBITION CAUSED BY ADRENALINE WAS PROLONGED. THE THRESHOLD DOSE WAS NOT LOWERED, BUT THE DURATION OF ACTION WAS INCREASED IN THE TISSUE FROM THYROXINE-TREATED ANIMALS. THIS AUTHOR CLAIMED THAT ATHYROID ANIMALS SHOULD BE USED IF A CONTROL OF CIRCULATING THYROID HORMONES WAS TO BE EXPECTED (70).

TRENDELENSBURG, ON THE OTHER HAND, OBSERVED THAT DUODENUM FROM THYROID-FED RABBITS RESPONDED TO NORADRENALINE OR ADRENALINE AT A DOSE WHICH WAS THE THIRD OF THE EFFECTIVE DOSE FOR THE NORMAL DUODENUM (50).

IT HAS BEEN FOUND THAT, IN HYPERTHYROID PATIENTS, THE BLOOD LEVEL OF FREE FATTY ACIDS IS ELEVATED (71). DEBONS HAD SHOWN THAT THE INJECTION OF ADRENALINE TO TRIIODOTHYRONINE-TREATED RATS HAD NO EFFECT ON FREE FATTY ACID RELEASE (72). HOWEVER, WHEN EPIDIDYMAL

FAT WAS REMOVED AND PLACED IN A MEDIUM SUPPLEMENTED WITH ADRENALINE AND INCREASE IN FREE FATTY ACID RELEASE WAS OBSERVED WITH HYPERTHYROID TISSUES COMPARED TO NORMAL. THE RESPONSE TO ADRENALINE IN FREE FATTY ACID RELEASE WAS MUCH HIGHER IN TISSUES FROM HYPERTHYROID ANIMALS THAN THAT IN TISSUES FROM NORMAL ONES. THREE HOURS AFTER A SINGLE INJECTION OF TRIIODOTHYRONINE, THE ADDITION OF ADRENALINE TO THE MEDIUM DID NOT ELICIT A LARGER RESPONSE IN TISSUE FROM THESE ANIMALS. BUT TWELVE HOURS FOLLOWING THREE INJECTIONS OF TRIIODOTHYRONINE, A VERY MARKED INCREASE IN FREE FATTY ACID RELEASE BY ADRENALINE WAS SEEN. IN 1963, DEYKIN AND VAUGHAN CONFIRMED DEBONS' FINDINGS (73). EPIDIDYMAI FAT FROM TRIIODOTHYRONINE-TREATED RATS EXHIBITED A LARGER RESPONSE TO ADRENALINE THAN THAT OBTAINED WITH NORMAL RATS ; THE LIPOLYTIC EFFECT OF ADRENALINE WAS MEASURED AS THAT AMOUNT OF GLYCEROL LIBERATED IN THE INCUBATION MEDIUM. SIMILAR RESULTS WERE REPORTED BY FELT ET AL. (75, 74). THESE AUTHORS, USING MESENTERIC FAT FROM NORMAL OR THYROID-FED FEMALE RATS, OBSERVED A GREATER RELEASE OF FREE FATTY ACIDS FROM THE TISSUE OF THE LATTER GROUP IN RESPONSE TO THE ADDITION OF ADRENALINE TO THE MEDIUM. IN THIS SERIES OF EXPERIMENTS, ADRENALINE STORES HAD BEEN DEPLETED BY PREVIOUS TREATMENT OF THE ANIMALS WITH A SYMPATHOLYTIC DRUG : PHENTOLAMINE. MORE RECENTLY, ROSENFELD AND ROSENBERG CONFIRMED THE RESULTS PRESENTED BY THE PREVIOUS INVESTIGATORS (76). HOWEVER, WHEN THE AUTHORS EXPRESSED THE INCREASE IN FREE FATTY ACID RELEASE BY ADRENALINE AS A PERCENT OF THE RELEASE WITHOUT ADRENALINE IN THE

MEDIUM. THE DIFFERENCE BETWEEN THE TISSUE FROM NORMAL AND THAT FROM THYROXINE-TREATED RATS WAS NOT SIGNIFICANT ANYMORE. ROSENFELD AND ROSENBERG CONCLUDED THAT THYROXINE DID NOT HAVE A DIRECT EFFECT ON THE ADRENALINE-INDUCED RELEASE OF FREE FATTY ACID.

SIMILAR CONTRADICTIONARY RESULTS HAVE BEEN REPORTED BY VARIOUS INVESTIGATORS FOR THE EXPERIMENTS DEALING WITH ISOLATED TISSUES FROM HYPOTHYROID ANIMALS. MAC SAWYER AND BROWN STUDIED THE EFFECT OF ADRENIN (*) ON THE WHOLE HEART OF THE CAT. EACH CAT WAS ITS OWN CONTROL. AFTER THE HEART OF THE CAT HAD BEEN COMPLETELY DENERVATED IT WAS PERFUSED THROUGH THE CORONARY ARTERY WITH ADRENIN. THE CAT WAS THEN THYROIDECTOMIZED AND THE INJECTION REPEATED AFTER A CERTAIN TIME HAD ELAPSED. FOLLOWING SURGICAL REMOVAL OF THE THYROID GLAND, THE RESPONSE OF THE DENERVATED HEART TO EXOGENOUS ADRENIN WAS DECREASED. THERE WAS STILL SOME RESPONSE TO ADRENIN AND, MOREOVER, THE DOSE NECESSARY TO ELICIT THAT RESPONSE WAS UNCHANGED (77) SIMILAR RESULTS WERE OBTAINED WITH HEARTS FROM THYROIDECTOMIZED CATS OR GUINEA-PIGS. THE DOSE OF ADRENALINE NECESSARY TO ELICIT AN INCREASE IN HEART RATE AND STROKE VOLUME WAS IDENTICAL FOR TISSUES FROM THYROIDECTOMIZED AND NORMAL ANIMALS. THE INCREASE WAS LESS MARKED IN THE FORMER THAN IN THE LATTER GROUP (62).

IN ISOLATED ATRIA, THE DOSE-RESPONSE CURVE TO ADRENALINE HAD THE SAME SHAPE WHETHER THE TISSUES WERE ISOLATED FROM ¹³¹IODINE-PRETREATED OR NORMAL RATS. THE CURVE WAS SLIGHTLY LOWER IN THE CASE

(*) ADRENIN WAS A CRUDE EXTRACT OF ADRENAL MEDULLA.

OF ATHYROID ATRIA SHOWING THAT THE RESPONSE OF THESE TISSUES TO ADRENALINE WAS DIMINISHED. THE THRESHOLD DOSE OF THE BIOGENIC AMINE WAS UNCHANGED WHEN ATRIA FROM ¹³¹IODINE-PRETREATED ANIMALS WERE USED, COMPARED TO THAT NECESSARY FOR ATRIA FROM THE NORMAL CONTROLS (63). A SLIGHT DECREASE IN THE SENSITIVITY OF ISOLATED MYOCARDIUM TO ADRENALINE WAS OBSERVED IN ¹³¹IODINE-PRETREATED RATS. THE DIFFERENCE OBSERVED WAS NOT SIGNIFICANT (78).

IN ISOLATED GUINE CAROTID ARTERY. SMITH OBSERVED A SHORTENING IN THE DURATION OF THE RESPONSE TO ADRENALINE IN THE HYPOTHYROID ANIMAL. THE RESPONSE WAS DIMINISHED AS A FUNCTION OF TIME RATHER THAN AS A FUNCTION OF THE AMPLITUDE (79). THE MINIMAL EFFECTIVE DOSE OF NORADRENALINE TO ELICIT A RESPONSE OF THE AORTA WAS HIGHER IN THE THYROIDECTOMIZED THAN IN THE NORMAL RABBIT (65). THE MAXIMAL RESPONSE, TO NORADRENALINE, HOWEVER, WAS SIGNIFICANTLY LARGER IN THE FORMER THAN IN THE LATTER CASE. THE RESPONSE OF THE SPLEEN FROM THE SAME ANIMALS WAS ALSO DECREASED FOR THE TISSUES FROM THE THYROIDECTOMIZED ANIMALS, AND THE THRESHOLD DOSE ELEVATED.

DEBONS ET AL. REPORTED NO EFFECT OF ADRENALINE ON THE RELEASE OF FREE FATTY ACIDS FROM EPIDIDYMAL FAT OF PROPYLTHIOURACIL-TREATED RATS. THE RESPONSE TO ADRENALINE WAS NOT PRESENT, SINCE A SIMILAR AMOUNT OF FREE FATTY ACIDS WAS RELEASED INTO THE MEDIUM REGARDLESS OF WHETHER OR NOT IT WAS SUPPLEMENTED WITH ADRENALINE (72). THE AUTHORS CONCLUDED THAT THYROID HORMONES WERE NECESSARY FOR THE RESPONSE OF FAT TISSUE TO ADRENALINE. FELT ET AL. CONFIRMED

THESE RESULTS IN THEIR STUDIES ON THYROIDECTOMIZED FEMALE RATS. THE RELEASE OF FREE FATTY ACIDS FROM MESENTERIC FAT WAS SLIGHTLY INCREASED BY ADDITION OF ADRENALINE TO THE MEDIUM, BUT THE HIGH STANDARD DEVIATION OBTAINED DID NOT INDICATE A SIGNIFICANT EFFECT OF ADRENALINE (75, 74). DEYKIN AND VAUGHAN REPEATED DEBONS' EXPERIMENTS. THEIR RATS WERE MADE HYPOTHYROID BY TREATMENT WITH PROPYLTHIOURACYL (73). WHEN THEY MEASURED THE RELEASE OF GLYCEROL FROM EPIDIDYMAL FAT NO DIFFERENCE WAS OBSERVED IN THE PRESENCE OR IN THE ABSENCE OF ADRENALINE. ROSENFELD AND ROSENBERG DID FIND A RESPONSE OF EPIDIDYMAL FAT FROM THYROIDECTOMIZED RATS (76). THE RESPONSE INDUCED BY ADRENALINE WAS IDENTICAL TO THAT ELICITED IN TISSUES FROM NORMAL CONTROLS. TO EXPLAIN THE CONTRADICTIONS BETWEEN THEIR RESULTS AND THE PREVIOUS ONES, THESE INVESTIGATORS CLAIMED THAT PROPYLTHIOURACYL COULD HAVE A DIRECT EFFECT ON FAT TISSUE. THEY BASED THEIR CLAIMS ON THE FACT THAT PROPYLTHIOURACYL HAD BEEN SHOWN TO HAVE PERIPHERAL EFFECTS.

2) EFFECTS OF CATECHOLAMINES AS A FUNCTION OF EXOGENOUS THYROXINE IN THE ISOLATED TISSUE.

NO STUDIES HAVE BEEN MADE ON THE EFFECT OF THYROXINE IN VITRO ON THE HEART OR HEART SLICES. MOST OF THE RESULTS FOUND IN THE LITERATURE HAVE BEEN DONE ON SMOOTH MUSCLE, OF EITHER THE ARTERIES OR THE SMALL INTESTINE.

IN 1956, SMITH OBSERVED THAT THE RESPONSE TO ADRENALINE, ABSENT IN THE CAROTID ARTERY FROM THYROIDECTOMIZED SWINE, WAS

RESTORED BY ADDITION OF THYROXINE TO THE PERFUSION MEDIUM (80). THAT THE HIGH PH OF THE MEDIUM WAS NOT RESPONSIBLE FOR THIS RETURN TO NORMAL. WAS SHOWN BY THE FACT THAT WHEN THYROXINE WAS NOT PRESENT, HIGH PH ALONE DID NOT ELICIT A RETURN TO NORMAL RESPONSE. THE CONCENTRATION OF THYROXINE USED WAS NOT WELL DEFINED, SINCE PART OF THE HORMONE WAS NOT IN SOLUTION. THE RESPONSE TO ADRENALINE WAS INCREASED IN BOTH AMPLITUDE AND DURATION IN THE PRESENCE OF THYROXINE. IN 1960, SHERMAN AND FALLON INVESTIGATED THE EFFECT OF EXOGENOUS TRIIODOTHYRONINE ON THE ADRENALINE-INDUCED CONTRACTION OF AORTIC STRIPS. A SINGLE CONCENTRATION OF ADRENALINE (1×10^{-7} M) WAS ASSAYED FOR A SINGLE CONCENTRATION OF TRIIODOTHYRONINE (1×10^{-6} M). TRIIODOTHYRONINE ALONE WAS WITHOUT EFFECT. ON LONG DURATION CONTRACTIONS, TRIIODOTHYRONINE WAS ALSO WITHOUT EFFECT, EVEN IN THE PRESENCE OF ADRENALINE. HOWEVER, IN SHORT DURATION EXPERIMENTS, IN THE PRESENCE OF TRIIODOTHYRONINE, THE RESPONSE TO ADRENALINE, WAS INCREASED IN AMPLITUDE AND IN DURATION (81). BARKER FOUND THAT THE RESPECTIVE ACETIC ACID ANALOGUES OF TRIIODOTHYRONINE AND THYROXINE POTENTIATED THE RESPONSE TO ADRENALINE OF THORACIC AORTA (83). SODIUM IODIDE AND DIIODOTYROSINE WERE WITHOUT EFFECT. THESE AUTHORS SUGGESTED THAT THE EFFECT OF TRIIODOTHYRONINE WAS DUE TO CHELATION OF COPPER. BECAUSE THE CATALYTIC EFFECT OF COPPER DURING ADRENALINE OXIDATION IS WELL ESTABLISHED (82), THEY CLAIMED THAT BY PREVENTING ADRENALINE OXIDATION, TRIIODOTHYRONINE WAS INCREASING THE ACTUAL CONCENTRATION OF ADRENALINE IN THE MEDIUM. THEREFORE, MORE ADRENALINE

WAS AVAILABLE FOR THE TISSUE. SHIDA ET AL. REPEATED THE EXPERIMENTS WITH THORACIC AORTA STRIPS FROM RABBITS. NO ENHANCEMENT OF EITHER ADRENALINE OR NORADRENALINE RESPONSE OF THE AORTA WAS OBSERVED IN THE PRESENCE OF THYROXINE, OR IN THE PRESENCE OF TRIIODOTHYRONINE, WHEN THE CONCENTRATION OF COPPER IN THE MEDIUM WAS AS LOW AS 1.5 μ G PER LITER. HOWEVER, WHEN THE MEDIUM WAS SUPPLEMENTED WITH COPPER, THYROXINE WAS FOUND TO POTENTIATE THE RESPONSE TO ADRENALINE. AN INCREASE IN AMPLITUDE AND IN DURATION WAS OBSERVED. THESE AUTHORS UTILIZED A CHELATING AGENT FOR COPPER : ETHYLENE DIAMINE TETRAACETIC ACID (EDTA) AND FOUND IT MIMICKED THE EFFECT OF THYROXINE. THESE INVESTIGATORS CONCLUDED THAT, UNDER THE EXPERIMENTAL CONDITIONS USED, THYROXINE AND EDTA EFFECTS WERE IDENTICAL. THEY POINTED OUT THAT EXTRAPOLATION TO IN VIVO EFFECTS OF THYROID HORMONES WAS SOMEWHAT IMPAIRED BY THE FACT THAT THE CONCENTRATION OF FREE COPPER IN THE TISSUE IS VERY LOW, IF ANY.

THE IN VITRO EFFECT OF THYROXINE ON THE RESPONSE OF SMALL INTESTINE TO ADRENALINE WAS INVESTIGATED BY THIBAUT IN 1948. IN A SERIES OF EXPERIMENTS WITH RABBIT SMALL INTESTINE STRIPS, THE AUTHOR WAS ABLE TO SHOW THAT THYROXINE POTENTIATED THE INHIBITORY EFFECT OF ADRENALINE ON THE SMALL INTESTINE. IT WAS SHOWN THAT SEVERAL REQUIREMENTS WERE NECESSARY TO ELICIT THIS POTENTIATION. THE LEVEL OF ENDOGENOUS THYROXINE WAS CRITICAL AND THE AUTHOR SUGGESTED THAT ONLY TISSUE FROM THYROIDECTOMIZED ANIMALS SHOULD BE USED. A PREINCUBATION WITH THYROXINE WAS NECESSARY BEFORE THE TISSUE EXHIBITED ANY

POTENTIATION OF ADRENALINE ACTION. WHEN THESE TWO REQUIREMENTS WERE FULFILLED, AN INCREASE IN BOTH AMPLITUDE AND DURATION OF THE RESPONSE TO ADRENALINE WAS OBTAINED (84, 85). SEVERAL AUTHORS (68, 69) HAD TRIED PREVIOUSLY TO SHOW ANY EFFECT OF THYROID HORMONES ON THE RESPONSE OF THE SMALL INTESTINE TO ADRENALINE. THIBAUT CLAIMED THAT THE FAILURE OF THESE INVESTIGATORS WAS PRIMARILY DUE TO THE FACT THAT THE ANIMALS USED HAD NOT BEEN THYROIDECTOMIZED. SHIDA ET AL. ATTEMPTED TO REPRODUCE THE WORK OF THIBAUT AND ASSOCIATES BUT, IN THE PRELIMINARY ASSAYS, THEY FOUND THAT SMALL INTESTINE WAS NOT A TISSUE SUITABLE FOR THE STUDY OF THE EFFECTS OF EXOGENOUS CATECHOLAMINES. THEY WERE UNABLE TO OBTAIN REPRODUCIBLE RESPONSES TO ADRENALINE, EVEN IN THE ABSENCE OF THYROXINE IN THE MEDIUM (83).

WHEN THIBAUT EXAMINED THE EFFECT OF THYROXINE ON THE ADRENALINE-INDUCED CONTRACTION OF THE SPLEEN, SHE OBSERVED THAT THE AMOUNT OF ADRENALINE NECESSARY TO INDUCE A RESPONSE WAS DIMINISHED BY HALF WHEN THYROXINE WAS ALSO PRESENT IN THE MEDIUM. RABBIT UTERUS WAS EVEN MORE SENSITIZED BY THYROXINE, SINCE THE THRESHOLD DOSE OF ADRENALINE WAS DIMINISHED TEN TIMES WHEN THYROXINE WAS ADDED TO THE INCUBATION MEDIUM. THIS SERIES OF EXPERIMENTS WAS PERFORMED WITH TISSUES FROM THYROIDECTOMIZED RABBITS (86, 87).

CONCLUSION OF PART I.

THERE IS DEFINITE EVIDENCE THAT IN HYPERTHYROID PATIENTS THE PHYSIOLOGICAL ACTION OF ADRENALINE AND NORADRENALINE ON THE

CARDIOVASCULAR SYSTEM AND ON BASAL METABOLISM IS AUGMENTED. ON THE OTHER HAND, IN HYPOTHYROIDISM, THE EFFECT OF EXOGENOUS CATECHOLAMINES IS DECREASED. FROM THE FOREGOING DISCUSSION, IT CAN BE SEEN THAT THE EXPERIMENTAL ATTEMPTS TO REPRODUCE THE SYMPTOMS OBSERVED BY CLINICIANS HAVE BEEN MORE OR LESS SUCCESSFUL. THE INCREASE IN OXYGEN CONSUMPTION FOLLOWING ADRENALINE INJECTION HAS BEEN FOUND IN ALL INVESTIGATIONS TO BE LARGER IN THYROID-FED OR THYROXINE-TREATED ANIMALS. SIMILARLY, THE INCREASE IN OXYGEN CONSUMPTION OF THE ISOLATED HEART HAS BEEN FOUND LARGER IN THE CASE OF THYROID-FED ANIMALS. IN ATHYROID ANIMALS, THE EXPERIMENTAL RESULTS WERE LESS CONSISTENT. SINCE SOME AUTHORS OBSERVED A DECREASED RESPONSE TO EXOGENOUS CATECHOLAMINES, WHILE OTHERS OBTAINED NO RESPONSE WHATSOEVER.

HOWEVER, WHEN OTHER PARAMETERS WERE STUDIED, SUCH AS ADRENALINE-INDUCED TACHYCARDIA, PRESSOR EFFECTS, HYPERGLYCEMIA OR FREE FATTY ACID RELEASE, THE DATA REPORTED BY VARIOUS GROUPS OF WORKERS ARE NOT CONCORDANT. AMONG THE GROUPS WHO HAVE OBSERVED A SENSITIZATION OF THESE SYSTEMS IN HYPERTHYROID ANIMALS, THERE ARE CONTRADICTORY RESULTS CONCERNING THE MANNER BY WHICH THE SENSITIZATION OCCURRED. SOME AUTHORS FOUND THE ADRENALINE THRESHOLD DOSE TO BE DECREASED, WHILE OTHERS DID NOT FIND ANY CHANGE IN THE THRESHOLD DOSE BUT RATHER AN INCREASE IN THE DURATION AND AMPLITUDE OF THE RESPONSE AMONG THE HYPERTHYROID ANIMALS. EXPERIMENTS IN VITRO REFLECT THE SAME CONTRADICTION. IN HYPOTHYROIDISM, THE ACTIONS OF CATECHOLAMINES AS TESTED BY MEASURING TACHYCARDIA, HYPERGLYCEMIA, PRESSOR EFFECTS OR FREE

FATTY ACID RELEASE HAVE BEEN FOUND UNCHANGED BY SOME INVESTIGATORS OR DIMINISHED BY A FEW OTHERS.

VERY FEW EXPERIMENTS HAVE BEEN DONE TO INVESTIGATE THE ACTIONS OF ADRENALINE OR NORADRENALINE ON SMOOTH MUSCLES, IN HYPERTHYROID AND HYPOTHYROID ANIMALS. THE RESULTS OBTAINED DURING THESE STUDIES HAVE NOT BEEN IN AGREEMENT.

EXCEPT FOR THE STUDIES ON THE OXYGEN CONSUMPTION, THERE IS NO REPORT IN THE LITERATURE, CONCERNING SYSTEMATIC INVESTIGATIONS ON THE OTHER EFFECTS OF CATECHOLAMINES IN THE WHOLE ANIMAL, OR IN ISOLATED TISSUE, IN CASE OF THYROID GLAND IMPAIRMENT. THE EVIDENCE HAS BEEN COLLECTED FROM ISOLATED EXPERIMENTS, MADE IN VARIOUS CONDITIONS, WITH ANIMALS OF DIFFERENT SPECIES. THE IMPAIRMENT OF THE THYROID GLAND WAS BROUGHT ABOUT BY VERY DIFFERENT METHODS. IN SOME OF THE EXPERIMENTS, THE ANIMALS WERE MADE HYPERTHYROID BY THYROID-FEEDING, IN OTHERS THYROXINE OR TRIIODOTHYRONINE INJECTIONS OF DIFFERENT DOSES, BY DIFFERENT ROUTES, FOR DIFFERENT PERIODS OF TIME WERE USED AS A MODE OF INDUCING HYPERTHYROIDISM. THE SAME VARIABILITY IN INDUCING HYPOTHYROIDISM HAS BEEN OBSERVED.

ALTHOUGH THERE IS NO DOUBT THAT THYROID HORMONES AND EXOGENOUS CATECHOLAMINES ARE INTERRELATED IN THEIR ACTIONS IN THE INTACT ANIMALS, THE CONFLICTING RESULTS REPORTED IN THE LITERATURE DO NOT PERMIT A DEFINITE CONCLUSION AS TO THE WAY IN WHICH THESE HORMONES ARE INTERFERING WITH EACH OTHER. IT WOULD APPEAR THAT MORE SYSTEMATIC STUDIES, IN A GIVEN SPECIES, ON A GIVEN PARAMETER WOULD

BE NECESSARY TO THROW SOME LIGHT ON THIS INTERACTION. THE BIOCHEMICAL EVIDENCES THAT THYROID HORMONES AND CATECHOLAMINES ARE RELATED IN SOME WAY WILL NOW BE PRESENTED IN PART I I OF THIS REVIEW.

PART II. THE EFFECTS OF THYROID HORMONES ON THE BIOLOGICAL INACTIVATION OF EXOGENOUS CATECHOLAMINES

THE INTRODUCTION IN BIOCHEMISTRY OF RADIOACTIVE MOLECULES WITH HIGH SPECIFIC ACTIVITY, HAS PERMITTED THE STUDY OF MECHANISMS NOT UNRAVELED UNTIL THEN. BECAUSE ADRENALINE OR NORADRENALINE LABELED WITH TRITIUM WERE AVAILABLE, THE INACTIVATION PATHWAYS OF THESE BIOGENIC AMINES HAVE BEEN THOROUGHLY INVESTIGATED. IT WOULD APPEAR THAT TWO MAIN ROUTES ARE TAKEN, IN PHYSIOLOGICAL CONDITIONS. THE FIRST WHICH APPEARS TO BE FIRST IN TIME RATHER THAN IN IMPORTANCE IS THE RAPID INACTIVATION OF EXOGENOUS CATECHOLAMINES BY BINDING TO SITES (88). THE SITES HAVE NOT BEEN IDENTIFIED, BUT IT IS POSTULATED THAT THEY ARE MAINLY PROTEINS (89). THE SECOND WAY OF INACTIVATION IS THROUGH THE ENZYMES SPECIFIC FOR CATECHOLAMINE DEGRADATION: CATECHOL-O-METHYL TRANSFERASE AND MONOAMINE OXIDASE. THYROID HORMONES MAY HAVE A POTENTIATING EFFECT BY INTERFERING WITH EITHER MECHANISMS.

IT HAS BEEN POSTULATED THAT THYROID HORMONES OCCUPIED THE BINDING SITES PREVENTING THE RAPID INACTIVATION OF CATECHOLAMINES AND THUS PROLONGING THEIR EFFECTS. THE INHIBITION, BY THYROXINE, OF ENZYMES RESPONSIBLE FOR CATECHOLAMINE DEGRADATION WOULD ALSO POTENTIATE THE EFFECT OF CATECHOLAMINES. IN THE LATTER CASE, HOWEVER, SINCE THE ENZYMIC ACTION APPEARS TO BE SECONDARY IN TIME, ONE WOULD EXPECT AN INCREASE IN DURATION OF ACTION RATHER THAN A DECREASE IN THRESHOLD AS COULD BE EXPECTED IN THE FORMER CASE. THYROXINE, IN LARGE AMOUNTS,

HAS BEEN FOUND TO PREVENT PROTEIN SYNTHESIS (91). IT MAY THUS EXERT ITS POTENTIATION BY PREVENTING THE SYNTHESIS OF EITHER THE BINDING PROTEIN OR THE DEGRADATIVE ENZYMES.

THE COMBINED USE OF BLOCKING DRUGS AND RADIOACTIVE CATECHOLAMINES HAS PERMITTED THE DIFFERENTIATION OF THE VARIOUS BINDING SITES IN THE CELL (90, 91-95). NUMEROUS STUDIES HAVE BEEN PERFORMED DURING THE LAST FEW YEARS, ESPECIALLY USING TRITIATED NORADRENALINE. BESIDE THE RECEPTOR BINDING SITES IT SEEMS THAT, IN THE CELL OTHER BINDING SITES FOR NORADRENALINE ARE PRESENT. THE STUDIES (96, 97, 99), WITH TYRAMINE, HAVE REVEALED THAT THIS SYMPATHOMIMETIC DRUG ACTS BY LIBERATING NORADRENALINE OR ADRENALINE FROM STORAGE BINDING SITES. HOWEVER, A CERTAIN AMOUNT OF CATECHOLAMINES CAN STILL BE BOUND IN THE CELL, WHILE TYRAMINE HAS NO MORE MIMETIC ACTION. FROM THESE FINDINGS THE AUTHORS CONCLUDED THAT IN THE TISSUE THERE EXISTS TWO KINDS OF STORAGE SITES. IN ONE CASE, THE HALF-LIFE OF THE AMINE IS QUITE SHORT. IT IS THE STORAGE SITE WHICH IS DEPLETED BY TYRAMINE. AT THE SECOND SITE, THE AMINE IS BOUND IN A FIRMER LINKAGE AND IS NOT RELEASED BY TYRAMINE. THE EFFECT OF THYROID HORMONES HAS BEEN POSTULATED TO TAKE PLACE AT EITHER STAGE. IT HAS BEEN VERY DIFFICULT TO SHOW EXACTLY WHERE THYROID HORMONES ARE ACTING BECAUSE THERE IS RELATIVELY LITTLE SYSTEMATIC WORK DONE ON THE DOSE-RESPONSE CURVE FOR EITHER THYROXINE OR TRIIODOTHYRONINE. TIME-COURSE EXPERIMENTS ARE NOT AVAILABLE EITHER. THE EFFECT OF TYRAMINE ON NORADRENALINE STORES HAS BEEN SHOWN,

BECAUSE TIME-COURSE STUDIES ON THE RELEASE OF NORADRENALINE AS A FUNCTION OF TYRAMINE EFFECT HAVE BEEN DONE. SINCE NO SUCH STUDY IS AVAILABLE IN THE CASE OF THYROID HORMONES, DEFINITE CONCLUSIONS CANNOT BE DRAWN ON THEIR EFFECTS. IT WOULD SEEM THAT THE TWO SITES OF BINDING FOR STORAGE ARE REPLENISHED FROM CIRCULATING CATECHOLAMINES. OR AT LEAST, THE EXOGENOUS NORADRENALINE CANNOT BE DIFFERENTIATED FROM ENDOGENOUS CATECHOLAMINE AT THIS POINT.

THE EFFECTS OF THYROID HORMONES ON THE UPTAKE AND BINDING OF EXOGENOUS CATECHOLAMINES WILL BE DISCUSSED IN THE FIRST SECTION. IN THE SECOND SECTION OF THIS DISCUSSION, THE EFFECT OF THYROID HORMONES ON CATECHOL-O-METHYL TRANSFERASE AND MONOAMINE OXIDASE WILL BE PRESENTED.

A. THE EFFECT OF THYROID HORMONES ON THE UPTAKE AND BINDING OF EXOGENOUS CATECHOLAMINES

WHEN STUDYING THE EFFECT OF THYROID HORMONES ON THE DEGRADATION OF EXOGENOUS CATECHOLAMINES ONE ELIMINATES ONE OF THE FACTORS WHICH MAY BE RESPONSIBLE FOR POTENTIATION OF CATECHOLAMINES. THYROID HORMONES COULD POSSIBLY SENSITIZE A TISSUE TO CATECHOLAMINES BY INCREASING THE RATE OF FORMATION OF THESE AMINES, OR BY EXERTING A DIRECT EFFECT ON THE STORAGE GRANULES. BY STUDYING THE EFFECTS OF THYROID HORMONES ON THE RESPONSE OF TISSUES TO ADRENALINE, ONE TESTS THE DIRECT EFFECT OF THYROIDINE AT THE RECEPTOR LEVEL. ONE HAZARD IN THE USE OF EXOGENOUS RADIOACTIVE MATERIAL, IS THAT EXOGENOUS AMINES MAY NOT REFLECT THE ACTUAL METABOLISM OF ENDOGENOUS AMINES.

IF ON THE OTHER HAND, THEY DO REFLECT THE ACTUAL METABOLISM OF ENDOGENOUS CATECHOLAMINES, THE POSSIBILITY OF AN EXCHANGE BETWEEN HOT AND COLD MATERIAL IS NOT ELIMINATED. VERY FEW AUTHORS, IF ANY, HAVE MEASURED THE SPECIFIC ACTIVITY OF THE MATERIAL ISOLATED FROM TISSUES AFTER ADRENALINE INJECTION. SECONDLY, AND THIS IS ESPECIALLY TRUE WHEN TRITIATED ADRENALINE IS CONCERNED, THE POSSIBILITY OF ISOTOPE EFFECTS ON THE PENETRATION OF THE AMINE HAS NOT BEEN ELIMINATED. IF, AS IT SEEMS ADRENALINE PENETRATES INTO THE CELL BY BOTH A PASSIVE DIFFUSION AND AN ACTIVE TRANSPORT (104), WHEN SMALL CONCENTRATIONS OF ADRENALINE ARE USED, ONLY THE ACTIVE TRANSPORT MECHANISM WILL ACT. THE ISOTOPE EFFECT MAY BE VERY IMPORTANT IN THIS CASE. ISOTOPE EFFECTS MAY ALSO ENTER THE PICTURE WHEN THE ENZYMIC DEGRADATION OR BINDING OF THE AMINES IS STUDIED. THIS AGAIN IS ESPECIALLY TRUE FOR THE TRITIATED COMPOUND. ELIMINATION OF POSSIBLE ISOTOPE EFFECTS WOULD BE POSSIBLE, OR AT LEAST, CONFIRMATION OF ISOTOPE EFFECTS COULD BE MADE IF ONE WAS COMPARING ^3H - AND ^{14}C -LABELED COMPOUNDS. IT IS DIFFICULT TO USE ^{14}C LABELED CATECHOLAMINE IN IN VIVO EXPERIMENTS, BECAUSE THE SPECIFIC ACTIVITY OF THE COMMERCIAL COMPOUNDS IS TOO LOW TO PERMIT THE USE OF SENSITIVE METHODS. THIS PROBLEM APPEARS INSOLUBLE AT THE PRESENT TIME. HOWEVER IT IS NECESSARY TO BEAR IT IN MIND WHEN CONSIDERING THE DATA WHICH WILL NOW BE PRESENTED.

THE INJECTION OF LABELED CATECHOLAMINES IS FOLLOWED BY A BINDING OF RADIOACTIVE MATERIAL IN THE TISSUE. THIS RADIOACTIVE MATERIAL HAS BEEN IDENTIFIED AS MAINLY UNCHANGED CATECHOLAMINES.

IT IS FOUND IN THE TISSUE LONG AFTER THE PHYSIOLOGICAL EFFECTS OF EITHER AMINE HAVE DISAPPEARED. IT WAS ON THESE FINDINGS THAT SEVERAL AUTHORS BASED THEIR HYPOTHESIS THAT BINDING IS ONE MODE OF INACTIVATION OF CATECHOLAMINES (88, 98). THE UPTAKE OF EXOGENOUS CATECHOLAMINES HAS BEEN SHOWN TO BE INVERSELY PROPORTIONAL TO THE CONCENTRATION OF CATECHOLAMINES OUTSIDE THE TISSUE (99). AT LOW CONCENTRATIONS OF ADRENALINE OR NORADRENALINE, THE ACTIVE MECHANISM IS MORE IMPORTANT, WHILE AT HIGH CONCENTRATIONS THE PASSIVE DIFFUSION APPEARS TO BE THE PRIMARY MECHANISM. TISSUES HAVE BEEN SHOWN TO CONCENTRATE CATECHOLAMINES EVEN AT HIGH EXTERNAL CONCENTRATIONS OF THE AMINES (98, 158).

LEDUC ET AL. WERE THE FIRST TO STUDY THE UPTAKE AND BINDING OF CATECHOLAMINES IN HYPERTHYROID ANIMALS. THESE AUTHORS USED IODINATED-CASEIN-FED RATS AND STUDIED THE DISTRIBUTION OF ADRENALINE FOLLOWING AN INTRAMUSCULAR INJECTION OF 125 MICROGRAMS OF ADRENALINE. THEY INVESTIGATED THE DISTRIBUTION OF ADRENALINE FIVE AND TEN MINUTES AFTER THE INJECTION, IN THE HEART, LIVER, SPLEEN, ADRENALS AND BLOOD OF NORMAL AND HYPERTHYROID RATS. IN ALL THESE TISSUES EXCEPT BLOOD, THEY IDENTIFIED BOTH ADRENALINE AND NORADRENALINE FLUORIMETRICALLY. IN THE HEART, THE CONCENTRATION OF ADRENALINE AFTER INJECTION WAS IDENTICAL FOR BOTH HYPERTHYROID AND NORMAL ANIMALS. HOWEVER, AS IN THE HEART OF HYPERTHYROID RATS, THE LEVEL OF ADRENALINE BEFORE THE INJECTION WAS HIGHER THAN THAT IN THE HEART OF NORMAL RATS, THE INCREASE AFTER THE INJECTION WAS THEREFORE

RELATIVELY SMALLER IN THE HYPERTHYROID ANIMALS. IN THE LIVER, THE INCREASE IN ADRENALINE CONCENTRATION FOLLOWING THE INJECTION WAS MARKEDLY LARGER AMONG THE HYPERTHYROID RATS. THERE WAS NO CHANGE IN THE ADRENALINE CONTENT OF THE ADRENALS, BUT THE CONCENTRATION OF ADRENALINE, WHEN EXPRESSED PER MG OF NITROGEN, WAS GREATER IN THE HYPERTHYROID GROUP. FOLLOWING THE INJECTION OF ADRENALINE, THE NORADRENALINE CONTENT OF THE SPLEEN WAS MARKEDLY INCREASED IN BOTH GROUPS (101).

WURTMANN ET AL. FOLLOWED THE TIME-COURSE OF THE DISAPPEARANCE OF TRITIATED ADRENALINE IN NORMAL, HYPER- AND HYPOTHYROID RATS. THE CATECHOLAMINES WERE INJECTED INTRAVENOUSLY. FORTY MINUTES AFTER THE INJECTION OF A PHYSIOLOGICAL DOSE OF ^3H -CL-ADRENALINE, THERE WAS A SIGNIFICANTLY LOWER CONCENTRATION OF RADIOACTIVE MATERIAL IN THE HEART OF THE HYPERTHYROID ANIMALS. AFTER IDENTIFICATION, THIS RADIOACTIVE MATERIAL WAS FOUND TO CONSIST ALMOST ENTIRELY OF UNCHANGED ADRENALINE. THE DISTRIBUTION OF TRITIATED NORADRENALINE WAS ALSO STUDIED IN THE COURSE OF THESE EXPERIMENTS. THE BINDING OF NORADRENALINE WAS NOT AFFECTED BY THE THYROID STATE OF THE ANIMALS (102).

IN THE WHOLE MOUSE. AXELROD AND TOMCHICK DID NOT FIND ANY DIFFERENCE IN THE RATE OF DISAPPEARANCE OF RADIOACTIVE ADRENALINE BETWEEN THYROXINE-PRETTREATED AND NORMAL ANIMALS. THE MEASUREMENTS WERE PERFORMED TEN MINUTES AFTER THE SUBCUTANEOUS INJECTION OF RADIOACTIVE ADRENALINE, AT A DOSE OF THREE MICROGRAMS PER MOUSE (103).

DENGLER AND TITUS STUDIED THE UPTAKE AND BINDING OF ISOTOPIC NORADRENALINE IN RAT HEARTS (104). TEN MINUTES AFTER INJECTION OF PHYSIOLOGICAL DOSES OF THE AMINE (6.6 UG/KG), THE UPTAKE OF RADIOACTIVE MATERIAL IN THE HEART FROM HYPERTHYROID RATS WAS 57% OF THAT FOUND IN THE HEART FROM NORMAL CONTROLS. HOWEVER, WHEN THE RADIOACTIVE MATERIAL WAS IDENTIFIED, IT WAS FOUND THAT IN THE HEART FROM NORMAL CONTROLS, ONLY 37.3% WAS NORADRENALINE. ADRENALINE WAS FOUND TO COMPRISE 19.6%. IN THE HEART FROM HYPERTHYROID ANIMALS, 92.1% OF THE RADIOACTIVE MATERIAL WAS ACCOUNTED FOR AS NORADRENALINE. THE HYPERTHYROID HEART APPEARED TO HAVE LOST THE CAPABILITY TO SYNTHESIZE ADRENALINE FROM NORADRENALINE SINCE NONE WAS FOUND. DURING IN VITRO INCUBATION OF RAT HEART SLICES WITH RADIOACTIVE DL-NORADRENALINE, SLICES FROM HYPERTHYROID HEART TOOK UP ONLY 70% AS MUCH RADIOACTIVE MATERIAL AS THE SLICES FROM NORMAL CONTROLS. THE AUTHORS CONCLUDED THAT THYROXINE PREVENTS THE UPTAKE OF NORADRENALINE.

IT APPEARS THAT THE CONCENTRATION OF CATECHOLAMINES FOUND IN THE TISSUES FROM HYPERTHYROID ANIMALS IS DEPENDENT UPON THE TIME OF INVESTIGATION AFTER THE INJECTION. AS SHOWN BY WÜRTHANM ET AL. (102), THE DISAPPEARANCE OF EXOGENOUS CATECHOLAMINES DOES NOT FOLLOW THE SAME PATTERN IN THE HYPERTHYROID ANIMAL AS THAT IN THE NORMAL. TWO MINUTES FOLLOWING INJECTION OF ADRENALINE THE CONCENTRATION OF THE UNCHANGED AMINE IS HIGHER IN THE HEART FROM HYPERTHYROID RATS THAN IN THE HEART OF NORMAL CONTROLS. FORTY MINUTES AFTER THE INJECTION, BECAUSE THE RATE OF DISAPPEARANCE IS FASTER IN THE HYPERTHYROID

ANIMALS, THE LEVEL OF UNCHANGED ADRENALINE IS LOWER IN THE HEART OF THE HYPERTHYROID ANIMALS THAN IN THE HEART OF THE CONTROLS. BECAUSE VERY FEW FIGURES ARE AVAILABLE ON THE UPTAKE AND BINDING OF EXOGENOUS ADRENALINE OR NORADRENALINE IN THE HYPERTHYROID ANIMAL, CONCLUSIONS ARE DIFFICULT TO DRAW. FURTHER WORK IS NECESSARY TO ASSESS THE ACTUAL IMPORTANCE OF BINDING AND UPTAKE ON THE POTENTIATION OF CATECHOLAMINE ACTION BY THYROID HORMONES.

B. THE EFFECT OF THYROID HORMONES ON CATECHOL-O-METHYL TRANSFERASE AND ON MONOAMINE OXIDASE.

AS POINTED OUT EARLIER, THE USE OF RADIOISOTOPES HAS ALLOWED THE ELUCIDATION OF THE METABOLIC PATHWAYS FOR THE INACTIVATION OF CATECHOLAMINES. THERE IS NO DOUBT THAT TWO ENZYMES ARE MAINLY RESPONSIBLE FOR THE DEGRADATION OF CATECHOLAMINES. MONOAMINE OXIDASE WAS THE FIRST ENZYME TO BE RECOGNIZED AS BEING INVOLVED IN THE OXIDATIVE DEAMINATION OF EITHER ADRENALINE OR NORADRENALINE. THIS ENZYME HAS NOT BEEN PURIFIED YET AND IT IS NOT COMPLETELY CERTAIN WHETHER IT IS A SINGLE OR A MULTIPLE ENZYME SYSTEM (105-107). MORE RECENTLY, A SECOND ENZYME (CATECHOL-O-METHYL TRANSFERASE) HAS BEEN IDENTIFIED WHICH METHYLATES NORADRENALINE AND ADRENALINE ON THE META HYDROXYL GROUP OF THE CATECHOL RING. THIS ENZYME HAS BEEN PARTIALLY PURIFIED (127).

THE RELATIVE IMPORTANCE OF THESE ENZYMES APPEARS TO DEPEND UPON THE SPECIES OF ANIMALS. IN MAN, METHYLATION OF ADRENALINE AND NORADRENALINE APPEARS TO BE THE MOST IMPORTANT PATHWAY

FOR THE DEGRADATION OF CATECHOLAMINES. IN THE RATS, OXIDATIVE DEAMINATION WOULD APPEAR TO BE MORE IMPORTANT. THE IMPORTANCE OF THE ENZYMES MAY ALSO DEPEND UPON THE ORGAN STUDIED IN A GIVEN SPECIES (108). RECENTLY, IT HAS BEEN POSTULATED THAT CATECHOL-O-METHYL TRANSFERASE IS THE ENZYME DEGRADING FREE CATECHOLAMINES. MONOAMINE OXIDASE WOULD METABOLIZE THE CATECHOLAMINES WHICH HAVE BEEN BOUND TO THE BINDING SITES (109,110).

THE ACTIVITY OF BOTH ENZYMES, IN HYPERTHYROID OR HYPOTHYROID ANIMALS, HAD BEEN MEASURED IN VARIOUS ORGANS : HEART, LIVER AND KIDNEY. MOREOVER, SOME KINETICS FOR CATECHOL-O-METHYL TRANSFERASE ACTIVITY IN THE PRESENCE OF THYROXINE ANALOGUES HAVE BEEN PUBLISHED (129-131). THE IMPORTANCE OF THYROID HORMONES ON THE INACTIVATION OF BOTH ENZYMES IS STILL A SUBJECT OF CONTROVERSY.

THERE ARE TWO APPROACHES TO THE INVESTIGATION OF THE EFFECTS OF THYROID HORMONES ON THE ENZYMES INACTIVATING CATECHOLAMINES. THE FIRST ONE IS TO MEASURE, IN VITRO THE ACTIVITY OF THESE ENZYMES PREPARED FROM ANIMALS IN VARIOUS THYROID STATES. THE SECOND APPROACH IS TO IDENTIFY THE METABOLITES IN THE WHOLE ANIMAL AFTER CATECHOLAMINE INJECTION. THIS SECOND PROCESS MEASURES THE RELATIVE ACTIVITY OF BOTH ENZYMES. THE FORMER MEASURES THE ABSOLUTE ACTIVITY OR RATHER THE POTENTIAL ACTIVITY OF THE ENZYMES.

CATECHOLAMINES HAVE BEEN SHOWN BEYOND DOUBT TO FOLLOW TWO MAIN ROUTES OF DEGRADATION (111). CATECHOL-O-METHYL TRANSFERASE (COMT) COMBINES A METHYL GROUP TO ONE OF THE HYDROXYL GROUP OF THE CATECHOL RING. IT IS THE META HYDROXYL GROUP WHICH IS PREFEREN-

TIALLY METHYLATED. MONOAMINE OXIDASE (MAO) OXIDATIVELY DEAMINATES EITHER ADRENALINE OR NORADRENALINE TO YIELD AN ALDEHYDE. THE ALDEHYDE IS SPONTANEOUSLY CONVERTED INTO EITHER AN ALCOHOL OR AN ACID. EACH METABOLITE MAY IN TURN BE AFFECTED BY THE OTHER ENZYMES.

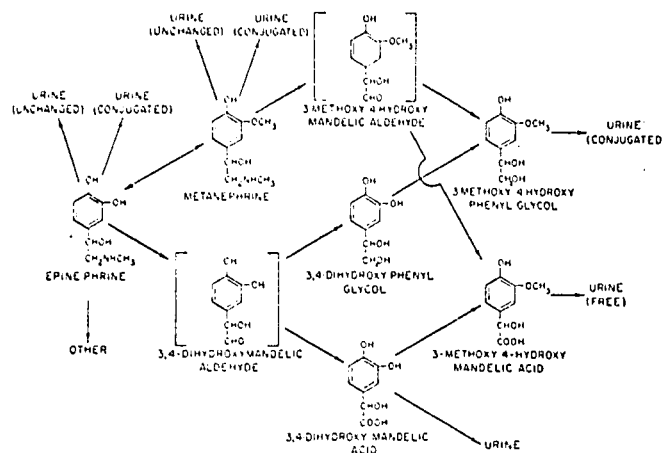


FIG. 1. Pathways for the metabolism of epinephrine

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THYROID HORMONE EFFECT MAY EITHER AFFECT COMT ACTIVITY OR MAO ACTIVITY OR BOTH OF THEM. ALTHOUGH THE USE OF INHIBITORS FOR MAO HAVE SHOWN A DECREASE IN THE FORMATION OF METABOLITES, THIS DECREASE HAS NOT BEEN CONCOMITANT WITH A PROLONGATION OR AN INCREASE IN THE RESPONSE OF TISSUES TO ADRENALINE OR NORADRENALINE (112, 113). ON THE OTHER HAND, THERE APPEARS TO BE A RELATIONSHIP BETWEEN COMT INHIBITION AND POTENTIATION OF ADRENALINE (111). IN VIVO, IT HAS BEEN SHOWN THAT ONE ENZYME MAY COMPENSATE FOR THE OTHER, AND THE LACK OF EFFECT DURING INHIBITION OF MAO MAY VERY

WELL BE ACCOUNTED FOR BY THIS SUPPLEMENTARY MECHANISM. ONE SHOULD ALSO TAKE INTO ACCOUNT THE FACT THAT THE INJECTION ROUTE OF CATECHOLAMINES IS VERY IMPORTANT. SINCE IN THE LIVER, MAO AND COMT ACTIVITIES HAVE BEEN SHOWN TO BE LARGE WHEN THE BLOOD IS GOING THROUGH THE LIVER BEFORE REACHING THE TARGET OF TISSUE IN WHICH ADRENALINE ACTION IS GOING TO BE MEASURED, PART OF THE EXOGENOUS CATECHOLAMINE WILL BE DESTROYED BEFORE IT HAD TIME TO REACH THE TISSUE (116).

SPINKS WAS THE FIRST TO SHOW IN 1952, THAT THERE WAS A SMALL DECREASE IN THE ACTIVITY OF AORTIC MONOAMINE OXIDASE AMONG THYROID-FED RABBITS. THE DECREASE, HOWEVER, WAS NOT STATISTICALLY SIGNIFICANT COMPARED TO THE AORTIC MONOAMINE OXIDASE ACTIVITY OF THE NORMAL CONTROLS (114). SPINKS AND BURN, IN THE SAME YEAR REPORTED THE EFFECT OF THYROID FEEDING ON THE LIVER MONOAMINE OXIDASE ACTIVITY OF RATS AND RABBITS. MONOAMINE OXIDASE ACTIVITY WAS DECREASED IN RABBIT LIVER BY THYROID FEEDING. IN THYROIDECTOMIZED RATS AND RABBITS, THE MONOAMINE OXIDASE ACTIVITY OF THE LIVER WAS INCREASED (115). TRENDELEBURG ALSO FOUND THAT THE LIVER FROM THYROID-FED RABBITS EXHIBITED A LOWER MONOAMINE OXIDASE ACTIVITY THAN THAT OF THE NORMAL RABBIT. THE DECREASE IN THE ACTIVITY EXPRESSED PER WEIGHT OF WET LIVER WAS SMALL BUT STATISTICALLY SIGNIFICANT (52). LEDUC ET AL., MEASURING THE CONCENTRATION OF ADRENALINE IN VARIOUS ORGANS, FOLLOWING ADRENALINE INJECTION, OBSERVED THAT HEARTS FROM IODINATED-CASEIN-FED

ANIMALS ACCUMULATED THE UNCHANGED AMINE. THEY CONCLUDED THAT THE METABOLISM OF THIS AMINE WAS IMPAIRED IN HYPERTHYROID ANIMALS (101).

USING THE SAME MANOMETRIC TECHNIQUE, AND THE SAME SUBSTRATE AS THOSE USED BY SPINKS AND BURN, WESTERMANN WAS UNABLE TO CONFIRM THEIR EXPERIMENTAL FINDINGS. THIS AUTHOR MEASURED THE MONOAMINE OXIDASE ACTIVITY IN THE LIVER OF MICE AND RATS PRETREATED FOR THREE TO FIVE DAYS WITH L-TRIODOOTHYRONINE. NO DIFFERENCE WAS FOUND IN THE LIVER ACTIVITY OF THE TRIODOOTHYRONINE-PRETREATED AND NORMAL ANIMALS (117). HOLTZ ET AL. CONFIRMED WESTERMANN'S RESULTS. THEY MEASURED THE MONOAMINE OXIDASE ACTIVITY IN THE LIVER OF RATS, GUINEA-PIGS AND RABBITS PRETREATED WITH TRIODOOTHYRONINE OR THYROXINE. IN FACT, THESE AUTHORS FOUND A SMALL BUT NOT SIGNIFICANT INCREASE IN THE ENZYMIC ACTIVITY OF THE LIVER FROM HYPERTHYROID ANIMALS (118). IN RATS WHICH HAD BEEN FED DESICATED THYROID FOR ONE MONTH, ZILE AND LARDY OBSERVED A SIGNIFICANT DECREASE IN LIVER MONOAMINE OXIDASE ACTIVITY. THYROXINE DID NOT HAVE A DIRECT INHIBITORY EFFECT ON THE ENZYME. THE LEVEL OF CIRCULATING ADRENALINE AND NORADRENALINE WAS ALSO FOUND TO BE INCREASED. NO DIFFERENCE IN THE LIVER MONOAMINE OXIDASE ACTIVITY WAS SEEN BETWEEN THYROIDECTOMIZED AND NORMAL RATS (119).

NOVICK STUDIED THE EFFECT OF BOTH D- AND L-TRIODOOTHYRONINE AND OF THYROID-FEEDING ON THE HEART MONOAMINE OXIDASE OF THE RATS AT VARIOUS AGES. D-TRIODOOTHYRONINE HAD NO EFFECT. IN YOUNG RATS, L-TRIODOOTHYRONINE WAS THE MOST EFFICIENT. A 204 % INCREASE IN THE

ENZYMIC ACTIVITY OF THE HEART WAS OBSERVED WITH THIS COMPOUND. IN THE ADULT GROUP, L-TRIODOOTHYRONINE AND THYROID-FEEDING HAD THE SAME EFFECT IN INCREASING THE HEART MONOAMINE OXIDASE ACTIVITY. NEITHER TREATMENT WAS FOUND TO HAVE AN EFFECT ON LIVER MONOAMINE OXIDASE ACTIVITY (120).

ZILE HAD PREVIOUSLY REPORTED THE RESULTS OBTAINED WHEN STUDYING THE EFFECT OF THYROXINE AND THYROID HORMONE ANALOGUES ON THE ACTIVITY OF MONOAMINE OXIDASE IN THE LIVER, HEART AND BRAIN OF RATS. THE SPECIFIC ACTIVITY OF LIVER MONOAMINE OXIDASE WAS DECREASED IN ANIMALS WHICH HAD BEEN PRETREATED WITH THYROXINE, TRIODOOTHYRONINE, TRIODOOTHYROACETIC ACID OR THYROXAMINE. IN THE HEART OR BRAIN, NO DIFFERENCE IN ENZYMIC ACTIVITY WAS SEEN BETWEEN TREATED AND NORMAL ANIMALS. THE AUTHOR POINTED OUT THAT THE METHOD USED WAS NOT VERY SENSITIVE, AND SMALL DIFFERENCES MAY NOT HAVE BEEN DETECTED (121).

SKILLEN ET AL. STUDIED THE EFFECTS OF THYROID IMPAIRMENT ON THE HEART MAO OF MALE AND FEMALE RATS. A SIGNIFICANTLY GREATER MAO ACTIVITY WAS FOUND IN THE HEART OF NORMAL FEMALES COMPARED TO NORMAL MALES. WHILE THYROID FEEDING INCREASED MAO ACTIVITY OF FEMALE HEART IT HAD NO EFFECT ON MALE HEART. ON THE OTHER HAND, PROPYLTHIOURACYL PRETREATMENT DECREASED THE MAO ACTIVITY OF MALE HEARTS BUT DID NOT AFFECT THE MAO ACTIVITY OF FEMALE HEARTS. THE AUTHORS CONCLUDED THAT THE EFFECT OF THYROID STATE ON HEART MAO ACTIVITY WAS DEPENDENT UPON THE SEX OF THE ANIMALS (122).

WURTMANN ET AL. TESTED THE LIVER MONOAMINE OXIDASE ACTIVITY IN MALE AND FEMALE RATS AS A FUNCTION OF THEIR THYROID STATE. THESE AUTHORS CONFIRMED SKILLEN'S RESULTS. HOWEVER, THEY FOUND THAT AFTER MASSIVE DOSES OF THYROXINE (500 UG/DAY, FOR 5 DAYS), THE LIVER MAO ACTIVITY WAS DECREASED IN THE FEMALE GROUP (102). MORE RECENTLY, BARRIEUX, PROULX AND D'ORIO CONFIRMED SPINKS' FINDINGS ON THE DECREASE IN LIVER MONOAMINE OXIDASE OF IODINATED-CASEIN-FED MALE RATS (123).

SINCE THE ROLE OF MONOAMINE OXIDASE IN THE BIOLOGICAL INACTIVATION OF CATECHOLAMINES APPEARS, AT LEAST IN MANY TARGET ORGANS, TO BE QUITE SECONDARY, IT SEEMS DIFFICULT TO CONNECT THE INFLUENCE OF THYROID HORMONES ON THIS ENZYME WITH THEIR POTENTIATION OF CATECHOLAMINE EFFECTS.

CATECHOL-O-METHYL TRANSFERASE APPEARS TO BE MORE IMPORTANT IN THE BIOLOGICAL DISPOSAL OF CATECHOLAMINES. THIS ENZYME HAS BEEN DISCOVERED ONLY RECENTLY (124, 127), BUT ALREADY SOME DATA ARE AVAILABLE ON THE EFFECT OF THYROID HORMONES ON ITS ACTIVITY. CATECHOL-O-METHYL TRANSFERASE HAS BEEN SHOWN RECENTLY TO BE THE ENZYME PRIMARILY RESPONSIBLE FOR THE DEGRADATION OF CATECHOLAMINES. ALTHOUGH METANEPHRINE HAS BEEN SHOWN TO POSSESS SOME ACTIVITY (125,126), ITS EFFICIENCY IS BY NO MEANS COMPARABLE TO THAT OF THE NON-METHYLATED ADRENALINE. CATECHOL-O-METHYL TRANSFERASE HAS BEEN FOUND IN ALL THE TISSUES STUDIED. BECAUSE ITS ACTION IS RAPID, IT SEEMS THAT THIS ENZYME MAY BE RESPONSIBLE FOR THE BIOLOGICAL INACTIVATION

OF CATECHOLAMINES.

BECAUSE OF THE CONFLICTING RESULTS CONCERNING THE EFFECTS OF THYROXINE ON MAO ACTIVITY, D'ICRIO ET AL., HAVING FOUND THAT ADRENALINE METABOLISM WAS DECREASED IN HYPERTHYROID RATS, DECIDED TO INVESTIGATE THE POSSIBLE INHIBITION OF COMT BY THYROID HORMONES (128). USING PROTOCATECHUIC ACID AS THE SUBSTRATE, THE AUTHORS MEASURED THE FORMATION OF ITS METHYLATED DERIVATIVE (VANILIC ACID) IN RAT LIVER HOMOGENATE. IN A SERIES OF EXPERIMENTS THYROXINE WAS ADDED DIRECTLY TO THE INCUBATION MEDIUM. IT WAS FOUND TO HAVE NO EFFECT ON THE ACTIVITY OF COMT. FOLLOWING THYROXINE INJECTION, THE LIVER COMT ACTIVITY WAS FOUND SIGNIFICANTLY DECREASED WHEN COMPARED TO THAT OF THE NORMAL LIVER. WHEN RADIOACTIVE ADRENALINE WAS INJECTED TO RATS, A GREATER AMOUNT OF EXCRETED UNCHANGED MATERIAL WAS FOUND IN THE THYROXINE TREATED GROUP. THE AMOUNT OF METANEPHRINE WAS ALSO SIGNIFICANTLY LOWER IN THAT GROUP. THE AUTHORS POINTED OUT THAT A LONG PRETREATMENT WITH THYROID HORMONES WAS REQUIRED TO OBTAIN A DECREASE IN ENZYME ACTIVITY. THE POTENTIATION OF CATECHOLAMINE ACTIONS BY THYROID HORMONES, ON THE CONTRARY, SEEMED TO BE IMMEDIATE. WURTMANN ET AL. REPEATED THE EXPERIMENTS OF D'ICRIO BUT MEASURED THE ACTIVITY OF CATECHOL-O-METHYL TRANSFERASE IN A DIFFERENT MANNER. COMT IS AN ENZYMIC SYSTEM REQUIRING THE PRESENCE OF S-ADENOSYL METHIONINE AS DONOR FOR THE METHYL GROUP. WURTMANN ET AL. MEASURED COMT ACTIVITY IN A MEDIUM SUPPLEMENTED WITH S-ADENOSYL METHIONINE (102). THEY DID NOT FIND ANY

CHANGE IN HEART COMT ACTIVITY IN THE HYPERTHYROID ANIMALS. THESE FINDINGS WERE CONFIRMED RECENTLY BY BARRIEUX, PROULX AND D'ORIO (123). THE SPECIFIC ACTIVITY OF LIVER COMT WAS DECREASED IN THE HYPERTHYROID RATS. HOWEVER, WHEN THE ACTIVITY OF THE ENZYME WAS EXPRESSED PER WHOLE HEART, NO CHANGE WAS SEEN. D'ORIO AND MAVRIDES WORKING WITH PARTIALLY PURIFIED ENZYMES FROM RAT LIVER STUDIED THE EFFECT, IN VITRO, OF VARIOUS THYROXINE ANALOGUES ON THE ACTIVITY OF COMT (129-131). THEY INVESTIGATED THE KINETICS OF THE ENZYME AND OBSERVED THAT SOME OF THE THYROXINE ANALOGUES WERE INHIBITORS OF THE ENZYME. THYROXINE, AT THE LOW CONCENTRATION USED IN THESE EXPERIMENTS, WAS WITHOUT EFFECT. TRIIODOTHYRONINE WAS FOUND TO BE A VERY POOR INHIBITOR OF THE ENZYME. THESE AUTHORS CONCLUDED THAT THE POTENTIATION, IN VIVO, OF THE PHYSIOLOGICAL ACTIONS OF CATECHOLAMINES BY THYROID HORMONES COULD NOT BE ACCOUNTED FOR BY THE INHIBITION OF CATECHOL-O-METHYL TRANSFERASE.

CONCLUSION OF PART II.

THERE IS GENERAL AGREEMENT THAT SOME OF THE PHYSIOLOGICAL EFFECTS OF CATECHOLAMINES ARE INCREASED IN HYPERTHYROID ANIMALS. THIS POTENTIATION OF CATECHOLAMINE ACTION IS NOT ALWAYS REVERSED IN HYPOTHYROID ANIMALS. THE BIOCHEMICAL ATTEMPTS TO SHOW THAT THIS POTENTIATION IS MEDIATED THROUGH THE INHIBITION OF THE TWO ENZYMES MAINLY RESPONSIBLE FOR THE BIOLOGICAL DEGRADATION OF CATECHOLAMINES HAVE FAILED TO GIVE CONCLUSIVE RESULTS. MONOAMINE

OXIDASE ACTIVITY IS DECREASED IN THE ORGANS OF HYPERTHYROID ANIMALS. THIS ENZYME, HOWEVER, HAS BEEN SHOWN NOT TO BE DIRECTLY RESPONSIBLE FOR THE RAPID DISAPPEARANCE OF THE PHYSIOLOGICAL EFFECTS OF CATECHOLAMINES. CATECHOL-O-METHYL TRANSFERASE, THE ENZYME APPARENTLY INVOLVED IN THE RAPID INACTIVATION OF CATECHOLAMINES IN VIVO, HAS BEEN FOUND UNCHANGED OR SLIGHTLY DECREASED IN THE LIVER AND HEART OF HYPERTHYROID ANIMALS. THYROID HORMONES HAVE NO DIRECT EFFECT ON THIS ENZYME IN VITRO. IT WOULD APPEAR THAT THYROID HORMONES POTENTIATE SOME OF THE EFFECTS OF CATECHOLAMINES THROUGH A DIFFERENT ROUTE : THE RAPID BINDING OF THE BIOGENIC AMINES TO TISSUE, RESULTING IN THE DISAPPEARANCE OF THE PHYSIOLOGICAL EFFECTS OF EXOGENOUS CATECHOLAMINES COULD BE AFFECTED DIRECTLY BY THYROID HORMONES. RECENT FINDINGS HAVE SHOWN THAT BINDING TO TISSUE IS PRIMARILY RESPONSIBLE FOR THE RAPID DISAPPEARANCE OF THE RESPONSE TO ADRENALINE.

FOR THESE REASONS, IN THE WORK TO BE PRESENTED HERE, AN ATTEMPT HAS BEEN MADE TO STUDY, IN THE WHOLE RAT, THE DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE ORGANS FOLLOWING A SINGLE INJECTION OF TRITIATED ADRENALINE OR NORADRENALINE. THIS STUDY WAS MADE AS A FUNCTION OF THE THYROID STATE OF THE RAT. THE EFFECT OF THYROXINE, BOTH IN VIVO AND IN VITRO, ON THE UPTAKE, BINDING AND O-METHYLATION OF TRITIATED ADRENALINE WAS INVESTIGATED IN STRIPS OF SMALL INTESTINE FROM RATS.

EXPERIMENTAL RESULTS

PART I. THE DISTRIBUTION OF RADIOACTIVE MATERIAL IN RATS IN VARIOUS THYROID STATES FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ^3H -ADRENALINE OR ^3H -NORADRENALINE.

I. MATERIALS AND METHODS

A) MATERIALS

ALL THE ANIMALS USED IN THESE EXPERIMENTS WERE ADULT, MALE, SPRAGUE-DAWLEY RATS.

L-THYROXINE SODIUM SALT, FROM SIGMA CHEMICALS, WAS USED FOR INDUCING HYPERTHYROIDISM. IT WAS DISSOLVED IN 50 % PROPYLENE GLYCOL AT A FINAL CONCENTRATION OF 7.19 Ml.

ETHYL ETHER, ANHYDROUS, FROM FISHER, WAS THE ANESTHETIC UTILIZED DURING THYROIDECTOMY OF THE RATS.

NEBUTAL WAS PURCHASED FROM ABBOT LAB., MONTRÉAL, IN STERILE SOLUTION OF 50 MG PER ML. IT WAS USED PRIOR TO THE MEASUREMENT OF OXYGEN CONSUMPTION, OR PRIOR TO THE INTRAVENOUS INJECTION OF RADIOACTIVE CATECHOLAMINE.

DL-NORADRENALINE-7- ^3H HYDROCHLORIDE AND DL-ADRENALINE-7- ^3H HYDROCHLORIDE WERE OBTAINED FROM NEW ENGLAND NUCLEAR CORP., BOSTON,

MASS.. THE COMPOUNDS WERE IN 0.1 N ACETIC ACID, IN AMPOULES SEALED UNDER NITROGEN. THE SPECIFIC ACTIVITY AVERAGED 9.55 CURIES PER MM FOR THE FORMER AMINE, AND 9.55 CURIES PER MM OR 6.1 CURIES PER MM FOR THE LATTER.

PPQ (2,5-DIPHENYLOXAZOLE) AND DIMETHYL-POPAP (1,4-BIS-2-(4-METHYL-5-PHENYLOXAZOLYL)-BENZENE), SCINTILLATION GRADE, FROM PACKARD INSTRUMENT COMPANY, INC., WERE USED TO PREPARE THE LIQUID FOR SCINTILLATION COUNTING.

THE METABOLISM APPARATUS, FROM PHIPPS & BIRD, INC. RICHMOND, VA., MODEL #71-2241, WAS USED FOR MEASUREMENT OF THE OXYGEN CONSUMPTION.

THE COUNTING OF RADIOACTIVITY WAS CARRIED OUT IN A LIQUID SCINTILLATION COUNTER, NUCLEAR CHICAGO MODEL 703, AT 4°C.

B) METHODS

IN ALL THE EXPERIMENTS THE ANIMALS WEIGHED BETWEEN 190 AND 290 GRAMS.

1) PREPARATION OF ANIMALS :

GROUP A. - THIS GROUP WAS SUBDIVIDED INTO SUBGROUPS. THEY CONSISTED OF RATS WEIGHING 120-130 GRAMS AT THE BEGINNING OF THE EXPERIMENTS. IN THE FIRST SUBGROUP, THE RATS WERE CONSIDERED AS NORMAL CONTROL ANIMALS AND KEPT FOR THREE WEEKS ON A NORMAL DIET AND WATER AD LIBITUM. THE RATS FROM THE SECOND SUBGROUP WERE MADE ATHYROID BY SURGICAL REMOVAL OF THE THYROID GLAND, WHILE UNDER ETHER ANESTHESIA.

THESE ANIMALS WERE THEN KEPT FOR THREE WEEKS ON NORMAL DIET AND WATER AD LIBITUM. AT THE END OF THREE WEEKS, THE WEIGHT AND BASAL METABOLISM OF THE CONTROL AND ATHYROID RATS WERE DETERMINED AND SERVED AS A MEASURE OF THYROID FUNCTION IMPAIRMENT.

GROUP B. - IN THIS GROUP, THE RATS WEIGHED AROUND 200 GRAMS AT THE BEGINNING OF THE EXPERIMENTS. AFTER THEIR WEIGHT AND OXYGEN CONSUMPTION HAD BEEN DETERMINED, THE RATS WERE INJECTED SUBCUTANEOUSLY, DAILY FOR FIVE DAYS, WITH L-THYROXINE : 0.119 μ M IN 0.100 ML OF 50 % PROPYLENE GLYCOL. ON THE SIXTH DAY, THE WEIGHT AND OXYGEN CONSUMPTION WERE RECORDED AGAIN.

2) MEASUREMENT OF OXYGEN CONSUMPTION :

ALL THE RATS WERE FASTED OVERNIGHT, BEFORE OXYGEN CONSUMPTION WAS MEASURED. THE DETERMINATION OF BASAL METABOLISM WAS CARRIED OUT ON ONE RAT AT A TIME. THE METABOLIC APPARATUS WAS PARTIALLY FILLED WITH SODA LIME TO ABSORB MOISTURE AND CARBON DIOXIDE. THE RAT WAS THEN WEIGHED AND INJECTED INTRAPERITONEALLY WITH NEMBUTAL AT A DOSE OF 20 MG PER KG OF BODY WEIGHT. TWENTY MINUTES LATER, THE RAT WAS PLACED IN THE CHAMBER AND OXYGEN WAS SUBBLED INTO THE APPARATUS FOR FIVE MINUTES. FIVE MINUTE EQUILIBRATION WAS ALLOWED. AT THE END OF THIS PERIOD A CALIBRATED TUBE CONTAINING ONE DROP OF SOAP SOLUTION WAS INSERTED IN THE AIR OUTLET OF THE METABOLIC APPARATUS. THE RATE AT WHICH THE SOAP BUBBLE WAS DISPLACED IN A 5 ML VOLUME WAS MEASURED. AT LEAST FIVE SUCCESSIVE MEASUREMENTS WERE MADE ON EACH ANIMAL. THE AVERAGE OF THE FIVE VALUES WAS TAKEN AS THE TIME

NECESSARY FOR THE RAT TO BURN 5 ML OF OXYGEN. THIS WAS CONVERTED TO THE VOLUME OF OXYGEN CONSUMED IN ONE MINUTE. TAKING INTO ACCOUNT THE WEIGHT OF THE ANIMAL, THE OXYGEN CONSUMPTION PER MINUTE PER KILOGRAM OF BODY WEIGHT WAS CALCULATED. CORRECTIONS WERE MADE TO BRING ALL THE RESULTS TO A TEMPERATURE OF 30°C IN THE CHAMBER.

3) INTRAVENOUS INJECTION OF ³H-DL-NORADRENALINE OR

³H-CL-ADRENALINE :

THE RATS WERE STARVED OVERNIGHT, BUT RECEIVED WATER AD LIBITUM. AFTER THE WEIGHT OF THE ANIMAL HAD BEEN RECORDED, AN INTRAPERITONEAL INJECTION OF NEMBUTAL (20 MG PER KG OF BODY WEIGHT) WAS GIVEN. THIRTY MINUTES LATER, 0.100 ML OF TRITIATED NORADRENALINE OR ADRENALINE WAS INJECTED INTO THE TAIL VEIN, USING A 1.0 ML INSULIN SYRINGE. THIS INJECTION REQUIRED ABOUT TWO MINUTES. AFTER COMPLETION OF THE INJECTION THE SPINAL CORD WAS CUT. THE TIME INTERVAL BETWEEN INJECTION AND SACRIFICE OF THE RAT WAS VARIED WITH EACH SERIES OF EXPERIMENTS.

4) PREPARATION OF TISSUE SAMPLES :

IMMEDIATELY AFTER SEVERING THE SPINAL CORD, BLOOD WAS DRAWN FROM THE ABDOMINAL AORTA AND THE VOLUME MEASURED IN A GRADUATED CYLINDER. IT WAS THEN POURED INTO A COLD LUSTEROID CENTRIFUGE TUBE AND PLACED IN ICE. THE TISSUES WERE REMOVED IN THE FOLLOWING ORDER : KIDNEYS, LIVER, SPLEEN, HEART AND A SAMPLE OF SKELETAL MUSCLE FROM THE RIGHT HIND LEG. IMMEDIATELY AFTER REMOVAL, EACH ORGAN WAS PLACED IN ICE-COLD ISOTONIC SODIUM CHLORIDE. WHEN ALL THE ORGANS HAD BEEN

REMOVED, EACH ORGAN IN TURN WAS BLOTTED ON FILTER PAPER, CLEANED AND WEIGHED ON A TORSION BALANCE. THE ORGAN WAS THEN PUT INTO ICE-COLD 0.1N HQ, TO GIVE A FINAL CONCENTRATION OF 1/4.

5) HOMOGENIZATION AND CENTRIFUGATION :

ALL THE SUBSEQUENT OPERATIONS WERE PERFORMED AT 4°C. THE TISSUE, AFTER SOAKING FOR ABOUT 30 MINUTES IN COLD HQ WAS TRANSFERRED INTO A CONICAL ALL GLASS HOMOGENIZER, AND HOMOGENIZED. AFTER THE VOLUME OF THE TOTAL HOMOGENATE HAD BEEN RECORDED THE SAMPLE WAS PLACED IN A LUSTEROID CENTRIFUGATION TUBE IN ICE. WHEN ALL THE TISSUES HAD BEEN HOMOGENIZED AND PLACED IN THE CENTRIFUGE TUBES, THEY WERE CENTRIFUGED IN A SERVALL REFRIGERATED CENTRIFUGE (MODEL SS-3, ROTOR SM-24) FOR FORTY MINUTES AT 12,350 G. THE VOLUME OF THE SUPERNATANT WAS MEASURED. THE PELLETT WAS DISCARDED. FROM THEN ON, ALL THE SAMPLES WERE RUN IN DUPLICATE.

6) DETERMINATION OF THE RADIOACTIVITY CONTENT :

THE SCINTILLATION LIQUID WAS PREPARED ACCORDING TO KIRSHNER ET AL. (127). IT CONTAINED 30 % ABSOLUTE ETHANOL, 0.02 % DIMETHYL-POPOP AND 0.5 % PPO IN TOLUENE. THIS SOLVENT CAN HOLD 2 % WATER IN SOLUTION. FIFTY µL OF SUPERNATANT FROM EACH SUPERNATANT WERE PLACED IN EACH OF TWO LIQUID SCINTILLATION VIALS CONTAINING 10 ML OF SCINTILLATION LIQUID. THE VIALS WERE PUT INTO THE COUNTER REFRIGERATOR TEN MINUTES BEFORE BEING COUNTED IN ORDER TO PERMIT EQUILIBRATION AT 4°C. THE RADIOACTIVITY CONTENT WAS DETERMINED FOR A FIXED PERIOD OF TIME WHICH WAS PRESET ACCORDING TO THE ACTIVITY OF THE

SAMPLE. THE PRESET TIME NEVER EXCEEDED THIRTY MINUTES. THE HIGH VOLTAGE OF THE COUNTER WAS SET AT 1275 VOLTS FOR THE GATE AND 1250 VOLTS FOR THE DATA. THESE VALUES HAD BEEN DETERMINED BY A STANDARD CURVE, USING TRITIATED TOLUENE AS RADIOACTIVITY SOURCE AND PPC-POPOP IN PURE TOLUENE AS THE SOLVENT.

7) EFFICIENCY OF THE COUNTER :

THE EFFICIENCY OF THE COUNTER WAS MEASURED USING THE INTERNAL STANDARD METHOD. AFTER THE VIALS CONTAINING THE UNKNOWN HAD BEEN COUNTED, A KNOWN VOLUME OF STANDARD ^3H -TOLUENE WAS ADDED INTO EACH VIAL. THE RADIOACTIVITY OF THE VIAL WAS COUNTED AGAIN. FROM THIS SECOND READING, THE READING OBTAINED IN THE FIRST DETERMINATION WAS SUBTRACTED. THE RESIDUAL COUNTS PER MINUTES CORRESPONDED TO THE AMOUNT OF RADIOACTIVITY DETECTED DUE TO STANDARD ^3H -TOLUENE. THE RATIO OF COUNTS PER MINUTE DETECTED BY THE COUNTER OVER THE ACTUAL DESINTEGRATIONS PER MINUTE OF THE STANDARD SAMPLE GAVE THE EFFICIENCY OF THE COUNTER. UNDER THESE EXPERIMENTAL CONDITIONS THE EFFICIENCY OF THE APPARATUS WAS QUITE STABLE AND AVERAGE 7 %.

B) CALCULATION OF RESULTS :

SINCE THE EFFICIENCY OF THE COUNTER FOR BOTH THE ADRENALINE OR NORADRENALINE STOCK SOLUTION AND UNKNOWN WAS IDENTICAL, NO CORRECTION WAS MADE TO CALCULATE THE RESULTS IN ABSOLUTE VALUES. EACH TIME A NEW BATCH OF ^3H -CATECHOLAMINE WAS OBTAINED, THE SPECIFIC ACTIVITY WAS GIVEN IN CURIES PER MG. IN ORDER TO FIND THE EQUIVALENT OF THE COUNTS/MIN DETECTED IN THE COUNTER TO THE CURIE THE STOCK SOLUTION WAS

TESTED. A 10 μ L ALIQUOT OF THIS SOLUTION WAS DILUTED TILL 10 ML WITH 0.01N ACETIC ACID, GIVING SOLUTION A. TEN μ L OF SOLUTION A WERE PLACED IN EACH OF FOUR SCINTILLATION VIALS CONTAINING 10 ML OF SCINTILLATION LIQUID. THE RADIOACTIVITY CONTENT WAS THEN MEASURED UNDER THE CONDITIONS PREVIOUSLY DESCRIBED. THE FOLLOWING EQUATION PERMITTED TO CALCULATE THE EQUIVALENT OF NM OF EITHER AMINE :

$$\frac{(C/MIN / 10\mu L \text{ OF SOLUTION A}) \times 10,000 \times 0.100}{10} = C/MIN / 11 \text{ NM}$$

$$\frac{C/MIN}{11 \text{ NM}} = C/MIN / \text{NM} \quad \text{CORRECTING FACTOR TO}$$

TRANSFORM THE NUMBER OF COUNTS PER MINUTE OBTAINED IN OUR SUPERNATANT INTO NM (NANOLES) OF EITHER ^3H -ADRENALINE OR ^3H -NORADRENALINE.

ALTHOUGH THE CATECHOLAMINE WAS NOT IDENTIFY, THE RESULTS WERE EXPRESSED IN NM OF UNCHARGED INJECTED AMINE. FOR EACH SAMPLE, THE TWO READINGS OF THE TWO DUPLICATE VIALS WERE AVERAGED TO YIELD THE NUMBER OF COUNTS PER MINUTE PER ALIQUOT. THIS NUMBER WAS THEN DIVIDED BY THE VOLUME OF THE ALIQUOT AND MULTIPLIED BY THE TOTAL VOLUME OF THE SUPERNATANT. THIS FINAL RESULT WAS TAKEN AS THE EXPRESSION OF THE RADIOACTIVITY CONTENT OF THE WHOLE ORGAN. THE COUNTS PER MINUTE PER ORGAN WERE DIVIDED BY THE WEIGHT OF THE ORGAN TO GIVE THE NUMBER OF COUNTS PER MINUTE PER GRAM OF WET TISSUE. THE CORRECTING FACTOR FOR COUNT PER MINUTE TO NM WAS THEN APPLIED.

DISCUSSION

THE APPARATUS USED FOR THE MEASUREMENT OF OXYGEN CONSUMPTION

PRESENTED SOME DISADVANTAGES. BECAUSE OF THE DIAMETER OF THE CHAMBER, THE SIZE OF THE ANIMAL COULD NOT EXCEED 300 GRAMS, OTHERWISE THE RAT SUFFOCATED. IF THE RUBBER STOPPER WAS NOT WELL SEALED, LEAKAGE OF AIR CAUSED ERROR IN THE MEASUREMENT OF GAS CONSUMPTION. OCCASIONALLY THE SODA LIME DRIED THE TUBE WHERE THE SOAP BUBBLE WAS TRAVELING, AND THE BUBBLE WOULD BREAK. BECAUSE OF THIS, ALL RATS WERE NOT IN THE CHAMBER FOR THE SAME PERIOD OF TIME. THE USE OF AN ANESTHETIC COULD NOT BE AVOIDED. WHEN THE RAT WAS NOT UNDER ANESTHESIA, IT MOVED AND THE READINGS WERE ERRATIC. THE SENSITIVITY OF THE ANIMALS TO NEMBUTAL DIFFERED. IT WAS FOUND THAT ATHYROID ANIMALS WERE MORE DEEPLY ANESTHETIZED THAN EITHER OF THE OTHER ONES. THE LETHAL DOSE AND EFFICIENT DOSE RANGE APPEARED TO BE LOWERED AMONG THE THYROXINE-TREATED GROUP.

WHEN, HOWEVER, ONE WAS AWARE OF THE DISADVANTAGES, THE METABOLIC APPARATUS WAS VERY CONVENIENT FOR ROUTINE MEASUREMENTS. THE RESULTS OBTAINED WERE REPRODUCIBLE AND THE MANIPULATIONS WERE MINIMAL.

THE USE OF TRITIATED CATECHOLAMINES WAS PREFERRED TO THAT OF THE CORRESPONDING ¹⁴C CARBON-LABELED MATERIAL, BECAUSE COMPOUNDS WITH HIGH SPECIFIC ACTIVITY WERE AVAILABLE. IT WAS THUS POSSIBLE TO WORK WITH PHYSIOLOGICAL DOSES OF CATECHOLAMINES AND STILL POSSIBLE TO DETECT CONSIDERABLE AMOUNT OF RADIOACTIVITY IN SMALL ALIQUOTS. THE USE OF TRITIUM PRESENTED SOME DRAWBACKS. FIRST OF ALL, THE EFFICIENCY OF THE LIQUID SCINTILLATION COUNTER IS LOW FOR TRITIUM.

LONGER PRESET TIME IS REQUIRED TO COUNT ANY SAMPLE. BECAUSE THE EFFICIENCY IS LOWER, THE SENSITIVITY OF THE INSTRUMENT IS DECREASED, SMALL DIFFERENCES ARE NOT APPARENT. SECONDLY, WHEN HIGH CONCENTRATIONS OF RADIOACTIVITY ARE PRESENT IN THE SCINTILLATION VIALS, IT HAS BEEN OBSERVED THAT THE RADIOACTIVITY DETERMINATIONS APPEAR TO DECREASE WITH TIME (133). PART OF THIS ARTEFACT SEEMS TO BE DUE TO QUENCHING OF THE SCINTILLATOR BY THE OXYGEN OF THE AIR (134). BECAUSE TRITIUM EMITS WEAK BETA PARTICLES, INTERNAL QUENCHING MAY ALSO BE PARTLY RESPONSIBLE FOR THIS. FINALLY, IT WOULD APPEAR THAT SOME OF THE RADIOACTIVITY IS ABSORBED ON THE SIDES OF THE VIALS (133). FOR ALL THESE REASONS, THE USE OF TRITIUM LEADS TO MORE VARIABLE RESULTS THAN THAT OF ¹⁴CARBON. HOWEVER, THE ADVANTAGE OF USING TRITIUM-LABELED CATECHOLAMINES IS THE HIGH SPECIFIC ACTIVITY.

AS PREVIOUSLY STATED, IT WAS FOUND THAT NEMBUTAL AS AN ANESTHETIC HAD DISADVANTAGES. IT WAS FOUND THAT THE EFFICIENT ANESTHETIC DOSE AND THE LETHAL DOSE WERE VERY CLOSE. THIS WAS A PROBLEM WITH LARGER RATS BUT NEMBUTAL WAS FOUND TO BE VERY EFFICIENT IN RATS UNDER 200 GRAMS. THE DEGREE OF ANESTHESIA WAS ALSO AFFECTED BY THE THYROID STATE OF THE ANIMALS (135). IT WAS FOUND THAT ETHER ANESTHESIA WAS NOT OF A LONG ENOUGH DURATION FOR SOME EXPERIMENTS, THEREFORE IT WAS NECESSARY TO USE NEMBUTAL IN THESE CASES. HOWEVER, FOR SHORT EXPERIMENTS, ETHER WAS USED.

FOR THE INTRAVENOUS INJECTION OF CATECHOLAMINES, A TIGHT RUBBER BAND WAS PLACED ON THE TAIL OF THE ANIMAL TO CAUSE A SWELLING.

OF THE VEIN. SOME OF THE ANIMALS, ALTHOUGH THEY WERE UNDER ANESTHESIA WERE FOUND TO REACT VIOLENTLY TO SUCH A TREATMENT.

THE USE OF ALCOHOL IN THE LIQUID FOR SCINTILLATION COUNTING, ACCORDING TO KIRSHNER ET AL. (132), WAS FOUND CONVENIENT FOR THE TYPE OF EXPERIMENTS DESCRIBED HERE. THE PRESENCE OF ALCOHOL PRODUCES A CERTAIN QUENCHING OF THE SAMPLE (136). HOWEVER, SINCE THE QUENCHING THUS CAUSED WAS HIGH, ADDING WATER DID NOT CAUSE FURTHER QUENCHING. THUS UNDER THE EXPERIMENTAL CONDITIONS, DOUBLING THE QUANTITY OF WATER OR ADDING ACID TO THE LIQUID DID NOT CAUSE A DIFFERENCE IN THE EFFICIENCY AS MEASURED BY INTERNAL STANDARD.

THE CHANNEL RATIO METHOD TO DETERMINE THE QUENCHING OF THE SAMPLES WAS NOT APPLICABLE, WHERE LOW RADIOACTIVITY WAS MEASURED. THE SENSITIVITY OF THE COUNTER VARIED WITH TIME AND FOR SOME UNKNOWN REASON THE RATIO WAS FOUND INVERSED. ON THE OTHER HAND, THE INTERNAL STANDARD METHOD YIELDED REPRODUCIBLE RESULTS UNDER THE EXPERIMENTAL CONDITIONS USED.

AS THE WORK PROGRESSED IT WAS FOUND THAT THE EXTRACTION WITH 0.1 N HCL WAS NOT AS EFFICIENT AS EXTRACTION WITH 0.4N PERCHLORIC ACID. THE FORMER WAS THEREFORE DISCONTINUED AND IN THE LAST SERIES OF EXPERIMENTS 0.4 N PERCHLORIC ACID WAS EMPLOYED INSTEAD.

II. RESULTS

A) THE EFFECT OF THYROID HORMONES ON THE TOTAL BODY WEIGHT AND OXYGEN CONSUMPTION OF THE RAT :

IN TABLE I, ARE GIVEN THE TOTAL BODY WEIGHTS AND OXYGEN CONSUMPTION IN VARIOUS THYROID STATES. THERE IS A SIGNIFICANT DECREASE IN THE OXYGEN CONSUMPTION OF THYROIDECTOMIZED RATS COMPARED TO UNTREATED CONTROLS. THE DECREASE IN OXYGEN CONSUMPTION IS QUITE REPRODUCIBLE AS SHOWN BY THE VALUE OF THE STANDARD DEVIATION. TREATMENT WITH L-THYROXINE INCREASED SIGNIFICANTLY THE OXYGEN CONSUMPTION OF THE RATS, AS CAN BE SEEN BY COMPARING THE VALUE BEFORE THYROXINE TREATMENT WITH THAT AFTER THYROXINE TREATMENT. A PAIRED COMPARISON WAS MADE IN THIS CASE SINCE EACH RAT WAS ITS OWN CONTROL.

THERE IS A SLIGHTLY LOWER BODY WEIGHT AMONG THE THYROIDECTOMIZED ANIMALS COMPARED TO THAT OF THE NORMAL ONES. THESE RATS GAINED LESS WEIGHT THAN THE NORMAL CONTROLS. IN THE THYROXINE-TREATED RATS, THERE IS AN ABSOLUTE DECREASE IN THE TOTAL BODY WEIGHT. THE DECREASE IN TOTAL BODY WEIGHT IS HIGHER THAN IT APPEARS HERE, SINCE NORMAL CONTROLS WOULD HAVE GAINED SOME WEIGHT DURING THE FIVE DAY DELAY.

TABLE I
 THE EFFECT OF THYROID STATE
 ON THE TOTAL BODY WEIGHT AND ON THE OXYGEN CONSUMPTION OF THE RAT

THYROID STATE	NUMBER OF RATS	TOTAL BODY WEIGHT GRAM	OXYGEN CONSUMPTION ML O ₂ /HR/100 G B.W.
EUTHYROID	9	247 ± 36.4	18.9 ± 3.19
THYROIDECTOMIZED	7	215 ± 24.6	8.9 ± 2.46
% CHANGE		- 12.9 %	- 52.9 %
THYROXINE TREATED			
BEFORE THYROXINE	4	222 ± 20.0	15.9 ± 0.93
AFTER THYROXINE	4	208 ± 13.0	24.9 ± 5.0
% CHANGE		- 6.31 %	+ 56.6 %

THE RESULTS ARE EXPRESSED AS THE MEAN ± S.D.

B) THE DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE RAT,
FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ³H-NORADRENALINE :

IN TABLE II, ARE PRESENTED THE RESULTS OBTAINED WITH RATS IN VARIOUS THYROID STATES GIVEN A SINGLE INTRAVENOUS INJECTION OF ³H-NORADRENALINE. THE AMOUNT OF RADIOACTIVE MATERIAL FOUND IN THE ORGANS IS EXPRESSED IN COUNTS/MINUTE PER GRAM OF WET TISSUE. THE AMOUNT OF ³H-NORADRENALINE INJECTED WAS 11 NM PER RAT; THE TIME ELAPSED BETWEEN COMPLETION OF THE INJECTION AND SACRIFICE OF THE ANIMAL WAS 10 MINUTES. EACH FIGURE REPRESENTS THE DATA OBTAINED WITH A SINGLE ANIMAL. IT CAN BE SEEN THAT INDIVIDUAL RESULTS ARE EXTREMELY SCATTERED.

IN TABLE III, THE RESULTS SHOWN IN TABLE II ARE EXPRESSED IN TERMS OF NM OF ³H-NORADRENALINE PER GRAM OF WET TISSUE. ALTHOUGH THE RADIOACTIVE MATERIAL WAS NOT ACTUALLY IDENTIFIED THIS SEEMED TO BE A CONVENIENT AND REPRESENTATIVE WAY OF EXPRESSING THE RESULTS. IN THIS TABLE III, THE FIGURES ARE MEAN ± STANDARD ERROR. BECAUSE OF THE HIGH VALUES IN THE STANDARD ERROR, NO STATISTICAL ANALYSIS OF THE RESULTS IS POSSIBLE. IT SEEMS, HOWEVER, THAT BOTH THYROXINE-TREATED AND NORMAL RATS CONCENTRATE ³H-NORADRENALINE IN ABOUT THE SAME QUANTITY. IN THE THYROIDECTOMIZED RAT, THE AMOUNT OF RADIOACTIVE MATERIAL FOUND IN ALL ORGANS EXCEPT SKELETAL MUSCLE, IS HIGHER THAN THAT FOUND IN BOTH THE NORMAL AND THYROXINE-TREATED ANIMALS. THE WEIGHT OF THE ORGANS IN THE THYROIDECTOMIZED RAT WAS LOWER THAN THAT IN THE NORMAL OR THYROXINE-TREATED ONE. THE AMOUNT OF RADIOACTIVE MATERIAL IN THE BLOOD OF THYROIDECTOMIZED RATS IS LOWER, THAN IN THE BLOOD OF THYROXINE-TREATED RATS HIGHER, THAN THAT IN THE BLOOD FROM THE NORMAL CONTROLS.

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DISTRIBUTION OF RADIOACTIVE MATERIAL IN RAT ORGANS FOLLOWING A SINGLE INTRAVENOUS INJECTION

OF ³H-DL-NORADRENALINE HYDROCHLORIDE

THYROID STATE	RAT #	BLOOD	HEART	KIDNEY	LIVER	SK. MUSCLE	SPLEEN
EUTHYROID	1	29,500	276,400	230,900	201,000	11,200	120,500
	2	15,800	62,400	59,400	39,900	2,800	16,900
	3	-	50,500	50,600	25,600	2,200	24,700
	4	4,400	110,100	62,000	62,200	6,300	4,800
THYROIDECTOMIZED	1	3,500	38,800	39,900	26,300	1,200	19,400
	2	21,600	459,600	330,400	192,600	28,400	115,200
	3	10,600	378,700	289,700	224,100	19,600	93,800
THYROXINE TREATED	1	36,600	272,300	169,700	182,200	22,700	85,300
	2	15,700	258,300	211,200	198,800	25,900	117,700
	3	7,700	24,000	36,100	33,000	0	16,200

³H-NORADRENALINE INJECTED PER RAT : 6,860,000 COUNTS PER MINUTE.

TABLE III

DISTRIBUTION OF RADIOACTIVE MATERIAL IN RAT ORGANS FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ³H-DL-NORADRENALINE HYDROCHLORIDE

THYROID STATE	NUMBER OF RATS	BANCHOLES OF ³ H-NORADRENALINE PER G OF NET TISSUE					
		BLOOD	HEART	KIDNEY	LIVER	SK. MUSCLE	SPLEEN
EUTHYROID	4	0.026 ± 0.012	0.200 ± 0.085	0.161 ± 0.071	0.131 ± 0.066	0.087 ± 0.0033	0.067 ± 0.043
THYROIDECTOMIZED	3	0.018 ± 0.009	0.469 ± 0.212	0.352 ± 0.149	0.236 ± 0.100	0.026 ± 0.012	0.122 ± 0.046
THYROXINE TREATED	3	0.032 ± 0.014	0.296 ± 0.132	0.222 ± 0.086	0.220 ± 0.125	-	0.116 ± 0.049

THE VALUES ARE THE MEAN ± THE STANDARD ERROR OF THE MEAN.

C) THE DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE RAT,
FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ³H-ADRENALINE :

IN TABLE IV, THE DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE NORMAL AND THYROIDECTOMIZED RAT IS SHOWN AS A FUNCTION OF TIME. THE RESULTS, AS MENTIONED PREVIOUSLY FOR ³H-NORADRENALINE, ARE EXPRESSED AS NM OF ³H-ADRENALINE PER GRAM OF WET TISSUE, ALTHOUGH NO ATTEMPT WAS MADE TO IDENTIFY THE RADIOACTIVE MATERIAL. THE VALUES SHOWN ARE MEAN ± STANDARD ERROR, FOR THE NORMAL ANIMAL. FOR THE THYROIDECTOMIZED RATS, AS THE NUMBER OF EXPERIMENTS WAS VERY LOW, INDIVIDUAL VALUES ARE GIVEN.

IN THE NORMAL RAT, THERE IS A DECREASE IN THE CONCENTRATION OF RADIOACTIVE MATERIAL IN BLOOD, HEART, KIDNEY AND SPLEEN AS A FUNCTION OF TIME. THERE IS AN INCREASE IN THE LIVER AND SKELETAL MUSCLE CONCENTRATION AS A FUNCTION OF TIME. AS ALREADY MENTIONED FOR THE EXPERIMENTS WITH ³H-NORADRENALINE, THE HIGH VALUES OF THE STANDARD ERROR, DID NOT PERMIT ANY STATISTICAL ANALYSIS.

IN THYROIDECTOMIZED RATS, THERE IS A LARGER AMOUNT OF RADIOACTIVITY IN ALL ORGANS THAN THERE IS AT THE SAME TIME IN THE NORMAL CONTROL. AFTER SIXTY MINUTES, THE DIFFERENCES ARE TOO HIGH FOR ANY INTERPRETATION OF THE RESULTS.

TEN MINUTES AFTER THE SINGLE INTRAVENOUS INJECTION OF ³H-ADRENALINE, THE AMOUNT OF RADIOACTIVITY IN THE ORGANS OF THYROIDECTOMIZED OR NORMAL RATS WAS SIMILAR TO THAT AMOUNT FOUND IN THE NORMAL AND THYROIDECTOMIZED ANIMAL AFTER A SINGLE INJECTION OF ³H-NORADRENALINE.

TABLE IV

DISTRIBUTION OF RADIOACTIVE MATERIAL IN RAT ORGANS FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ³H-DL-ADRENALINE HYDROCHLORIDE

THYROID STATE	NUMBER OF RATS	NANOGRAMS OF ³ H-ADRENALINE PER GRAM OF WET TISSUE					
		BLOOD	HEART	KIDNEY	LIVER	SK. MUSCLE	SPLEEN
EUTHYROID *	5	0.012 ± 0.0037	0.222 ± 0.125	0.125 ± 0.055	0.125 ± 0.075	0.012 ± 0.0070	0.074 ± 0.042
THYROIDECTOMIZED * THYROIDECTOMIZED *	2	0.059 0.023	0.487 0.967	0.519 0.555	0.318 0.413	0.018 0.022	0.173 0.518
EUTHYROID **	3	0.0020 ± 0.0014	0.112 ± 0.086	0.045 ± 0.029	0.061 ± 0.052	0.0053 ± 0.0014	0.043 ± 0.030
EUTHYROID ***	8	0.011 ± 0.0024	0.051 ± 0.040	0.052 ± 0.015	0.179 ± 0.056	0.0076 ± 0.0027	0.017 ± 0.0034
THYROIDECTOMIZED*** THYROIDECTOMIZED***	2	0.023 0.004	0.065 0.026	0.153 0.011	0.314 0.037	0.025 0.002	0.054 0.009

THE RESULTS ARE THE MEAN ± S.E., EXCEPT FOR THOSE EXPERIMENTS WHERE ONLY TWO RATS WERE USED, THE INDIVIDUAL VALUES ARE GIVEN IN THE LATTER CASE.

* RATS KILLED 10 MIN AFTER THE INJECTION ** 15 MIN AFTER THE INJECTION *** 60 MIN AFTER THE INJECTION.

III. DISCUSSION

THE INCREASE IN BASAL METABOLISM AMONG HYPERTHYROID ANIMALS HAS BEEN OBSERVED FOR A LONG TIME (1). THE DECREASE IN OXYGEN CONSUMPTION IN HYPOTHYROIDISM HAS BEEN FOUND SIMILARLY IN MAN AS WELL AS IN OTHER SPECIES. THESE VARIATIONS OF BASAL METABOLISM AS A FUNCTION OF THE THYROID STATE HAVE BEEN TAKEN ADVANTAGE OF, AND FOR THIS REASON THIS HAS BEEN CHOSEN AS THE INDEX OF THYROID GLAND IMPAIRMENT, IN THIS SERIES OF EXPERIMENTS. WHETHER THIS CHANGE IS BROUGHT ABOUT BY THYROID HORMONE DIRECT ACTION, OR INDIRECTLY THROUGH THE SYMPATHETIC SYSTEM DOES NOT PREVENT THIS TEST TO BE OF VALUE. IT CAN BE SEEN IN THE THYROIDECTOMIZED RATS, THAT A SIGNIFICANT DECREASE IN OXYGEN CONSUMPTION IS OBSERVED THREE WEEKS AFTER SURGICAL REMOVAL OF THE THYROID GLAND. THE DECREASE IN BASAL METABOLISM CANNOT GO BEYOND A CERTAIN LIMIT, SO THAT THE TEST IS NOT A MEASURE OF ATHYROIDISM, BUT RATHER A MEASURE OF LOWER THYROID ACTIVITY. IN THIS SERIES OF EXPERIMENTS, NO ATTEMPT WAS MADE TO ASCERTAIN THAT TOTAL ATHYROIDISM WAS REACHED. THERE IS STILL, AT THE PRESENT TIME, SOME DISAGREEMENT ON WHETHER THYROID GLAND IS THE ONLY SITE OF THYROID HORMONE SYNTHESIS (137). FOR THIS REASON, A DECREASE IN OXYGEN CONSUMPTION WAS JUDGED A SATISFACTORY MEASUREMENT OF DECREASED THYROID FUNCTION.

IT HAS BEEN CLAIMED THAT THYROXINE-TREATED ANIMALS EXHIBIT RATHER A "THYROTOXIC" PICTURE THAN A CHRONIC HYPERTHYROIDISM. THIS IS ESPECIALLY SHOWN WHEN A DECREASE IN BODY WEIGHT ACCOMPANIES THE

INCREASE IN OXYGEN CONSUMPTION. SINCE THE POTENTIATION OF CATECHOLAMINE EFFECTS IS ESPECIALLY NOTICED DURING THE "THYROTOXIC STORM", IT WAS THOUGHT THAT THE EXPERIMENTS WERE NOT INVALIDATED BY THE FACT THAT THE RATS WERE THYROTOXIC, ALTHOUGH NO EVIDENCE OF THAT STATE WAS CONCLUSIVELY PRESENT.

DISTRIBUTION OF RADIOACTIVE MATERIAL IN THE RATS AFTER CATECHOLAMINE INJECTION :

THE LARGE VARIATIONS OBSERVED IN THIS SERIES OF EXPERIMENTS PREVENTED THE STATISTICAL TREATMENT OF THE DATA AND LIMIT SOMEWHAT THE DISCUSSION AND INTERPRETATION OF THE RESULTS. THESE VARIATIONS MAY BE DUE TO SEVERAL FACTORS OF TECHNICAL OR BIOLOGICAL ORIGINS.

1. FACTORS OF BIOLOGICAL ORIGINS :

THE RAT IS AN ANIMAL WHICH HAS BEEN SHOWN TO BE VERY SENSITIVE TO CATECHOLAMINES. THE INTRAVENOUS LETHAL DOSE OF ADRENALINE IS VERY LOW IN THE RAT COMPARED TO OTHER SPECIES SUCH AS MAN OR MOUSE (138,139). THE ANIMALS USED IN THESE EXPERIMENTS WERE OF EXTERNAL ORIGIN. IN THE COURSE OF THE WORK, IT BECAME OBVIOUS THAT ALTHOUGH THE RATS WERE OF THE SPRAGUE-DAWLEY STRAIN, THEY WERE NOT ALWAYS ORDERED FROM THE SAME SOURCE. THE RATS WERE KEPT TWO DAYS IN THE UNIVERSITY ANIMAL QUARTERS BEFORE USE, BUT THIS SHORT PERIOD WOULD NOT STANDARDIZE THE ANIMALS, IF THE CONDITIONS UNDER WHICH THEY WERE PREVIOUSLY KEPT WERE NOT SIMILAR. IT WAS NOT POSSIBLE TO CONTROL THE ENVIRONMENTAL CONDITIONS PRIOR THEIR ARRIVAL TO THE UNIVERSITY. ANOTHER DRAWBACK IN THESE EXPERIMENTS WAS DUE TO THE FACT

THAT THE WORK WAS EXTENDED OVER A PERIOD OF A YEAR. AT THE TIME OF THE EXPERIMENTS, THE ANIMALS WERE KEPT IN ROOMS NOT AIR-CONDITIONED. RATS ARE KNOWN TO BE VERY SENSITIVE TO HEAT. FURTHERMORE TEMPERATURE REGULATION OF THE BODY IS UNDER THE CONTROL OF THE ADRENALS (140). THE ANIMALS USED MAY HAVE BEEN IN DIFFERENT ADRENAL STATES, DEPENDING UPON THE SEASON DURING WHICH THE EXPERIMENTS WERE PERFORMED. THESE TWO BIOLOGICAL FACTORS APPEAR TO BE PARTIALLY RESPONSIBLE FOR THE HIGH VARIATIONS OBSERVED.

2. FACTORS OF TECHNICAL ORIGIN :

THE MAIN RESTRICTION IN THE USE OF TAIL VEIN AS THE MODE OF INTRAVENOUS INJECTION INTO THE RAT IS THAT THE COMPLETE PENETRATION OF THE INJECTED COMPOUND INTO THE BLOOD STREAM IS NOT CONTROLLED. CATECHOLAMINES HAVE TO BE INJECTED SLOWLY TO PREVENT UNDESIRABLE SIDE-ACTIONS SUCH AS THE INCREASE IN HEART RATE AND BLOOD PRESSURE. THIS SIDE ACTION MAY EVENTUALLY LEAD TO DEATH. THE NEEDLE MAY BE DISPLACED BY A SMALL MOVEMENT OF THE TAIL, LEADING TO A PARTIAL SUBCUTANEOUS INJECTION. THE INTRAVENOUS INJECTION CAN BE MADE THROUGH THE ABDOMINAL VENA CAVA OR THROUGH THE FEMORAL VEIN. BOTH THESE WAYS, HOWEVER, REQUIRE DEEP ANESTHESIA AND SURGERY. A LOSS OF BLOOD AND A DECREASE IN THE INTERNAL BODY TEMPERATURE ARE DIFFICULT TO AVOID UNDER THESE CONDITIONS. THE COMPENSATORY MECHANISMS ENTERING INTO ACTION, IT WAS THOUGHT THAT UNDER THESE CONDITIONS, THE ANIMAL WAS NOT IN A PHYSIOLOGICAL STATE ANYMORE. AS PHYSIOLOGICAL CONDITIONS, WERE DESIRABLE THE TAIL VEIN WAS CHOSEN AS THE ROUTE OF INJECTION.

THE SECOND FACTOR WHICH MAY HAVE BEEN RESPONSIBLE FOR THE LARGE VARIATIONS OBSERVED WAS THOUGHT TO BE THE ANESTHETIC. AS POINTED OUT EARLIER, THE REACTION OF THE RAT TO NEMBUTAL DEPENDS UPON ITS THYROID STATE. THE DIFFERENCES DUE TO A CHANGE IN THE SENSITIVITY OF THE RAT TO NEMBUTAL WOULD BE MORE OBVIOUS BETWEEN VARIOUS GROUPS, RATHER THAN AMONG THE RATS OF THE SAME GROUP. NEMBUTAL IS KNOWN TO BE BOUND TO PLASMA PROTEINS. SINCE THE DAY BEFORE THE EXPERIMENT THE RAT WAS INJECTED WITH NEMBUTAL FOR MEASUREMENT OF OXYGEN CONSUMPTION, THE LEVEL OF NEMBUTAL THE NEXT DAY WOULD DEPEND UPON THE AMOUNT STORED THE DAY BEFORE. A CUMULATIVE ACTION WOULD BE EXPECTED (141). THIS MAY EXPLAIN WHY SO MANY NORMAL OR THYROXINE-TREATED RATS DIED UNDER NEMBUTAL ANESTHESIA.

A THIRD FACTOR WHICH MAY HAVE BEEN RESPONSIBLE FOR THE LARGE VARIATIONS, WAS THE TIGHT RUBBER BAND ON THE TAIL OF THE ANIMAL. DEPENDING UPON THE DEPTH OF ANESTHESIA AND UPON THE LENGTH OF TIME NECESSARY TO ENTER INTO THE VEIN, THE ANIMAL REACTED DIFFERENTLY TO THE PAIN STIMULUS. ONE OR MORE OF THESE FACTORS MUST HAVE BEEN INVOLVED IN CAUSING LARGE VARIATIONS. AT NO TIME WAS IT POSSIBLE TO RELATE THE LOW OR HIGH RADIOACTIVITY CONTENT OF THE ORGANS TO A SINGLE OF THESE FACTORS.

BEARING IN MIND THE LACK OF STATISTICAL ANALYSIS, AND THE HIGH VALUES OF THE STANDARD ERRORS, A DISCUSSION OF THE IN VIVO RESULTS WILL BE PRESENTED.

FOLLOWING A SINGLE INTRAVENOUS INJECTION OF ³H-NORADRENALINE

INTO THE RAT, THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE BLOOD OF THYROXINE PRETREATED RATS WAS SIMILAR TO THAT IN THE BLOOD OF THE NORMAL CONTROLS ; IT WAS HIGHER IN ANY OF THE OTHER ORGANS STUDIED, IN THE THYROXINE-TREATED RATS THAN THAT IN THE NORMAL ONES. THIS FINDING MAY REFLECT AN ALTERED RATE OF UPTAKE OF THE CIRCULATING CATECHOLAMINE, OR AN INCREASED RATE OF BINDING OF THE EXOGENOUS CATECHOLAMINE IN THE TISSUE, OR A LESS RAPID RATE OF INACTIVATION OF THE AMINE. IN THE LIGHT OF WURTMANN'S FINDINGS THAT MOST OF THE ³H-NORADRENALINE INJECTED IS FOUND UNCHANGED IN THE HEART, FORTY MINUTES AFTER THE INJECTION (102), IT SEEMS THAT WE CAN ASSUME THAT A LARGE PART OF THE RADIOACTIVE MATERIAL IS UNCHANGED MATERIAL. IT APPEARS NECESSARY AT THIS POINT OF THE PROCEEDINGS TO DEFINE THE WORD " UPTAKE ", SINCE NO CURRENT AGREEMENT ON ITS MEANING SEEMS TO EXIST IN THE LITERATURE. IN THIS THESIS, UPTAKE IS DEFINED AS " THAT AMOUNT OF RADIOACTIVE MATERIAL DETECTED IN A TISSUE, AFTER THAT TISSUE HAS BEEN PLACED IN CONTACT WITH THE RADIOACTIVE CATECHOLAMINE FOR A GIVEN PERIOD OF TIME ". THE UPTAKE IS EXPRESSED AS THE CONCENTRATION OF UNCHANGED RADIOACTIVE AMINE PER GRAM OF WET TISSUE, ALTHOUGH NO ATTEMPT HAS BEEN MADE TO IDENTIFY IT AS SUCH.

IN THYROIDECTOMIZED RATS, THE UPTAKE OF ³H-NORADRENALINE SEEMS TO BE INCREASED, SINCE THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE BLOOD IS LOWER, AND THAT IN THE OTHER ORGANS HIGHER THAN THAT IN THE BLOOD AND ORGANS FROM NORMAL CONTROLS. BECAUSE THE WEIGHT OF

THE ORGANS FROM THYROIDECTOMIZED ANIMALS WAS LOWER THAN THAT OF THE NORMAL CONTROLS THE TOTAL AMOUNT OF RADIOACTIVE MATERIAL IN THE WHOLE ORGAN WAS HIGHER THAN THAT IN THE WHOLE ORGANS OF THE NORMAL CONTROLS. THE BINDING CAPACITY OF THYROIDECTOMIZED TISSUES APPEARS TO BE INCREASED COMPARED TO THAT OF THE NORMAL RATS.

THE PERCENTAGE OF THE INJECTED DOSE OF NORADRENALINE IN THE HEART OF BOTH NORMAL AND THYROXINE-TREATED OF THYROIDECTOMIZED ANIMALS WAS IDENTICAL TO THAT FOUND BY WURTMANN ET AL. (102). SUCH WAS ALSO THE CASE FOR THE AMOUNT OF RADIOACTIVE MATERIAL FOUND AFTER INJECTION OF RADIOACTIVE ADRENALINE.

THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE BLOOD IS IDENTICAL 10 MINUTES OR 60 MINUTES AFTER THE INTRAVENOUS INJECTION OF ADRENALINE TO NORMAL RATS. IN ALL THE OTHER TISSUES BUT LIVER AND SKELETAL MUSCLE, THERE IS A STEADY DECREASE IN THE CONCENTRATION OF ADRENALINE WITH TIME. BUT AS POINTED OUT PREVIOUSLY BY WURTMANN, IT IS INTERESTING TO NOTE THAT ONE HOUR AFTER A SINGLE INJECTION, THERE IS STILL CONSIDERABLE RADIOACTIVE MATERIAL IN THE HEART OF THE NORMAL RAT. ALTHOUGH THE AMOUNT OF RADIOACTIVE MATERIAL FOUND PER GRAM OF SKELETAL MUSCLE IS LOW, THIS TISSUE REPRESENTS A LARGE PERCENTAGE OF THE TOTAL BODY WEIGHT, SO THAT THE AMOUNT OF ADRENALINE IN THE WHOLE TISSUE REPRESENT PROBABLY A GREAT PERCENTAGE OF THE INJECTED DOSE. IN VIEW OF THE FINDINGS THAT ADRENALINE INJECTION LOWERS SKELETAL MUSCLE GLYCOGEN IT WOULD APPEAR INTERESTING TO INVESTIGATE FURTHER THE DISTRIBUTION OF ADRENALINE IN THIS TISSUE.

IN THE THYROIDECTOMIZED RATS, TEN MINUTES AFTER THE INJECTION OF ADRENALINE THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUES IS MUCH HIGHER THAN THAT IN THE NORMAL CONTROLS. THE UPTAKE AND BINDING OF ADRENALINE BY TISSUES FROM THYROIDECTOMIZED ANIMALS APPEAR INCREASED. THE LEVEL OF CIRCULATING CATECHOLAMINE IS ALSO ELEVATED AS SHOWN BY THE HIGHER LEVEL OF RADIOACTIVE MATERIAL IN THE BLOOD OF THYROIDECTOMIZED RATS. SIXTY MINUTES AFTER THE INJECTION, HOWEVER, THE LEVEL OF RADIOACTIVE MATERIAL HAS DECREASED AND IS INFERIOR TO THAT FOUND IN THE NORMAL RAT EXCEPT FOR THAT IN THE KIDNEY AND IN THE LIVER. BECAUSE THE CONCENTRATION OF RADIOACTIVE MATERIAL IS ELEVATED COMPARED TO THE NORMAL IN THE ORGANS METABOLIZING ADRENALINE, IT MAY BE ASSUMED THAT IN THE THYROIDECTOMIZED ANIMAL, THE ENZYMIC DEGRADATION OF EXOGENOUS ADRENALINE IS MORE IMPORTANT THAN IN THE NORMAL RAT. HOWEVER, THE DIFFERENCES BETWEEN THE TWO ANIMALS IS TOO HIGH TO PERMIT THE INTERPRETATION OF THE RESULTS.

FOR ALL THE REASONS STATED ABOVE, IT SEEMED NECESSARY TO USE A SIMPLER SYSTEM. THE WORK PURSUED ON THE ISOLATED SMALL INTESTINE WILL PRESENTED IN THE NEXT PART II.

PART II. THE EFFECT OF THYROXINE ON THE UPTAKE,
BINDING AND METABOLISM OF ^3H -ADRENALINE BY INTESTINAL STRIPS,
FOLLOWING A FIVE MINUTE INCUBATION WITH ^3H -DL-ADRENALINE .

1. MATERIALS AND METHODS

A) MATERIALS :

BESIDES THE CHEMICALS ALREADY MENTIONED IN PART I THE FOLLOWING ONES HAVE BEEN USED :

BOVINE SERUM ALBUMIN (FRACTION V, BIOLOGICAL) PURCHASED FROM MANN, N.Y..

THREE DIFFERENT BATCHES OF ^3H -ADRENALINE WERE USED WITH A SPECIFIC ACTIVITY OF : 5.76 CURIES PER MM OR 6.19 CURIES PER MM.

B) METHODS :

1) PREPARATION OF ANIMALS :

THE ANIMALS WERE PREPARED AS PREVIOUSLY DESCRIBED IN PART. I.

2) PREPARATION OF INCUBATION MEDIUM :

KREBS-RINGER BICARBONATE GLUCOSE, SUPPLEMENTED WITH ALBUMIN WAS USED AS THE INCUBATION MEDIUM. IT WAS PREPARED, PRIOR TO SACRIFICE OF THE ANIMALS FROM STOCK SOLUTIONS KEPT AT 5°C . AS SUGGESTED BY UMBREIT (142). FIFTEEN MILLILITERS OF A 20 % GLUCOSE SOLUTION WERE ADDED TO 190 ML OF BUFFER. ALBUMIN (630 MG) WAS THEN ADDED TO THE KREBS-RINGER BICARBONATE GLUCOSE AND ALLOWED TO DISSOLVE COMPLETELY. A KNOWN VOLUME OF ^3H -ADRENALINE SOLUTION, DEPENDING UPON THE FINAL CONCENTRATION OF

ADRENALINE DESIRED, WAS THEN ADDED TO THE MEDIUM. A GAS MIXTURE CONTAINING OXYGEN-CARBON DIOXIDE (95 % : 5 %) WAS SUBBLED AT THE SURFACE OF THE MEDIUM FOR A PERIOD OF 5 MINUTES. THE INCUBATION MEDIUM WAS THEN RAPIDLY DISTRIBUTED INTO ERLENMEYERS, 10 ML OF MEDIUM INTO EACH OF SIXTEEN 50ML-ERLENMEYERS. EACH ERLENMEYER WAS STOPPERED WITH A RUBBER STOPPER AS SOON AS THE MEDIUM HAS BEEN DELIVERED INTO IT. THE ERLENMEYERS CONTAINING THE MEDIUM WERE THEN PREINCUBATED AT 37° C IN A "DUBNOFF METABOLIC SHAKER", IN ORDER TO EQUILIBRATE THE MEDIUM AT 37° C.

3) PREPARATION OF INTESTINAL STRIPS

AFTER THE WEIGHT OF THE RATS TO BE KILLED HAD BEEN RECORDED, THE METHOD PROCEEDED IN THE FOLLOWING MANNER :

ONE THYROIDECTOMIZED RAT WAS DECAPITATED, THE STOMACH AND PART OF THE SMALL INTESTINE REMOVED AND PLACED ON ICE. ONE NORMAL RAT WAS THEN KILLED IN THE SAME WAY, ITS STOMACH AND SMALL INTESTINE REMOVED AND PLACED ON ICE. THE SMALL INTESTINE FROM THE THYROIDECTOMIZED ANIMAL WAS THEN CUT INTO 2 CM PIECES, AT A DISTANCE OF 4 CM FROM THE PYLORUS. THE STRIPS WERE THEN CUT OPEN, CLEANED OF FAT OR FECES AND PLACED INTO A BEAKER CONTAINING ICE-COLD ISOTONIC NAOL. THE SMALL INTESTINE FROM THE NORMAL RAT WAS TREATED IN AN IDENTICAL MANNER. EIGHT PIECES OF INTESTINAL TISSUE FROM THE THYROIDECTOMIZED RAT WERE PLACED INTO 4 BEAKERS CONTAINING ICE-COLD ISOTONIC NAOL., 2 STRIPS INTO EACH BEAKER. EIGHT PIECES FROM THE NORMAL RAT INTESTINE WERE PUT INTO 4 OTHER BEAKERS CONTAINING ICE-COLD ISOTONIC NAOL, 2 STRIPS IN EACH BEAKER. THE TIME

ELAPSED BETWEEN DISTRIBUTION OF THE INCUBATION MEDIUM INTO THE ERLKENMEYERS AND INCUBATION ZERO TIME NEVER EXCEEDED 20 MINUTES.

4) INCUBATION

THE PROCESS USED FOR INCUBATION WILL BE DESCRIBED IN DETAILS FOR A SINGLE STRIP. EACH STRIP WAS TREATED IN AN IDENTICAL MANNER, AT 0.25 MINUTE INTERVAL. THE STRIPS FROM THE THYROIDECTOMIZED RAT SMALL INTESTINE WERE FIRST TREATED. THE FIRST STRIP WAS BLOTTED ON FILTER PAPER AND AT TIME ZERO PUT INTO THE FIRST ERLKENMEYER. AT TIME 5 MIN, THE STRIP WAS REMOVED FROM THE ERLKENMEYER AND PLACED IN A BEAKER CONTAINING ISOTONIC SODIUM CHLORIDE, FOR RINSING. THE STRIP WAS TRANSFERRED TO A SECOND BEAKER CONTAINING ICE-COLD ISOTONIC NaCl, 0.25 MINUTES LATER. THEN A FINAL RINSING WAS PERFORMED IN A THIRD BEAKER 0.25 MINUTES LATER FOR 0.25 MINUTES. THE TISSUE WAS THEN PLACED ON A FILTER PAPER AND BLOTTED. WHEN ALL THE STRIPS FROM ONE RAT HAD BEEN COLLECTED ON THE FILTER PAPER AND BLOTTED, THEIR WEIGHT WAS RECORDED. THEY WERE THEN PLACED INTO A HOMOGENIZER CONTAINING 0.4 N PERCHLORIC ACID AND PUT IN THE DEEP-FREEZE.

THE NEXT EXPERIMENT WAS THEN STARTED FROM STEP 2, BUT THIS TIME THE NORMAL RAT WAS THE FIRST ONE TO BE KILLED.

THE WATER-BATH SHAKER WAS KEPT ON DURING THE PERIOD WHICH ELAPSED BETWEEN EQUILIBRATION OF THE ERLKENMEYERS UP TILL THE REMOVAL OF THE LAST OF THE SIXTEEN STRIPS.

5) HOMOGENIZATION

THE FROZEN SAMPLES FROM THE PREVIOUS EXPERIMENTS WERE

ALLOWED TO THAW AND HOMOGENIZATION WAS PERFORMED WITH AN ALL GLASS MOTOR DRIVEN POTTER-EVENLUJEN. THE HOMOGENATE WAS THEN TRANSFERRED INTO A GRADUATED CYLINDER AND THE HOMOGENIZER RINCED WITH ICE-COLD 0.4 N PERCHLORIC ACID. THE VOLUME OF HOMOGENATE PLUS RINSING WAS RECORDED. TWO ALIQUOTS OF 0.100 ML EACH WERE PLACED INTO EACH OF TWO SCINTILLATION VIALS. THE GRADUATED CYLINDER WAS THEN EMPTIED OF ITS CONTENT INTO A LUSTEROID CENTRIFUGE TUBE AND RINCED TWICE WITH ICE-COLD 0.4 N PERCHLORIC ACID. THE WASHINGS WERE ADDED TO THE LUSTEROID TUBE WHICH WAS THEN PUT INTO ICE. THE NEXT SAMPLE WAS THEN HOMOGENIZED.

6) EXTRACTION OF RADIOACTIVE MATERIAL

WHEN ALL THE SAMPLES HAD BEEN HOMOGENIZED AND ALIQUOTS FOR RADIOACTIVITY DETERMINATION SET ASIDE, THE CENTRIFUGE TUBES WERE CENTRIFUGED IN A REFRIGERATED SERVALL CENTRIFUGE AS DESCRIBED IN PART I. HOWEVER, AFTER THE FIRST SUPERNATANT HAD BEEN REMOVED, EACH PELLET WAS WASHED TWICE WITH 0.4 N PERCHLORIC ACID. THE TWO WASHINGS AND THE FIRST SUPERNATANT WERE COMBINED. THE RESULTING EXTRACT WAS PLACED IN THE DEEP-FREEZE AND WAS USED AT A LATER TIME FOR IDENTIFICATION OF ^3H -ADRENALINE AND ^3H -METANEPHRINE.

7) DETERMINATION OF RADIOACTIVITY CONTENT

INTO EACH OF THE SCINTILLATION VIALS CONTAINING 0.100 ML OF THE HOMOGENATE WERE ADDED 10 ML OF LIQUID FOR SCINTILLATION COUNTING. THE VIALS WERE THEN PLACED IN THE REFRIGERATION UNIT OF THE COUNTER AND ALLOWED TO EQUILIBRATE FOR 10 MINUTES. THE CONDITIONS OF COUNTING WERE : CHANNEL 1 : BASE-LEVEL 1, CHANNEL 2 : LEVEL 1-LEVEL 2 ;

HIGH VOLTAGE : GATE 1275 v, DATA 1175 v ; PRESET COUNTS : 1,000,000.
THE TIME PRESET FOR COUNTING DEPENDED UPON THE ACTIVITY OF THE SAMPLE
AND WAS SET ACCORDING TO THE ACTIVITY OF THE FIRST VIAL. IT VARIED FROM
ONE EXPERIMENT TO ANOTHER BUT USUALLY, 600 SECONDS OR 1,200 SECONDS
WERE THE DURATION OF EACH COUNTING. THE BACKGROUND WAS MEASURED BY
COUNTING A VIAL CONTAINING 10 ML OF SCINTILLATION LIQUID AND 0.100 ML
OF 0.4 N PERCHLORIC ACID. THE BACKGROUND WAS DETERMINED FOR AT LEAST
1,800 SECONDS. THE RADIOACTIVITY OF THE INCUBATION MEDIUM WAS MEASURED
ON FOUR 0.100 ML ALIQUOTS OF WHAT HAD REMAINED AFTER THE DISTRIBUTION
INTO THE ERLLENMEYERS.

8) IDENTIFICATION OF ^3H -METANEPHRINE AND ^3H -ADRENALINE

THE RADIOACTIVITY OF THE PERCHLORIC EXTRACT WAS MEASURED ON
0.200 ML ALIQUOTS BEFORE IDENTIFICATION OF ^3H -ADRENALINE OR
 ^3H -METANEPHRINE WERE PERFORMED. THE CONTENT OF RADIOACTIVITY IN 1 ML
OR 2 ML OF THE PERCHLORIC EXTRACT WAS THEN CALCULATED, DEPENDING ON
WHETHER 1 ML OR 2 ML ALIQUOTS WERE USED FOR THE IDENTIFICATION. THIS
RADIOACTIVITY WAS CONSIDERED AS THE AMOUNT OF TOTAL RADIOACTIVITY
USED FOR IDENTIFICATION.

^3H -ADRENALINE WAS IDENTIFIED BY THE METHOD OF AXELROD (143),
BUT AS SUGGESTED BY MISSALA (144), THE BATCH PROCESS RATHER THAN THE
COLUMN FOR ADSORPTION ON ALUMINA WAS USED.

^3H -METANEPHRINE WAS ISOLATED AND IDENTIFIED ACCORDING TO
THE PROCEDURE OF AXELROD (143).

9) CALCULATIONS OF RESULTS

TOTAL RADIOACTIVITY CONTENT OF THE TISSUE

AFTER THE RADIOACTIVE CONTENT OF THE ALIQUOTS FROM THE HOMOGENATE HAD BEEN RECORDED, THE DATA FROM TWO ALIQUOTS WERE AVERAGED, AND THE BACKGROUND SUBTRACTED. THE TOTAL RADIOACTIVITY IN THE HOMOGENATE WAS THEN CALCULATED BY TAKING THE VOLUME OF THE HOMOGENATE INTO ACCOUNT. THIS RESULT WAS CONSIDERED TO CORRESPOND TO THE AMOUNT OF RADIOACTIVE MATERIAL PRESENT IN THE TISSUE SAMPLE. IT WAS EXPRESSED IN COUNTS PER MINUTE. BY DIVIDING THIS RESULT BY THE WEIGHT OF THE SAMPLE ONE OBTAINED THE SPECIFIC RADIOACTIVITY IN COUNTS PER MINUTE PER GRAM OF TISSUE. BECAUSE BOTH THE AMOUNT OF ^3H -ADRENALINE AND THE COUNTS PER MINUTE OF THE INCUBATION MEDIUM WERE KNOWN, IT WAS POSSIBLE TO EXPRESS THE RADIOACTIVE CONTENT OF THE UNKNOWN AS THE NUMBER OF MOLES OF ^3H -ADRENALINE PER GRAM OF TISSUE.

^3H -ADRENALINE AND ^3H -METANEPHRINE

AFTER THE TOTAL RADIOACTIVITY OF THE SAMPLE HAD BEEN CALCULATED IN COUNTS PER MINUTE, THE RATIO OF RADIOACTIVITY IN THE SAMPLE TO THAT IN THE PERCHLORIC EXTRACT WAS CALCULATED. THE PERCENTAGE OBTAINED WAS THEN CALCULATED AS A FUNCTION OF THE MOLES OF ^3H -ADRENALINE OBTAINED IN THE HOMOGENATE AND EXPRESSED PER GRAM OF TISSUE. THE CORRECTIONS FOR A RECOVERY OF 80 % AND 50 % IN THE CASE OF ADRENALINE AND METANEPHRINE RESPECTIVELY, WERE APPLIED ON THE PERCENTAGE VALUES.

10) STATISTICAL ANALYSIS

GROUP COMPARISON AND ANALYSIS OF VARIANCE WITH INTERACTION

WERE THE TWO STATISTICAL METHODS USED IN COMPILING THE DATA. A VALUE OF P 0.01 WAS CHOSEN AS STATISTICALLY SIGNIFICANT.

DISCUSSION

IN THE IN VIVO EXPERIMENTS, MEASUREMENTS OF BASAL METABOLISM HAD SHOWN THAT THE METHODS USED TO INDUCED HYPERTHYROIDISM AND ATHYROIDISM WERE ADEQUATE. NO ATTEMPT WAS MADE IN THIS SERIES OF EXPERIMENTS TO DETERMINE OXYGEN CONSUMPTION. IT WAS THOUGHT THAT, SINCE THIS MEASUREMENT REQUIRED THE USE OF NEMBUTAL AS AN ANESTHETIC, THIS LAST FACTOR MAY INTRODUCE A VARIABLE. SINCE THIS COULD BE AVOIDED, THE BASAL METABOLISM WAS NOT MEASURED BUT THE BODY WEIGHT OF THE ANIMALS WAS CHECKED.

INCUBATION MEDIUM

KREBS-RINGER BICARBONATE HAS BEEN RECOMMENDED AS A SUITABLE BUFFER FOR IN VITRO STUDIES WITH ADRENALINE, SINCE PHOSPHATE CATALYSES THE OXIDATION OF ADRENALINE (145). GLUCOSE AND ALBUMIN WERE ADDED IN ORDER TO APPROACH AS MUCH AS POSSIBLE THE PHYSIOLOGICAL MEDIUM. MOREOVER, BECAUSE OF ITS COPPER CHELATING PROPERTIES ALBUMIN IN ALKALINE MEDIUM PREVENTS ADRENALINE OXIDATION (146). IN A DIALYSIS ANALYSIS, IT WAS FOUND THAT THE BINDING OF ADRENALINE TO ALBUMIN WAS NEGLIGIBLE. FIVE ML OF THE INCUBATION MEDIUM CONTAINING ³H-ADRENALINE (8.3×10^8) WERE PLACED INTO A DIALYSIS BAG. THE RADIOACTIVITY CONTENT OF THE SAMPLE HAD BEEN PREVIOUSLY DETERMINED ON 0.200 ML ALIQUOTS. THE DIALYSIS BAG WAS THEN PLACED IN A BEAKER CONTAINING 100 ML OF DISTILLED WATER, AND DIALYSIS ALLOWED TO TAKE PLACE AT COLD ROOM TEMPERATURE.

THE CONTENT OF THE BEAKER WAS CONTINUOUSLY STIRRED. THIRTY MINUTES LATER, THREE ALIQUOTS OF WATER (0.200ML EACH) WERE REMOVED AND RADIOACTIVITY CONTENT MEASURED. THIS WAS REPEATED TWICE, EVERY 30 MINUTES AND THE DIFFUSATE WAS THEN REPLACED BY FRESH DISTILLED WATER. THE DIALYSIS WAS PERFORMED FOR 16 HOURS, AT THE END OF WHICH THE RADIOACTIVITY CONTENT OF BOTH THE DIFFUSATE AND THE SOLUTION IN THE DIALYSIS BAG WAS MEASURED.

- TABLE V -

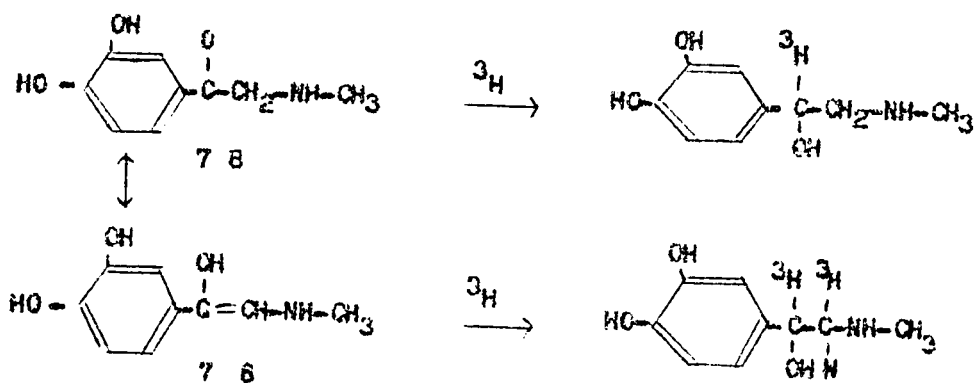
TIME	RADIOACTIVITY IN THE DIFFUSATE C/MIN	DIFFUSION	RADIOACTIVITY IN THE DIALYSATE C/MIN	UNDIFFUSED
0	0	0 %	154,600	100 %
30 MIN	74,500	48 %	-	
16 HRS	138,750	89 %	22,200	14 %

ON THE OTHER HAND, THE AFFINITY OF ALBUMIN FOR THYROXINE IS WELL ESTABLISHED (147, 148). THE ACTUAL CONCENTRATION OF FREE THYROXINE IN THE INCUBATION MEDIUM WAS INFERIOR TO THE TOTAL CONCENTRATION. HOWEVER, SINCE THE EXPERIMENTAL CONDITIONS WERE IDENTICAL NO CORRECTION WAS MADE FOR THE BINDING OF THYROXINE TO ALBUMIN.

³H. ADRENALINE

AS POINTED OUT EARLIER, THE USE OF TRITIUM AS A LABEL PERMITS THE OBTENTION COMMERCIALY OF MOLECULES WITH HIGH SPECIFIC ACTIVITY. IT IS THUS POSSIBLE TO USE PHYSIOLOGICAL CONCENTRATIONS OF ADRENALINE

WITH A MEASURABLE RADIOACTIVITY (749). IN THE EXPERIMENTAL CONDITIONS USED HERE SEVERAL FACTORS APPEARED TO HAMPER SOMEWHAT THE BENEFICIARY USE OF ^3H -ADRENALINE. THE COMMERCIAL PREPARATION OF ^3H -ADRENALINE WAS PERFORMED BY CATALYTIC TRITIATION OF ADRENALONE WHICH YIELDED ADRENALINE LABELED ON THE CARBON 7 AND ON THE HYDROXYL GROUP OF CARBON 7 WITH ^3H . SLOW EXCHANGE CHROMATOGRAPHY PERMITTED TO REMOVE THE LABEL ON THE HYDROXYL GROUP. NEW ENGLAND NUCLEAR CORP. COULD NOT ASCERTAIN THAT THE ENOL FORM OF ADRENALONE WAS NOT PRESENT DURING TRITIATION. THIS WOULD RESULT IN THE LABELING WITH ^3H OF BOTH CARBON 7 AND CARBON 6. PAPER CHROMATOGRAPHY OF THE COMPOUND PERMITTED TO IDENTIFY ADRENALINE BUT DID NOT GIVE ANY INFORMATION ABOUT THE SITE OF THE LABEL.



THREE BATCHES OF ^3H -ADRENALINE WERE USED IN THE COURSE OF THE EXPERIMENTS. ONE OF THEM HAD A SPECIFIC ACTIVITY OF 5.75 CURIES/MM, THE TWO OTHERS 6.19 CURIES/MM. ALTHOUGH CHROMATOGRAPH SHOWED THAT THE COMPOUNDS WERE PURE, THE ACTIVITY IN COUNTS PER MINUTE WAS MUCH HIGHER WITH THE

BATCHES WHERE THE SPECIFIC ACTIVITY AVERAGED 6.19 CURIES/ML. FOR THIS REASON, CORRECTION FACTORS WERE APPLIED ACCORDING TO THE COUNTS PER MINUTE DETECTED AND NOT ACCORDING TO THE MILLICURIES.

ANOTHER DRAWBACK IN THE USE OF TRITIUM AS A LABEL FOR ADRENALINE, RESIDED IN THE PLACE WHERE THE LABEL WAS PUT. OXIDATION OF ADRENALINE IN ALKALINE OR NEUTRAL MEDIUM GIVES THE FORMATION OF A RING WITH THE EVENTUAL ELIMINATION OF THE HYDROGEN ON THE CARBON 7. (146). THIS RESULTED IN THE LOSS OF THE LABEL ON THE MOLECULE AND THE FORMATION OF TRITIATED WATER. THIS TRITIATED WATER WOULD NOT BE DIFFERENTIATED WHEN MEASURING THE RADIOACTIVITY CONTENT OF THE TISSUE, FROM ADRENALINE AND ITS METABOLITES. IN ONE SERIES OF EXPERIMENTS, THE CONTENT OF $^3\text{H}_2\text{O}$ FORMED WAS MEASURED BY THE PROCEDURE OF LA BROUSSE (150). A CONTROL FLASK WITHOUT TISSUE WAS RUN IN THE SAME EXPERIMENTAL CONDITIONS THAT THE EXPERIMENTAL FLASKS. AFTER THE 5 MINUTE INCUBATION, THE TRITIATED WATER CONTENT OF THE SOLUTION WAS MEASURED IN THE FOLLOWING MANNER. THE RADIOACTIVITY CONTENT OF THE SOLUTION WAS MEASURED ON AN ALIQUOT; THE SOLUTION WAS THEN EVAPORATED TO DRYNESS, UNDER NITROGEN ATMOSPHERE AT 40°C . THE RESIDUE WAS TAKEN UP WITH WATER AND RADIOACTIVITY CONTENT DETERMINED ON THE RESIDUE AND ON THE DISTILLATE. THE CONCENTRATION OF ^3H -ADRENALINE IN THE STARTING SOLUTION WAS 3.3×10^{-8} M.

	COUNTS/MIN PER 0.100 ML	COUNTS/MIN/TOTAL VOLUME	% RECOVERY
SOLUTION	2,667	266,700	-
CONCENTRATE	2,363	238,300	89
DISTILLATE	230	32,200	12

NO CORRECTION WAS MADE FOR THIS ERROR WHEN CALCULATING THE UPTAKE SINCE THE AMOUNT OF WATER WHICH HAD PENETRATED INTO THE TISSUE WAS UNKNOWN. IT WAS THOUGHT, THAT THE EXPERIMENTAL CONDITIONS USED WERE IDENTICAL AND THAT THIS ERROR WOULD BE SIMILAR IN ALL THE CASES. THE STATISTICAL ANALYSIS WOULD TAKE THAT FACTOR INTO ACCOUNT.

II. RESULTS

A) THE EFFECT OF THYROXINE ON THE UPTAKE OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE

1) THE IN VIVO EFFECT OF THYROXINE

THE EFFECT OF THYROXINE ON THE UPTAKE OF ^3H -ADRENALINE BY INTESTINAL STRIPS HAS BEEN TESTED IN VIVO, BY INJECTING THYROXINE INTO RATS DAILY FOR SIX DAYS, BEFORE REMOVING THE INTESTINE FOR INCUBATION WITH ^3H -DL-ADRENALINE. IN THIS SERIES A, A SINGLE CONCENTRATION OF ^3H -DL-ADRENALINE IN THE MEDIUM WAS ASSAYED (6.3×10^{-8} M). THE RESULTS OF INDIVIDUAL EXPERIMENTS ARE PRESENTED IN TABLE VI. IN THIS SERIES OF EXPERIMENTS, TISSUES FROM SEVERAL EXPERIMENTS WERE POOLED TOGETHER AND THIS EXPLAINED THE VARIATIONS IN THE WEIGHTS OF THE TISSUES. IT WAS FOUND HOWEVER, THAT WHEN TISSUES FROM A SINGLE ANIMAL WERE USED, THE RESULTS WERE SIMILAR (EXPERIMENT # 2). FOR THIS REASON, IN THE NEXT EXPERIMENTS TO BE DESCRIBED TISSUES FROM A SINGLE ANIMAL WERE USED. IN THIS SERIES A, TWO DIFFERENT BATCHES OF ^3H -DL-ADRENALINE WERE USED. THEY HAD DIFFERENT SPECIFIC ACTIVITIES, AND THIS IS EVIDENT WHEN COMPARING THE UPTAKE EXPRESSED IN COUNTS PER MINUTE AND EXPRESSED IN MOLES PER GRAM. THE RESULTS OBTAINED WITH TISSUES FROM NORMAL OR THYROXINE-TREATED RATS ARE SIMILAR. THE STATISTICAL ANALYSIS OF THESE RESULTS IS PRESENTED ON PAGE

2) THE IN VITRO EFFECT OF THYROXINE

THE EFFECT OF THYROXINE ON THE UPTAKE OF ^3H -ADRENALINE BY

TABLE VI

THE INFLUENCE OF THYROXINE-PRETREATMENT ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS

AFTER A 5 MINUTE INCUBATION WITH ^3H -CL-ADRENALINE (^3H -CL-ADRENALINE (B.31C-8_H))

(SERIES A)

EXPERIMENT #	WEIGHT OF TISSUE GRAMS	VOLUME OF HOMOGENATE ML	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE 10 ¹¹ MOLES/G
			IN 0.100 ML OF HOMOGENATE COUNTS/MIN	CONTENT OF HOMOGENATE COUNTS/MIN	PER GRAM OF TISSUE COUNTS/MIN/G		
1 N*	1.005	4.5	132	5,940	5,910	1.5	
1 T4**	0.960	4.0	150	6,400	6,670	1.6	
2 T4	0.457	1.8	120	2,160	4,730	1.2	
2 T4	1.377	6.0	311	18,660	13,550	1.7	
3 T4	1.151	6.0	197	11,820	10,270	1.3	
4 N*	1.397	6.0	331	19,800	14,220	2.0	
4 T4	0.671	4.5	258	11,610	13,330	1.8	

* N : TISSUE FROM NORMAL RATS

** T4 : TISSUE FROM THYROXINE-PRETREATED RATS

INTESTINAL STRIPS WAS TESTED IN VITRO, BY SUPPLEMENTING THE INCUBATION MEDIUM WITH THYROXINE. THREE SERIES OF EXPERIMENTS WERE PERFORMED (B, C AND D), USING THREE DIFFERENT CONCENTRATIONS OF ^3H -DL-ADRENALINE IN THE MEDIUM (1.61×10^{-8} M, 8.3×10^{-8} M AND 16.1×10^{-8} M). IN THE THREE SERIES OF EXPERIMENTS, TISSUES FROM NORMAL AND THYROIDECTOMIZED RATS WERE USED.

EACH SERIES OF EXPERIMENTS COMPRISED FOUR SETS OF EXPERIMENTS WHERE THREE DIFFERENT CONCENTRATIONS OF THYROXINE WERE USED (0.02×10^{-10} M, 0.20×10^{-10} M AND 2.00×10^{-10} M). EACH SET OF EXPERIMENTS COMPRISED AT LEAST 3 INDIVIDUAL EXPERIMENTS.

A) SERIES B 1 CONCENTRATION OF ^3H -DL-ADRENALINE,
 1.61×10^{-8} M

THE INDIVIDUAL RESULTS FOR THE FOUR SETS OF EXPERIMENTS ARE PRESENTED IN TABLE VII, VII A, VII B AND VII C. THE STATISTICAL ANALYSIS OF THE RESULTS IS PRESENTED IN TABLE VII D.

UPTAKE IN THE ABSENCE OF THYROXINE IN THE MEDIUM

IN TABLE VII, ARE PRESENTED THE RESULTS OBTAINED IN INDIVIDUAL EXPERIMENTS WHEN NO THYROXINE WAS PRESENT IN THE MEDIUM. FIVE MINUTES AFTER INCUBATION WITH 1.61×10^{-8} M ^3H -DL-ADRENALINE, A DETECTABLE AMOUNT OF RADIOACTIVE MATERIAL IS PRESENT IN THE TISSUES FROM NORMAL AND THYROIDECTOMIZED ANIMALS. THE TISSUES FROM NORMAL RATS CONCENTRATE RADIOACTIVE MATERIAL IN THE SAME AMOUNT AS THE TISSUES FROM THYROIDECTOMIZED RATS. IN BOTH CASES INDIVIDUAL VARIATIONS ARE HIGH.

UPTAKE IN THE PRESENCE OF THYROXINE IN THE MEDIUM

THE ADDITION OF THYROXINE (0.02×10^{-10} M) TO THE INCUBATION

MEDIUM RESULTED IN A SLIGHT INCREASE IN THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUES FROM THYROIDECTOMIZED RATS, BUT DID NOT HAVE ANY EFFECT ON THE CONCENTRATION OF RADIOACTIVE MATERIAL BY TISSUES FROM NORMAL ANIMALS. IN EXPERIMENT #3, THE DIFFERENCE IN THE WEIGHT OF TISSUE BETWEEN THYROIDECTOMIZED AND NORMAL ANIMALS WAS DUE TO THE FACT THAT ONE TISSUE WAS NOT PROPERLY INCUBATED. THE SUM IS THAT OF 7 TISSUES, WHILE FOR THE OTHERS IT IS 8.

INCREASING THE AMOUNT OF THYROXINE TEN TIMES (0.20×10^{-10} M) DID NOT CHANGE THE AMOUNT OF RADIOACTIVE MATERIAL CONCENTRATED BY THE TISSUES OF EITHER NORMAL OR THYROIDECTOMIZED ANIMALS (TABLE VII B) IN TABLE VII C, THE EFFECT OF A CONCENTRATION OF THYROXINE 100 TIMES HIGHER ARE SHOWN. THE UPTAKE OF ^3H -ADRENALINE BY TISSUES FROM NORMAL RATS IS DECREASED, WHILE THE UPTAKE BY TISSUES FROM THYROIDECTOMIZED RATS IS NOT AFFECTED.

STATISTICAL ANALYSIS OF THE RESULTS :

THE STATISTICAL ANALYSIS OF THE RESULTS OF SERIES B ARE PRESENTED IN THE NEXT TABLE (TABLE VI: d). A GROUP COMPARISON HAS BEEN PERFORMED FOR EACH CONCENTRATION OF THYROXINE TO SEE WHETHER THERE WAS A DIFFERENCE IN UPTAKE BETWEEN THYROIDECTOMIZED AND NORMAL RATS. THERE IS NO DIFFERENCE IN THE UPTAKE OF ^3H -ADRENALINE BETWEEN TISSUES FROM THYROIDECTOMIZED AND NORMAL RATS, IN THE ABSENCE OF THYROXINE IN THE MEDIUM. THERE IS A SIGNIFICANTLY HIGHER UPTAKE BY THE TISSUES FROM THYROIDECTOMIZED RATS AT THE LOWEST CONCENTRATION OF THYROXINE ASSAYED (0.02×10^{-10} M). INCREASING TEN TIMES THE

TABLE VII

THE INFLUENCE OF THYROIDECTOMY ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE ($1.6 \cdot 10^{-8}\text{M}$)

(SERIES B)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE
			IN 0.100 ML OF HOMOGENATE		CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	
			COUNTS/MIN	COUNTS/MIN	COUNTS/MIN	COUNTS/MIN/G	
1 N ^o Td ^o	0.294	2.3	31	590	2,350	0.30	
	0.258	2.2	49	1,078	4,180	0.53	
2 N Td	0.226	2.3	42	966	4,270	0.54	
	0.220	2.4	35	600	2,630	0.33	
3 N Td	0.255	2.4	28	672	2,630	0.33	
	0.204	1.9	34	646	3,170	0.40	
4 N Td	0.243	2.5	22	550	2,260	0.28	
	0.188	2.1	40	840	4,470	0.56	

^o N : TISSUE FROM NORMAL RATS

^o Td : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E V I I A
 THE INFLUENCE OF ADDED THYROXINE (0.02×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (1.61×10^{-10} M)
 (SERIES B A)

EXPERIMENT #	WEIGHT OF TISSUE GRAM	VOLUME OF HOMOGENATE ML	RADIOACTIVE MATERIAL			
			IN 0.100 ML OF HOMOGENATE	CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	EXPRESSED AS ^3H -ADRENALINE
			COUNTS/MIN	COUNTS/MIN	COUNTS/MIN/G	10^{11} MOLES /G
1 Td **	0.232	2.0	46	920	3,970	0.50
2 Td	0.210	2.3	28	644	3,070	0.39
3 N *	0.258	2.8	26	728	2,820	0.36
	0.188	2.0	54	1,080	5,740	0.72
4 N Td	0.261	2.5	24	600	2,300	0.29
	0.255	2.4	40	960	3,760	0.47
5 N	0.255	2.4	28	672	2,640	0.33

* N : TISSUE FROM NORMAL RATS ** Td : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E V I I B

THE INFLUENCE OF ADDED THYROXINE (0.20×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (1.01×10^{-8} M)

(SERIES B B)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL						
			GRAM	ML	IN 0.100 ML. OF HOMOGENATE		CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	EXPRESSED AS ^3H -ADRENALINE
					COUNTS/MIN	COUNTS/MIN			
1 N [*] Td ^{**}	0.354	2.4	37	690	2,510	0.32	0.32 0.43		
	0.252	2.1	41	861	3,420	0.43			
2 N Td	0.253	2.0	30	600	2,370	0.30	0.30 0.54		
	0.232	1.9	52	988	4,260	0.54			
3 N Td	0.235	2.0	33	660	2,810	0.35	0.35 0.34		
	0.218	1.7	35	595	2,730	0.34			
4 N Td	0.308	2.8	30	840	2,730	0.34	0.34 0.44		
	0.213	2.0	37	740	3,470	0.44			
5 N	0.263	2.9	22	638	2,430	0.31	0.31		

* N : TISSUE FROM NORMAL RATS

** Td : TISSUE FROM THYROIDECTOMIZED RATS

TABLE VII C

THE INFLUENCE OF ADDED THYROXINE (2.0×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (1.61×10^3 M)

(SERIES B c)

EXPERIMENT #	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL						
			GRAM	ML	IN 0.100 ML OF HOMOGENATE		CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	EXPRESSED AS ^3H -ADRENALINE
					COUNTS/MIN	COUNTS/MIN/G			
1 N * Td **	0.280	2.0	23	460	1,640	0.21	0.21 0.30		
	0.310	2.2	34	748	2,410	0.30			
2 N Td	0.557	2.9	31	899	2,520	0.32	0.32 0.32		
	0.366	3.1	30	930	2,540	0.32			
3 N Td	0.315	2.0	31	620	1,980	0.25	0.25 0.49		
	0.221	2.2	39	858	3,880	0.49			
4 N Td	0.348	3.0	20	600	1,720	0.22	0.22 0.54		
	0.181	2.3	34	782	4,320	0.54			
5 N	0.280	2.8	25	700	2,500	0.32	0.32		

* N : TISSUE FROM NORMAL RATS

** Td : TISSUE FROM THYROIDECTOMIZED RATS

T A B L E V I I D

THE INFLUENCE OF THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (1.61×10^{-8} M)

GROUP COMPARISON

EXPERIMENT	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.		T	DEGREE OF FREEDOM	P
		NORMAL	THYROIDECTOMIZED			
B	0.00×10^{-10} M	0.36 ± 0.12	0.46 ± 0.11	1.235	6	< 0.3
B A	0.02×10^{-10} M	0.33 ± 0.035	0.52 ± 0.12	2.235	5	< 0.1
B B	0.20×10^{-10} M	0.32 ± 0.021	0.44 ± 0.082	3.258	7	< 0.02
B C	2.00×10^{-10} M	0.26 ± 0.053	0.41 ± 0.12	2.525	7	< 0.05

CONCENTRATION OF THYROXINE (0.20×10^{-10} M), OR A HUNDRED TIMES (2.0×10^{-10} M) RENDERS THE DIFFERENCE NON-SIGNIFICANT.

IN THE NORMAL GROUP, THE ADDITION OF THYROXINE TO THE MEDIUM RESULTS IN A DECREASED UPTAKE. IN THE THYROIDECTOMIZED GROUP, THE ADDITION OF THYROXINE TO THE MEDIUM PROVOKES FIRST AN INCREASE THEN A DECREASE IN THE UPTAKE.

B) SERIES C : CONCENTRATION OF $^3\text{H-DL-ADRENALINE}$, 8.3×10^{-8} M

THE RESULTS OBTAINED WITH INDIVIDUAL EXPERIMENTS WITH A CONCENTRATION OF $^3\text{H-DL-ADRENALINE}$ FIVE TIMES HIGHER (8.3×10^{-8} M) ARE PRESENTED IN TABLE VIII, VIII A, VIII B, VIII C.

UPTAKE IN THE ABSENCE OF THYROXINE IN THE MEDIUM :

IN TABLE VIII, THE EFFECTS OF ENDOGENOUS THYROXINE OF THE UPTAKE OF ADRENALINE ARE GIVEN. THE CONCENTRATION OF RADIOACTIVE MATERIAL IN TISSUES FROM BOTH NORMAL AND THYROIDECTOMIZED RATS IS HIGHER THAN THAT OBSERVED IN SERIES B. THERE IS A GREATER UPTAKE BY THYROIDECTOMIZED THAN BY NORMAL TISSUES.

UPTAKE IN THE PRESENCE OF THYROXINE :

ADDITION OF THYROXINE TO THE MEDIUM (0.02×10^{-10} M) APPEARS TO HAVE A SLIGHT EFFECT ON THE UPTAKE BY TISSUES FROM BOTH GROUPS (TABLE VIII A). TEN TIMES HIGHER A CONCENTRATION OF THYROXINE DOES INCREASE THE UPTAKE OF ADRENALINE BY TISSUES FROM NORMAL AND THYROIDECTOMIZED RATS. THE UPTAKE OF ADRENALINE IS STILL HIGHER FOR THE THYROIDECTOMIZED TISSUES (TABLE VIII B). INCREASING FURTHER THE CONCENTRATION OF THYROXINE, PRODUCED A FURTHER INCREASE IN THE UPTAKE BY THYROIDECTOMIZED AND NORMAL TISSUES (TABLE VIII C). BUT THE UPTAKE IS STILL

TABLE VIII

THE INFLUENCE OF THYROIDECTOMY ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (6.3×10^{-8} M)

(SERIES C)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE
			IN 0.100 ML. OF HOMOGENATE		CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	
			COUNTS/MIN	COUNTS/MIN			
1 H To	1.656 1.600	7.0 7.4	140 157	9,870 11,620	5,950 7,260	1.5 1.8	
2 H To	1.210 1.016	5.6 5.0	92 149	5,150 7,450	4,260 7,330	1.1 1.8	
3 H To	1.204 0.804	5.4 5.0	125 137	6,750 6,850	5,610 8,120	1.4 2.0	
4 H To	0.674 1.226	3.3 6.2	129 164	4,260 11,410	6,320 9,310	1.6 2.3	
5 H To	1.071 0.890	4.5 3.5	142 181	6,390 6,340	5,970 7,120	1.5 1.7	
6 H To	0.684 0.757	2.9 2.8	335 527	9,720 14,760	14,200 19,490	2.0 2.7	
7 H To	0.786 0.892	3.6 4.2	305 367	10,800 15,414	13,968 17,260	1.9 2.4	

3 H : TISSUE FROM NORMAL RATS

3 H To : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E V I I I A

THE INFLUENCE OF ADDED THYROXINE (0.02×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

(SERIES C A)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE
			IN 0.100 ML OF HOMOGENATE	CONTENT OF HOMOGENATE	PER GRAM OF TISSUE		
			COUNTS/MIN	COUNTS/MIN	COUNTS/MIN/G	10^3 MOLES / G.	
1 N To	0.266	1.9	186	3,570	13,430	1.7	
	0.284	2.0	321	6,420	22,610	2.8	
2 N TD	0.382	2.4	189	4,540	11,870	1.5	
	0.426	3.0	293	8,790	20,540	2.5	
3 N TD	0.246	2.2	170	3,740	10,810	1.4	
	0.338	2.4	234	5,620	16,620	2.1	
4 N TD	0.430	2.5	178	4,450	10,350	1.3	
	0.354	2.5	208	5,200	14,690	1.9	
5 N TD	0.394	2.5	230	5,750	14,590	1.6	
	0.328	2.5	251	6,260	19,130	2.4	

* N : TISSUE FROM NORMAL RATS

* * TO : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE VIII B

THE INFLUENCE OF ADDED THYROXINE (0.2×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

(SERIES C B)

EXPERIMENT	WEIGHT OF TISSUE GRAM	VOLUME OF HOMOGENATE ML	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE
			IN 0.100 ML OF HOMOGENATE COUNTS/MIN	CONTENT OF HOMOGENATE COUNTS/MIN	PER GRAM OF TISSUE COUNTS/MIN/G	10^{11} MOLES / G	
1 N* Td**	0.326	2.5	284	6,100	18,700	2.4	
	0.256	2.0	404	8,080	31,560	4.0	
2 N Td	0.300	2.3	211	4,850	16,180	2.0	
	0.236	2.5	226	5,650	23,940	3.0	
3 N Td	0.310	2.3	230	5,290	17,070	2.2	
	0.234	2.1	310	6,310	27,820	3.5	
4 N Td	0.374	2.3	198	5,540	14,820	1.9	
	0.218	2.1	303	6,360	29,190	3.7	
5 N Td	0.314	2.5	231	5,780	18,390	2.3	
	0.196	1.9	264	5,460	27,530	3.5	

* N : TISSUE FROM NORMAL RATS

** Td : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE VIII C

THE INFLUENCE OF ADDED TRYROXINE (2.0×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

(SERIES C C)

EXPERIMENT #	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL						
			GRAM	NL.	IN 0.100 NL. OF HOMOGENATE		PER GRAM OF TISSUE		EXPRESSED AS ^3H -ADRENALINE 10^{11} MOLES / G
					COURTS/MIN	COUNTS/MIN	CONTENT OF HOMOGENATE	COURTS/MIN/G	
1 T ₀ ^e	0.288	1.7	289	4,910	17,060	2.1			
2 T ₀	0.342	2.0	282	5,540	16,490	2.1			
3 T ₀	0.236	2.0	282	5,640	23,900	3.0			
3 T ₀	0.250	2.2	207	4,550	18,220	2.3			
4 H ^{ee}	0.337	2.6	217	5,640	16,740	2.1			
4 T ₀	0.270	2.0	234	4,680	17,330	2.2			
5 H T ₀	0.360	2.1	261	5,900	16,390	2.1			
5 T ₀	0.278	2.2	357	7,850	28,280	3.6			
6 H	0.312	2.5	286	7,150	22,920	2.9			
6 H	0.314	2.2	286	6,290	20,040	2.5			
7 H	0.036	3.1	208	6,440	19,170	2.4			

^e T₀ : TISSUE FROM THYROIDECTOMIZED RATS

^{ee} H : TISSUE FROM NORMAL RATS.

HIGHER IN THYROIDECTOMIZED ANIMALS.

STATISTICAL ANALYSIS OF THE RESULTS :

IN TABLE VIII D, THE STATISTICAL ANALYSIS FOR THE INDIVIDUAL RESULTS OF SERIES A AND C ARE GIVEN.

A GROUP COMPARISON BETWEEN NORMAL AND THYROIDECTOMIZED GROUPS AND BETWEEN THYROIDECTOMIZED AND THYROXINE-TREATED GROUPS IS PRESENTED. THERE IS NO DIFFERENCE IN THE UPTAKE BETWEEN NORMAL AND THYROXINE-TREATED RATS. THE DIFFERENCE IN THE UPTAKE BETWEEN THYROIDECTOMIZED AND NORMAL GROUPS IS NOT SIGNIFICANT. THERE IS A LARGER DIFFERENCE BETWEEN THYROIDECTOMIZED AND THYROXINE-TREATED GROUPS WHICH IS NOT SIGNIFICANT EITHER.

THE ADDITION OF THYROXINE RESULTS IN A STATISTICALLY SIGNIFICANT DIFFERENCE IN THE UPTAKE BETWEEN NORMAL AND THYROIDECTOMIZED RATS. IN THE THYROIDECTOMIZED RATS, THE UPTAKE IS HIGHER FOR THE TWO LOWEST CONCENTRATIONS OF THYROXINE (0.02×10^{-10} M AND 0.20×10^{-10} M); AT A CONCENTRATION OF THYROXINE OF 2.00×10^{-10} M, THE DIFFERENCE BETWEEN THE TWO GROUPS IS NOT SIGNIFICANT ANYMORE.

IN THE NORMAL GROUP, THE ADDITION OF THYROXINE TO THE INCUBATION MEDIUM INCREASES THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUE. IN THE THYROIDECTOMIZED GROUP, THERE IS AN INCREASE IN THE UPTAKE FOR THE FIRST TWO CONCENTRATIONS OF THYROXINE ASSAYED. THEN THERE IS A DECREASE IN THE UPTAKE; HOWEVER, IT DOES NOT FALL TO THE LEVEL OBSERVED IN THE ABSENCE OF THYROXINE.

T A B L E V I I I O

THE INFLUENCE OF THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS

AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

GROUP COMPARISON

EXPERIMENTS	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.			T	D.F.	P
		NORMAL	THYROIDECTOMIZED	T4-TREATED			
A, C	$0.00 \times 10^{-10}\text{M}$	1.6 ± 0.30	$2.1 \pm 0.37^*$	$1.6 + 0.32^{**}$	2.941*	14	< 0.02
C A	$0.02 \times 10^{-10}\text{M}$	1.5 ± 0.21	2.3 ± 0.35		2.381**	8	< 0.05
C B	$0.20 \times 10^{-10}\text{M}$	2.2 ± 0.21	3.5 ± 0.37		4.444	8	< 0.01
C C	$2.00 \times 10^{-10}\text{M}$	2.4 ± 0.34	$2.6 + 0.62$		6.842	8	< 0.01
					1.111	9	< 0.30

* GROUP COMPARISON BETWEEN NORMAL AND THYROIDECTOMIZED

** GROUP COMPARISON BETWEEN THYROIDECTOMIZED AND THYROXINE-TREATED.

c) SERIES D : CONCENTRATION OF ³H-DL-ADRENALINE,
16.1 x 10⁻⁸ M

THE INDIVIDUAL RESULTS FOR THIS SERIES OF EXPERIMENTS ARE SHOWN IN TABLE IX, IX A, IX B, IX C AND THE STATISTICAL ANALYSIS IN IX D.

UPTAKE IN THE ABSENCE OF THYROXINE :

IN TABLE IX, THE EFFECT OF ENDOGENOUS THYROXINE, ON THE UPTAKE OF ADRENALINE BY INTESTINAL STRIPS, AT A CONCENTRATION OF ³H-DL-ADRENALINE 2 TIMES THAT OF SERIES C ARE PRESENTED. IN BOTH THYROIDECTOMIZED AND NORMAL GROUPS, THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUES IS HIGHER THAN THAT OBSERVED IN THE PREVIOUS SERIES OF EXPERIMENTS. THE INCREASED UPTAKE IS MORE MARKED AMONG THE THYROIDECTOMIZED THAN AMONG THE NORMAL RATS.

UPTAKE IN THE PRESENCE OF THYROXINE :

THE ADDITION OF THYROXINE TO THE MEDIUM (TABLE IX A) DECREASES THE UPTAKE FOR BOTH GROUPS. THIS DECREASE IS MORE MARKED WITH THE TISSUES FROM THYROIDECTOMIZED ANIMALS. IN TABLE IX B, THE EFFECT, ON THE UPTAKE, OF TEN TIMES THE CONCENTRATION OF THYROXINE SHOWS AN INCREASE WHEN COMPARING TO THE PREVIOUS TABLE, BUT NO CHANGE OR A DECREASE WHEN COMPARING WITH NO THYROXINE IN THE MEDIUM. FURTHER INCREASE OF THYROXINE CONCENTRATION (TABLE IX C) DOES NOT PRODUCE ANY CHANGE IN THE UPTAKE.

STATISTICAL ANALYSIS OF THE RESULTS :

THE HIGHER UPTAKE BY THYROIDECTOMIZED TISSUES COMPARED TO NORMAL TISSUES, OBSERVED IN THE PREVIOUS SERIES OF EXPERIMENTS IS STILL OBTAINED HERE (TABLE IX D). THERE IS A SIGNIFICANT DIFFERENCE

T A B L E I X

THE INFLUENCE OF THYROIDECTOMY ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (15.1×10^{-8} M)

(SERIES D)

EXPERIMENT #	WEIGHT OF TISSUE GRAM	VOLUME OF HOMOGENATE ML	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE 10^{11} MOLES / G
			IN 0.100 ML OF HOMOGENATE		PER GRAM OF TISSUE		
			COUNTS/MIN	COUNTS/MIN	CONTENT OF HOMOGENATE	COUNTS/MIN/G	
1 N ^o To ^{oo}	0.394	2.9	328	9,510	24,140	3.0	
	0.314	1.9	665	12,640	40,240	5.1	
2 N ^o To	0.433	3.4	377	12,620	29,660	3.7	
	0.320	2.0	747	14,940	46,590	5.9	
3 N ^o To	0.392	2.7	328	8,060	22,250	2.8	
	0.296	2.0	560	11,200	37,840	4.8	
4 N ^o To	0.482	3.6	342	12,310	25,540	3.2	
	0.355	2.6	494	12,840	40,140	5.1	

^o N : TISSUE FROM NORMAL RATS

^{oo} To : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E I X A

THE INFLUENCE OF ADDED THYROXINE (0.02×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL
BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

(SERIES D A)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL					
			GRAM	ML	IN 0.100 ML OF HOMOGENATE	CONTENT OF HOMOGENATE	PER GRAM OF TISSUE	EXPRESSED AS ^3H -ADRENALINE
1 N*	0.389	2.5	342	8,550	21,980	3.0		
1 D**	0.352	2.6	304	7,900	20,470	2.8		
2 N	0.522	3.4	241	7,850	18,080	2.1		
2 D	0.440	3.3	395	13,040	29,630	4.1		
3 N	0.502	2.5	348	8,700	17,330	2.2		
4 R	0.370	2.2	313	6,890	18,610	2.4		
4 D	0.300	2.3	479	11,020	28,990	3.8		
5 N	0.480	2.3	420	9,660	19,960	2.6		
6 N	0.460	2.2	395	8,690	18,890	2.5		
6 D	0.446	2.5	714	17,850	40,030	5.0		
7 D	0.562	2.2	553	12,620	33,490	4.4		

* N : TISSUE FROM NORMAL RATS ** D : TISSUE FROM THYROIDECTOMIZED RATS

T A B L E 1 X B

THE INFLUENCE OF ADDED THYROXINE (0.20×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

(SERIES D 9)

EXPERIMENT	WEIGHT OF TISSUE	VOLUME OF HOMOGENATE	RADIOACTIVE MATERIAL					
			IN 0.100 ML OF HOMOGENATE		CONTENT OF HOMOGENATE		PER GRAM OF TISSUE	EXPRESSED AS
			COUNTS/MIN	COUNTS/MIN	COUNTS/MIN	COUNTS/MIN	COUNTS/MIN/G	^3H -ADRENALINE
1 N * TD **	0.368	2.7	273	7,370	20,030	2.6		
	0.332	2.5	439	10,980	33,060	4.3		
2 N TD	0.517	3.1	454	14,070	27,220	3.5		
	0.474	3.3	478	15,770	33,280	4.3		
3 N TD	0.288	2.4	359	8,140	20,250	3.7		
	0.346	2.7	480	12,960	37,460	4.9		
4 N TD	0.376	2.5	353	8,830	23,470	3.1		
	0.300	2.5	335	8,380	27,920	3.6		

* N : TISSUE FROM NORMAL RATS

** TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE IX C
 THE INFLUENCE OF ADDED THYROXINE (2.00×10^{-10} M) ON THE UPTAKE OF RADIOACTIVE MATERIAL
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)
 (SERIES D c)

EXPERIMENT	HEIGHT OF TISSUE GRAM	VOLUME OF HOMOGENATE ML	RADIOACTIVE MATERIAL				EXPRESSED AS ^3H -ADRENALINE 10^{11} MOLES/G
			IN 0.100 ML OF HOMOGENATE COUNTS/MIN	CONTENT OF HOMOGENATE COUNTS/MIN	PER GRAM OF TISSUE COUNTS/MIN/G		
1 N [*] Td ^{**}	0.327	2.4	378	9,070	27,740	3.6	
	0.305	2.4	441	10,580	34,700	4.5	
2 N Td	0.389	2.9	297	8,610	22,140	2.9	
	0.349	2.7	357	9,640	27,620	3.6	
3 N Td	0.311	2.8	255	7,140	22,960	3.0	
	0.277	2.2	637	14,010	50,590	6.6	
4 N Td	0.345	3.0	269	8,670	25,130	3.3	
	0.310	2.5	399	9,980	32,180	4.2	
5 Td	0.280	2.2	273	6,010	21,450	2.8	

* N : TISSUE FROM NORMAL RATS ** Td : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E I X D

THE INFLUENCE OF THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

GROUP COMPARISON

EXPERIMENT	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.		T	DEGREE OF FREEDOM	P
		NORMAL	THYROIDECTOMIZED			
D	0.00×10^{-10} M	3.2 ± 0.39	5.2 ± 0.47	6.667	6	< 0.01
D A	0.02×10^{-10} M	2.5 ± 0.32	4.0 ± 0.84	4.054	9	< 0.01
D B	0.20×10^{-10} M	3.2 ± 0.49	4.3 ± 0.53	3.056	6	< 0.02
D C	2.00×10^{-10} M	3.2 ± 0.32	4.3 ± 1.4	1.499	7	< 0.20

BETWEEN THE TWO GROUPS, IN THE ABSENCE OF THYROXINE, OR AT THE TWO LOWEST CONCENTRATIONS OF THYROXINE IN THE MEDIUM. HOWEVER, AT HIGH CONCENTRATION OF THYROXINE IN THE MEDIUM THE DIFFERENCE IS NOT SIGNIFICANT ANYMORE. THIS IS PROBABLY DUE TO THE FACT THAT AMONG THE THYROIDECTOMIZED RATS, THERE IS LARGE VARIATION.

IN THE NORMAL GROUP, THERE IS A DECREASE IN THE UPTAKE AFTER ADDITION OF THYROXINE, WHICH THEN INCREASES AGAIN TO THE LEVEL IN THE ABSENCE OF THYROXINE. IN THE THYROIDECTOMIZED GROUP, THE SAME PATTERN IS FOLLOWED, BUT THE LEVEL OF THE UPTAKE IN THE ABSENCE OF THYROXINE IS NEVER REACHED.

ANALYSIS OF VARIANCE WITH INTERACTION

THE EFFECT OF THYROXINE, ENDOGENOUS OR EXOGENOUS, THE EFFECT OF ADRENALINE AND THE CONJUGATED EFFECT OF THYROXINE AND ADRENALINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS, AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE HAS BEEN STUDIED BY A TREATMENT OF THE DATA OF THE THREE SERIES B, C, AND D. THE VALUES OBTAINED ARE PRESENTED IN TABLE X.

- EFFECT OF ENDOGENOUS THYROXINE. IN THE STATISTICAL TREATMENT, THE RESULTS OBTAINED WITH SERIES B, C, AND D, WHEN NO THYROXINE WAS PRESENT IN THE INCUBATION MEDIUM WERE COMPILED. THE VALUES OF P, FOR THYROXINE, ADRENALINE AND THYROXINE-ADRENALINE INTERACTION ARE SMALLER THAN 0.01. STATISTICALLY, THYROXINE IS SHOWN TO HAVE AN EFFECT, ALONE, ON THE UPTAKE OF RADIOACTIVE MATERIAL. ADRENALINE ALONE HAS ALSO AN EFFECT ON THE UPTAKE. THE EFFECT OF ADRENALINE,

IS HOWEVER, MUCH LARGER AS CAN BE SEEN WHEN LOOKING AT THE VALUE OF F WHICH IS MUCH HIGHER IN THE CASE OF ADRENALINE. ADRENALINE AND THYROXINE TOGETHER AFFECT THE UPTAKE OF RADIOACTIVE MATERIAL. HOWEVER, THE INTERACTION BETWEEN THE TWO GIVES A LOWER EFFECT THAN THAT OF THYROXINE OR ADRENALINE ALONE.

- EFFECT OF ADDED THYROXINE. IN THE STATISTICAL TREATMENT, THE RESULTS OF SERIES B, B A, B B, B C, C, C A, C B, C C, D, D A, D B, D C WERE COMPILED, FOR THE NORMAL RATS ON ONE HAND, AND FOR THE THYROIDECTOMIZED RATS ON THE OTHER HAND.

IN THE NORMAL RATS, THYROXINE ALONE DOES NOT HAVE AN EFFECT ON THE UPTAKE AS SHOWN BY THE VALUE OF P. ADRENALINE ALONE HAS A VERY LARGE EFFECT, NOT AS LARGE AS IN THE FIRST CASE. THYROXINE WHEN IN THE PRESENCE OF ADRENALINE HAS AN EFFECT.

IN THYROIDECTOMIZED RATS, THE UPTAKE IS SIMILARLY AFFECTED, BUT BOTH ADRENALINE AND THYROXINE-ADRENALINE EFFECTS ARE LESS MARKED. THE VALUES OF F ARE LOWER THAN THOSE OBTAINED WITH NORMAL RATS.

3) THE EFFECT OF THYROXINE AND ³H-DL-ADRENALINE ON THE CONCENTRATION OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS

THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS AS A FUNCTION OF THE CONCENTRATION OF ADRENALINE IN THE MEDIUM, AND AS A FUNCTION OF THYROXINE CONCENTRATION IN THE MEDIUM IS PRESENTED

T A B L E X

THE EFFECT OF THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY
INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE

ANALYSIS OF VARIANCE WITH INTERACTION

EFFECT OF ENDOGENOUS THYROXINE : SERIES B, C AND D.

SOURCE	D ⁰ F	F	P
THYROXINE	1 + 19	57.303	< 0.01
ADRENALINE	2 + 19	339.325	< 0.01
THYROXINE x ADRENALINE	2 + 19	21.910	< 0.01

EFFECT OF ADDED THYROXINE : SERIES B ABC, C ABC, D ABC.

- NORMAL RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 45	2.379	< 0.05
ADRENALINE	2 + 45	437.214	< 0.01
THYROXINE x ADRENALINE	6 + 45	12.060	< 0.01

- THYROIDECTOMIZED RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 43	1.075	> 0.05
ADRENALINE	2 + 43	185.374	< 0.01
THYROXINE x ADRENALINE	6 + 43	4.355	< 0.01

IN TABLE XI. EACH VALUE HAS BEEN CALCULATED BY DIVIDING THE MEAN VALUE PRESENTED IN TABLES VII D, VIII D AND IX D BY THE ADEQUATE VALUE FOR THE CONCENTRATION OF ADRENALINE IN THE MEDIUM. FOR THE THREE CONCENTRATIONS OF ADRENALINE ASSAYED, AND FOR ALL THE CONCENTRATIONS OF THYROXINE, THERE IS A HIGHER CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUES OF THYROIDECTOMIZED RATS.

IN THE NORMAL GROUP, WHEN THYROXINE IS ABSENT FROM THE MEDIUM, INCREASING THE CONCENTRATION OF ADRENALINE IN THE MEDIUM RESULTS IN A DECREASED CONCENTRATING MECHANISM. AT THE LOWEST CONCENTRATION OF ADRENALINE IN THE MEDIUM (1.61×10^{-8} M), ADDITION OF THYROXINE RESULTS IN A DECREASE IN THE CONCENTRATING MECHANISM. FOR THE CONCENTRATIONS OF ADRENALINE 5 AND 10 TIMES HIGHER, 0.02×10^{-10} M THYROXINE CAUSES A DECREASE IN THE CONCENTRATING MECHANISM. TEN OR 100 TIMES THAT CONCENTRATION OF THYROXINE RESULTS EITHER IN AN INCREASE, OR UNCHANGED CONCENTRATION.

IN THE THYROIDECTOMIZED GROUP, AT THE THREE CONCENTRATIONS OF ADRENALINE ASSAYED ($1.61, 8.3$ AND 16.1×10^{-8} M), IN THE ABSENCE OF THYROXINE IN THE MEDIUM, THERE IS A DECREASED THEN AN INCREASED CONCENTRATING MECHANISM. THE CONCENTRATING MECHANISM IS MORE EFFICIENT AT THE HIGHEST CONCENTRATION OF ADRENALINE IN THE MEDIUM. FOR THE TWO LOWER CONCENTRATIONS OF ADRENALINE, THE ADDITION OF THYROXINE RESULTS IN AN INCREASED

THEN DECREASED MECHANISM. THE PEAK, HOWEVER, IS NOT AT THE SAME LEVEL OF THYROXINE : 0.02×10^{-10} M APPEARS TO BE THE OPTIMUM CONCENTRATION OF THYROXINE WHEN ADRENALINE CONCENTRATION IS THE LOWEST (1.61×10^{-8} M) ; BY INCREASING 5 TIMES THE CONCENTRATION OF ADRENALINE, THE OPTIMUM CONCENTRATION OF THYROXINE BECOMES TEN TIMES HIGHER (0.20×10^{-10} M). FURTHERMORE, WHILE IN THE FORMER CASE, INCREASING THYROXINE CONCENTRATION RESULTS IN A DECREASED CONCENTRATING MECHANISM BELOW THE CONTROL LEVEL IN THE ABSENCE OF THYROXINE, IN THE LATTER CASE THERE IS A DECREASED LEVEL COMPARED TO THE OPTIMUM, BUT THIS LEVEL NEVER REACHED THE VALUE OBTAINED IN THE ABSENCE OF THYROXINE. AT THE HIGHEST CONCENTRATION OF ADRENALINE IN THE MEDIUM (16.1×10^{-8} M), THE ADDITION OF THYROXINE RESULTS IN A DECREASED CONCENTRATING POWER OF THE TISSUE, THEN A SMALL INCREASE, WHICH HOWEVER, NEVER ATTAINS THE VALUE OBSERVED IN THE ABSENCE OF THYROXINE IN THE MEDIUM. IT IS INTERESTING TO NOTE THAT AT THE TWO EXTREME CONCENTRATIONS OF ADRENALINE ASSAYED (1.61×10^{-8} M) AND (16.1×10^{-8} M), THE LOWER CONCENTRATION OF THYROXINE ASSAYED (0.02×10^{-10} M) SEEMS CRITICAL. WHEN THE CONCENTRATION OF ADRENALINE IS LOW, IT HAS AN INCREASING EFFECT ON THE CONCENTRATION MECHANISM. WHEN THE CONCENTRATION OF ADRENALINE IS TEN TIMES HIGHER IT EXHIBITS A DECREASING EFFECT.

TABLE XI
 THE EFFECT OF THYROXINE ON THE CONCENTRATING MECHANISM OF ³H-ADRENALINE BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ³H-OL-ADRENALINE

THYROXINE CONCENTRATION IN THE MEDIUM x 10 ¹⁰ M	$\frac{[\text{ }^3\text{H-ADRENALINE}]_T}{[\text{ }^3\text{H-ADRENALINE}]_M} \times 100$					
	³ H-ADRENALINE CONCENTRATION IN THE MEDIUM 1.61 x 10 ⁻⁸ M		³ H-ADRENALINE CONCENTRATION IN THE MEDIUM 5.3 x 10 ⁻⁸ M		³ H-ADRENALINE CONCENTRATION IN THE MEDIUM 16.1 x 10 ⁻⁸ M	
	NORMAL %	THYROIDECTOMIZED %	NORMAL %	THYROIDECTOMIZED %	NORMAL %	THYROIDECTOMIZED %
0	22.4	28.6	19.3	25.3	19.9	32.5
0.02	20.5	32.3	16.1	27.7	15.5	24.8
0.20	19.9	27.3	26.5	42.2	19.9	26.7
2.00	16.1	25.5	28.9	31.3	19.9	26.7

[³H-ADRENALINE]_T : RADIOACTIVE MATERIAL PRESENT IN THE TISSUE EXPRESSED AS ³H-ADRENALINE μ MOLES PER GRAM OF TISSUE
 [³H-ADRENALINE]_M : ³H-ADRENALINE PUT IN THE INCUBATION MEDIUM EXPRESSED IN MOLES PER ML OF MEDIUM.

B) THE EFFECT OF THYROXINE ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE

THE ADRENALINE WAS IDENTIFIED ON THE EXTRACTS FROM SERIES A, C AND D. THE LOW RADIOACTIVITY CONTENT IN SERIES B, DID NOT ALLOW THE IDENTIFICATION OF ^3H -ADRENALINE.

1) THE IN VIVO EFFECT OF THYROXINE

IN THIS SERIES OF EXPERIMENTS, THE IN VIVO EFFECT OF ^3H -ADRENALINE ON THE ^3H -ADRENALINE BINDING BY INTESTINAL STRIPS WAS TESTED. THE RESULTS OBTAINED DURING THIS STUDY ARE PRESENTED IN TABLE XII. FIVE MINUTES AFTER THE INCUBATION, THE PERCENTAGE OF ADRENALINE IS HIGH, IN THE THREE GROUPS ASSAYED. THERE IS NO APPARENT DIFFERENCE BETWEEN NORMAL, THYROIDECTOMIZED AND THYROXINE-TREATED GROUPS, WHERE THE CONCENTRATION OF BOUND ^3H -ADRENALINE IS CONCERNED.

2) THE IN VITRO EFFECT OF THYROXINE

A) SERIES C : CONCENTRATION OF ^3H -DL-ADRENALINE,
 $8.3 \times 10^{-8} \text{ M}$

IN THE NEXT THREE TABLES (XII A, XII B, AND XII C) THE INDIVIDUAL RESULTS IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF THYROXINE IN THE MEDIUM ARE SHOWN. THE ADDITION OF THYROXINE ($0.02 \times 10^{-10} \text{ M}$) TO THE MEDIUM (TABLE XII A) RESULTED IN A LOWERING OF THE PERCENTAGE OF ^3H -ADRENALINE IN THE TISSUE. IN BOTH NORMAL AND THYROIDECTOMIZED GROUPS, THE PERCENTAGE OF ^3H -ADRENALINE IS LOWERED COMPARED TO THAT IN THE ABSENCE OF THYROXINE. THE VARIATIONS IN THE PERCENTAGE ARE HIGHER IN THE NORMAL THAN IN THE THYROIDECTOMIZED GROUP. THE ABSOLUTE VALUES (MOLES PER GRAM) ARE HIGHER IN THE CASE OF

TABLE XII

THE EFFECT OF THYROXINE ON THE BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS

AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE (8.3 x 10⁻⁸ M)

(SERIES A & C)

EXPERIMENT #	RADIOACTIVE MATERIAL		CORRECTION FOR 80% RECOVERY %	³ H-ADRENALINE PER GRAM OF TISSUE 10 ¹¹ MOLES/G
	TOTAL PRESENT IN PERCHLORIC EXTRACT COUNTS/MIN	³ H-ADRENALINE EXTRACTED COUNTS/MIN		
5 N ^o	2,385	1,188	62.3	0.93
6 N	2,223	893	50.3	0.75
9 N	7,000	3,433	61.8	1.2
10 N	3,510	1,845	65.8	1.3
11 N	4,900	1,800	53.8	1.0
5 T ₀ **	2,038	1,325	81.3	1.4
10 T ₀	4,375	2,243	64.1	1.1
11 T ₀	4,900	2,583	65.3	1.7
6 T ₄ ***	2,325	1,160	62.4	1.0
7 T ₄	425	203	59.6	0.72
T ₄	6,175	3,445	52.6	1.0
8 T ₄	4,430	1,773	49.8	0.70
9 T ₄	4,660	2,010	54.0	0.97

** N : TISSUE FROM NORMAL RATS ** T₀ : TISSUE FROM THYROIDECTOMIZED RATS *** T₄ : TISSUE FROM THYROXINE-TREATED RATS.

TABLE XII A
 THE EFFECT OF ADDED THYROXINE (0.02×10^{-10} M) ON THE BINDING OF ^3H -ADRENALINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (6.3×10^{-8} M)
 (SERIES C A)

EXPERIMENT	RADIOACTIVE MATERIAL				CORRECTION FOR 80% RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -ADRENALINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN	%			
1 N*	860		220		25.6	0.54	
2 N	930		170		18.3	0.34	
3 N	860		247		28.7	0.50	
4 N	720		279		38.8	0.63	
5 N	1,265		334		26.4	0.59	
1 TD**	1,640		388		26.7	0.63	
2 TD	1,695		493		29.1	0.91	
3 TD	1,210		379		31.3	0.82	
4 TD	1,075		300		27.9	0.66	
5 TD	1,270		407		32.0	0.96	

* N : TISSUE FROM NORMAL RATS ** TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XII B
 THE EFFECT OF ADDED THYROXINE (0.20×10^{-10} M) ON THE BINDING OF ^3H -ADRENALINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (6.3×10^{-8} M)

(SERIES C B)

EXPERIMENT	RADIOACTIVE MATERIAL			CORRECTION FOR 60% RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT	^3H -ADRENALINE EXTRACTED	PERCENT OF TOTAL		
	COUNTS/MIN	COUNTS/MIN	%		
1 H*	1,160	528	45.5	56.9	1.4
2 N	1,020	458	44.9	56.1	1.1
3 N	1,090	422	38.7	48.4	1.1
4 N	950	374	39.4	49.3	0.94
5 N	1,040	498	47.6	59.9	1.4
1 T ^o	1,750	660	37.7	47.1	1.9
2 T ^o	1,100	476	43.3	54.1	1.6
3 T ^o	1,380	964	69.9	87.4	3.1
4 T ^o	1,310	594	45.3	56.6	2.1
5 T ^o	1,220	576	47.2	59.0	2.1

* H : TISSUE FROM NORMAL RATS

** T^o : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E X I I I c

THE EFFECT OF ADDED THYROXINE (2.00×10^{-10} M) ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

(SERIES C c)

EXPERIMENT #	RADIOACTIVE MATERIAL			CORRECTION FOR 80 % RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT		PERCENT OF TOTAL		
	COUNTS/MIN	^3H -ADRENALINE EXTRACTED COUNTS/MIN			
1 N *	970	390	40.2	50.3	1.1
2 N	1,030	468	45.4	56.8	1.2
3 N	1,170	520	44.4	55.5	1.6
4 N	1,200	568	47.3	59.1	1.5
5 N	880	404	46.0	57.5	1.4
1 Td **	1,150	568	49.4	61.8	1.3
2 Td	980	488	49.8	62.3	1.3
3 Td	1,120	472	42.1	52.6	1.6
4 Td	950	384	40.4	50.5	1.2
5 Td	900	462	51.3	64.1	1.4
6 Td	1,230	674	54.8	68.5	2.5

1 1 4 1

* N : TISSUE FROM NORMAL RATS

** Td : TISSUE FROM THYROIDECTOMIZED RATS.

THE THYROIDECTOMIZED GROUP, ALTHOUGH THIS DIFFERENCE WAS NOT APPARENT WHEN THE PERCENTAGE VALUES WERE CONSIDERED. THIS IS DUE TO THE FACT THAT THE UPTAKE WAS HIGHER IN THE SECOND GROUP. INCREASING TEN TIMES (TABLE XII B) THE CONCENTRATION OF THYROXINE PRODUCES AN INCREASE IN THE PERCENTAGE OF ADRENALINE IN THE TISSUE. THE SAME ACTION IS OBSERVED WHEN THE CONCENTRATION OF THYROXINE IS ELEVATED 100 TIMES (TABLE XII C). THE FACT NOTICED PREVIOUSLY IS ALSO OBVIOUS IN THE TWO CASES ; THE PERCENTAGE OF ³H-ADRENALINE IS IDENTICAL IN THE CASE OF NORMAL AND THYROIDECTOMIZED GROUPS, BUT THE ABSOLUTE VALUES ARE HIGHER IN THE LATTER GROUP. FOR THESE TWO CONCENTRATIONS OF THYROXINE, THE VARIATIONS ARE HIGHER, BETWEEN INDIVIDUAL EXPERIMENTS, FOR THE THYROIDECTOMIZED GROUP.

STATISTICAL ANALYSIS OF THE RESULTS :

IN TABLE XII B, STATISTICAL ANALYSIS FOR THE INDIVIDUAL DATA PRESENTED, IS GIVEN. THE EFFECT OF THYROXINE, IN VIVO, ON THE BINDING OF ³H-ADRENALINE DOES NOT APPEAR SIGNIFICANT AS SHOWN BY THE P VALUES OF 0.02 AND 0.05, WHEN COMPARING THYROXINE-TREATED VERSUS THYROIDECTOMIZED AND NORMAL VERSUS THYROIDECTOMIZED GROUPS. THE ADDITION OF THYROXINE TO THE MEDIUM, RESULTS IN A SIGNIFICANTLY HIGHER BINDING IN THE THYROIDECTOMIZED GROUP, AS SHOWN BY THE VALUES OF P INFERIOR AT 0.01 FOR THE TWO LOWER CONCENTRATIONS OF THYROXINE (0.02 AND 0.20 x 10⁻¹⁰ M). FURTHER INCREASE IN THYROXINE CONCENTRATION RESULTS IN THE DISAPPEARANCE OF THE SIGNIFICANT DIFFERENCE BETWEEN THE TWO GROUPS. AS NOTED PREVIOUSLY, FOR THE TWO GROUPS, THYROIDECTOMIZED AND NORMAL, ADDITION OF THYROXINE TO THE MEDIUM RESULTS IN A

T A B L E X I I I O

THE EFFECT OF THYROXINE ON THE BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS

AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE (8.3×10^{-8} M)

GROUP COMPARISON

EXPERIMENTS	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.		T	D.F.	P
		NORMAL	THYROIDECTOMIZED			
C A	0.00×10^{-10} M	1.0 \pm 0.22	1.4 \pm 0.30 *	2.548	6	< 0.05
				0.88 \pm 0.15**	3.590	6
C A	0.02×10^{-10} M	0.52 \pm 0.11	0.84 \pm 0.11	4.571	8	< 0.01
C B	0.20×10^{-10} M	1.2 \pm 0.21	2.2 \pm 0.57	3.704	6	< 0.01
C C	2.00×10^{-10} M	1.4 \pm 0.21	1.6 \pm 0.49	0.870	9	< 0.50

* GROUP COMPARISON BETWEEN NORMAL AND THYROIDECTOMIZED RATS

** GROUP COMPARISON BETWEEN THYROIDECTOMIZED AND THYROXINE-TREATED ANIMALS.

DECREASE IN ^3H -ADRENALINE BINDING. WHILE IN THE NORMAL GROUP, FURTHER ADDITION PROVOKES AN INCREASE IN BINDING COMPARED TO THAT OBSERVED IN THE ABSENCE OF THYROXINE IN THE MEDIUM, IN THE THYROIDECTOMIZED GROUP THERE IS AN OPTIMUM CONCENTRATION OF THYROXINE (0.20×10^{-10} M). FURTHER INCREASE IN THYROXINE CONCENTRATION CAUSES A FALL IN THE BINDING, THIS FALL HOWEVER, DOES NOT REACH THE LEVEL OBTAINED IN THE ABSENCE OF THYROXINE IN THE MEDIUM.

b) SERIES D : CONCENTRATION OF ^3H -DL-ADRENALINE,
 16.1×10^{-8} M

THE EFFECT OF DOUBLING THE CONCENTRATION OF ADRENALINE IN THE INCUBATION MEDIUM IS SHOWN IN THE NEXT TABLES XIII, XIII A, XIII B AND XIII C.

IN TABLE XIII, THE INDIVIDUAL RESULTS OBTAINED WITH NORMAL AND THYROIDECTOMIZED TISSUES, WHEN NO THYROXINE HAS BEEN ADDED TO THE MEDIUM ARE GIVEN. ALTHOUGH AT THAT CONCENTRATION OF ADRENALINE, THE UPTAKE IS HIGHER, IN BOTH GROUPS, THE PERCENTAGE OF BOUND ^3H -ADRENALINE IS LOW. THE PERCENTAGE OF BOUND ADRENALINE SEEMS SLIGHTLY HIGHER IN THE THYROIDECTOMIZED THAN IN THE NORMAL GROUP. WHEN THE RESULTS ARE EXPRESSED AS ABSOLUTE VALUES, THE BOUND ^3H -ADRENALINE IS MORE IMPORTANT IN THE THYROIDECTOMIZED GROUP.

ADDITION OF THYROXINE (0.02×10^{-10} M) TO THE INCUBATION MEDIUM (TABLE XIII A) FURTHER INCREASES THE DIFFERENCE OBSERVED BETWEEN THIS TWO GROUPS. THE PERCENTAGE OF BOUND ^3H -ADRENALINE AND THE ABSOLUTE CONTENT OF THE TISSUE ARE SUPERIOR IN THE THYROIDECTOMIZED

GROUP. ALTHOUGH THE VARIATIONS BETWEEN INDIVIDUAL RESULTS ARE HIGHER IN THE NORMAL GROUP, WHEN THE PERCENTAGE VALUES ARE CONSIDERED, EXPRESSING THE RESULTS IN MOLES OF ^3H -ADRENALINE RESULTS IN THE FINDING THAT THE VARIATIONS ARE HIGHER IN THE THYROIDECTOMIZED GROUP. IN TABLE XIII B, INDIVIDUAL RESULTS IN THE PRESENCE OF TEN TIMES THE CONCENTRATION OF THYROXINE ARE GIVEN. THE PERCENTAGE OF BOUND ^3H -ADRENALINE IS MUCH DECREASED, WHEN THE CONCENTRATION OF THYROXINE IN THE MEDIUM IS TEN TIMES INCREASED, IN THE CASE OF THYROIDECTOMIZED TISSUES. AN INVERSE EFFECT APPEARS TO BE FOUND IN THE NORMAL GROUP, SO THAT THE RELATIVE AMOUNT OF ^3H -ADRENALINE IS ALMOST SIMILAR AMONG THE TWO GROUPS. THIS SIMILARITY IS NOT SO EVIDENT IN THE ABSOLUTE VALUES, ESPECIALLY BECAUSE THERE IS A LARGE VARIATION IN THE NORMAL GROUP. SIMILAR FINDINGS ARE OBSERVED WHEN THYROXINE IS INCREASED TEN TIMES MORE (TABLE XIII C, 2.00×10^{-10} M). ALTHOUGH, THE RELATIVE CONCENTRATIONS OF ^3H -ADRENALINE ARE, IN THE THYROIDECTOMIZED GROUP, OF VERY SIMILAR VALUES, EXPRESSING THESE RESULTS IN ABSOLUTE AMOUNTS CAUSES A MARKED VARIATION IN INDIVIDUAL EXPERIMENTS.

STATISTICAL ANALYSIS OF THE RESULTS :

THESE VARIATIONS ARE REFLECTED IN THE STATISTICAL ANALYSIS OF THE RESULTS PRESENTED IN TABLE XIII D. THE STANDARD DEVIATIONS SHOW THAT THE VARIATIONS AMONG INDIVIDUAL RESULTS ARE HIGHER THAN THOSE OBSERVED AT HALF THE CONCENTRATION OF ADRENALINE IN THE MEDIUM. IN SPITE OF THESE LARGE VARIATIONS, THERE IS A SIGNIFICANT INCREASE IN

TABLE XIII
 THE EFFECT OF THYROIDECTOMY ON THE BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE (16.1 x 10⁻⁸ M)
 (SERIES D)

EXPERIMENT #	RADIOACTIVE MATERIAL				CORRECTION FOR 50% RECOVERY	³ H-ADRENALINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		³ H-ADRENALINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN	%			
1 N ^o	2,660		553	20.8	26.0	0.97	
2 N	3,340		884	26.4	33.1	1.2	
3 N	2,300		669	29.1	36.4	1.0	
4 N	3,100		757	24.4	30.5	0.98	
1 TD ^{oo}	4,420		1,280	29.0	36.3	1.9	
2 TD	5,100		1,374	26.8	33.6	2.0	
3 TD	3,480		1,065	30.6	38.3	1.8	
4 TD	3,800		983	25.9	32.4	1.7	

^o N : TISSUE FROM NORMAL RATS ^{oo} TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XIII A

THE EFFECT OF ADDED THYROXINE (0.02×10^{-10} H) ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

(SERIES D A)

EXPERIMENT ↓	RADIOACTIVE MATERIAL				CORRECTION FOR 80% RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -ADRENALINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN				
1 N °	2,740	625	22.8	28.5	0.63		
2 N	2,330	577	24.6	30.9	0.74		
3 N	3,050	574	28.7	35.8	0.93		
4 N	3,740	573	15.3	19.1	0.48		
1 TD **	1,860	819	44.0	55.1	2.3		
3 TD	3,930	951	24.2	30.3	1.2		
4 TD	6,280	2,456	39.1	48.9	2.5		
5 TD	4,260	1,221	28.7	35.8	1.6		

° N : TISSUE FROM NORMAL RATS

** TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XIII 9

THE EFFECT OF ADDED THYROXINE (0.20×10^{-10} M) ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

(SERIES D 10)

EXPERIMENT #	RADIOACTIVE MATERIAL			CORRECTION FOR 80% RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT	^3H -ADRENALINE EXTRACTED	PERCENT OF TOTAL		
	COUNTS/MIN	COUNTS/MIN	%		
1 N *	2,320	490	21.1	26.4	0.69
2 N	4,610	848	18.4	23.0	0.81
3 N	1,800	679	37.7	47.2	1.8
4 N	2,790	650	23.3	29.1	0.90
1 T0 **	3,750	980	26.1	32.7	1.4
2 T0	4,520	1,205	26.7	33.3	1.4
3 T0	3,570	982	27.5	34.4	1.7
4 T0	2,900	761	24.2	30.2	1.1

* N : TISSUE FROM NORMAL RATS

** T0 : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E X I I I c

THE EFFECT OF ADDED THYROXINE (2.00×10^{-10} M) ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

(SERIES D c)

EXPERIMENT	RADIOACTIVE MATERIAL				CORRECTION FOR 80% RECOVERY	^3H -ADRENALINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -ADRENALINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN				
1 N *	2,700	585	21.8	27.3	0.98		
2 N	1,800	678	37.7	47.1	1.4		
3 N	2,070	631	30.5	38.1	1.1		
4 N	2,550	558	21.9	27.4	0.90		
1 TD **	3,570	853	23.9	29.9	1.4		
2 TD	1,895	523	27.6	34.5	1.2		
3 TD	3,410	597	29.2	36.5	2.4		
4 TD	2,940	748	25.4	31.8	1.3		

* N : TISSUE FROM NORMAL RATS

** TD : TISSUE FROM THYROIDECTOMIZED RATS.

³H-ADRENALINE BINDING IN THE THYROIDECTOMIZED GROUP WHEN COMPARED TO THE NORMAL ONE, IN THE ABSENCE OF EXOGENOUS THYROXINE. THE P VALUE IS INFERIOR TO 0.01.

THE ADDITION OF THYROXINE (0.02×10^{-10} M) DOES NOT PREVENT THIS INCREASE, P VALUE SMALLER THAN 0.01, BUT IT DECREASES IT, AS CAN BE SEEN BY A LOWER VALUE OF T. FURTHER ADDITION OF THYROXINE TO THE MEDIUM RESULTS IN THE DISAPPEARANCE OF A SIGNIFICANT DIFFERENCE.

IN THE NORMAL GROUP, ADDITION OF THYROXINE TO THE MEDIUM PRODUCES, FIRST A DECREASE (Da) THEN AN INCREASE IN THE AMOUNT OF ³H-ADRENALINE BOUND. THE INCREASE REACHES AND OVERCOMES THE LEVEL OBSERVED IN THE ABSENCE OF THYROXINE IN THE MEDIUM.

IN THE THYROIDECTOMIZED GROUP, THERE IS ALSO A DECREASE THEN INCREASE IN THE AMOUNT OF ³H-ADRENALINE BOUND AS A FUNCTION OF THYROXINE CONCENTRATION IN THE MEDIUM. HOWEVER, THE INCREASE IS ONLY RELATIVE, SINCE IN THE PRESENCE OF THYROXINE HIGHEST CONCENTRATION (2.0×10^{-10} M) THE AMOUNT OF ³H-ADRENALINE PER GRAM OF TISSUE DOES NOT REACH THAT OBTAINED IN THE ABSENCE OF THYROXINE IN THE MEDIUM.

ANALYSIS OF VARIANCE WITH INTERACTION

THE INDIVIDUAL EFFECT OF THYROXINE AND ADRENALINE AND THE CONJUGATED EFFECT OF BOTH HORMONES ON THE BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE HAS BEEN STUDIED STATISTICALLY (TABLE XIV). THE EFFECT OF ENDOGENOUS

TABLE XIII D
 THE EFFECT OF THYROXINE ON THE BINDING OF ³H-ADRENALINE BY INTRESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE (16.1 x 10⁻⁸ M)

GROUP COMPARISON

EXPERIMENTS	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN ± S. D.		T	DEGREE OF FREEDOM	P
		NORMAL	THYROIDECTOMIZED			
D	0.00 x 10 ⁻¹⁰ M	1.0 ± 0.16	1.9 ± 0.12	9.137	6	0.01
D A	0.02 x 10 ⁻¹⁰ M	0.70 ± 0.19	1.9 ± 0.60	3.797	6	0.01
D B	0.20 x 10 ⁻¹⁰ M	1.0 ± 0.48	1.4 ± 0.25	1.465	6	0.20
D C	2.00 x 10 ⁻¹⁰ M	1.1 ± 0.21	1.6 ± 0.54	1.695	6	0.20

THYROXINE ON THE BINDINGS OF ^3H -ADRENALINE IS CALCULATED USING THE RESULTS OBTAINED WITH THYROIDECTOMIZED AND NORMAL RATS, AT THE TWO CONCENTRATIONS OF ADRENALINE ASSAYED. THE VALUE OF P, FOR THYROXINE, INFERIOR AT 0.01 SHOWS, THAT IN VIVO, THYROXINE CONCENTRATION, OR RATHER THYROXINE ABSENCE HAS A MARKED EFFECT ON ^3H -ADRENALINE BINDING. THE NATURE OF THE EFFECT IS NOT SEEN HERE, BUT BY LOOKING AT THE HIGHER VALUES OBTAINED IN THE THYROIDECTOMIZED GROUPS (TABLE XII D AND XIII D), IT MAY BE DEDUCTED : THYROIDECTOMY INCREASES THE AMOUNT OF ^3H -ADRENALINE BOUND BY THE TISSUES. THE EFFECT OF ADRENALINE ALONE OR OF ADRENALINE AND THYROIDECTOMY ON THE BINDING OF ^3H -ADRENALINE IS NOT SIGNIFICANT. WHATEVER THE CONCENTRATION OF ADRENALINE ASSAYED, THE BINDING BY INTESTINAL STRIPS WILL BE ALWAYS HIGHER IN THYROIDECTOMIZED ANIMALS.

THE INDIVIDUAL EFFECT OF ADDED THYROXINE OR ADRENALINE AND THE CONJUGATED EFFECT OF EXOGENOUS ADRENALINE AND THYROXINE HAVE BEEN STUDIED IN THE NORMAL AND IN THE THYROIDECTOMIZED GROUPS.

IN THE NORMAL GROUP, THYROXINE ALONE HAS AN EFFECT ON THE BINDING OF ^3H -ADRENALINE, BY INTESTINAL STRIPS. DEPENDING UPON THE CONCENTRATION OF THYROXINE IN THE MEDIUM (TABLE XII D AND XIII D), THE PRESENCE OF THYROXINE WILL RESULT IN AN INCREASE OR A DECREASE IN BINDING. ADRENALINE ALONE OR ADRENALINE PLUS THYROXINE DO NOT AFFECT THE BINDING AS SHOWN BY THE VALUE OF P SUPERIOR TO 0.01. IT IS OF INTEREST TO NOTE THAT THE EFFECT OF THYROXINE AND ADRENALINE IS IDENTICAL TO THAT OBSERVED IN VIVO. THYROXINE CONCENTRATION ALONE

T A B L E X I V

THE EFFECT OF THYROXINE AND ADRENALINE ON THE BINDING OF ³H-ADRENALINE
BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE

ANALYSIS OF VARIANCE WITH INTERACTION

EFFECT OF ENDOGENOUS THYROXINE : SERIES C AND D

SOURCE	D ⁰ F	F	P
THYROXINE	1 + 12	37.300	< 0.01
ADRENALINE	1 + 12	4.678	< 0.05
THYROXINE x ADRENALINE	1 + 12	2.927	> 0.05

EFFECT OF ADDED THYROXINE : SERIES C ABC AND D ABC

- NORMAL RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 33	44.080	< 0.01
ADRENALINE	1 + 33	3.673	> 0.05
THYROXINE x ADRENALINE	3 + 33	0.612	> 0.05

- THYROIDECTOMIZED RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 27	2.261	> 0.05
ADRENALINE	1 + 27	1.500	> 0.05
THYROXINE x ADRENALINE	3 + 27	7.056	< 0.01

HAS AN EFFECT. BUT WHILE IN THE ABSENCE OF THYROXINE THE EFFECT IS ALWAYS IN THE SAME DIRECTION,--DECREASED BINDING IN THE PRESENCE OF THYROXINE, IN THE NORMAL RAT THE CONCENTRATION OF EXOGENOUS THYROXINE MAY HAVE EITHER A DECREASING OR AN INCREASING EFFECT ON THE BINDING. THE VALUES OF P ARE CONSISTENT WITH THE ASSUMPTION THAT THERE IS NO INTERACTION BETWEEN THYROXINE AND ADRENALINE IN THE BINDING. ADRENALINE CONCENTRATION DOES NOT AFFECT EITHER THE BINDING OF EXOGENOUS ADRENALINE.

IN THE THYROIDECTOMIZED RATS, THE BINDING OF ^3H -ADRENALINE IS NOT AFFECTED BY EITHER THYROXINE ALONE OR ADRENALINE ALONE (P VALUES GREATER THAN 0.01). HOWEVER, BOTH HORMONES TOGETHER DO AFFECT THE BINDING OF ^3H -ADRENALINE. DEPENDING UPON THE RELATIVE CONCENTRATIONS OF EACH HORMONE THE EFFECT WILL BE ADDITIVE OR OPPOSITE (TABLE XII D AND XIII D).

C) THE EFFECT OF THYROXINE ON THE FORMATION OF ^3H -METANEPHRINE BY INTACT STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -ADRENALINE

THE EFFECT OF THYROXINE ON METANEPHRINE FORMATION HAS BEEN STUDIED IN VIVO, WITH ONE CONCENTRATION OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM (8.3×10^{-8} M) (SERIES A) AND IN VITRO WITH TWO CONCENTRATIONS OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM (8.3 AND 16.1×10^{-8} M) (SERIES C AND D RESPECTIVELY).

1) THE IN VIVO EFFECT OF THYROXINE

IN TABLE XV, THE EFFECT, IN VIVO, OF THYROXINE ON THE

FORMATION OF ^3H -METANEPHRINE, IS SHOWN FOR INDIVIDUAL EXPERIMENTS. THE PERCENTAGE OF ^3H -METANEPHRINE FORMED IS LOW IN ALL THE GROUPS. BUT THERE IS NO DIFFERENCE IN THE RELATIVE AMOUNT OF ^3H -METANEPHRINE AMONG THE THREE GROUPS OF ANIMALS : NORMAL, THYROIDECTOMIZED OR THYROXINE-TREATED. THE ABSOLUTE AMOUNT OF ^3H -METANEPHRINE IS A LITTLE HIGHER FOR THE THYROIDECTOMIZED GROUP.

2) THE IN VITRO EFFECT OF THYROXINE

A) SERIES C : CONCENTRATION OF ^3H -DL-ADRENALINE,
 8.3×10^{-8} M

THE ADDITION OF THYROXINE (0.02×10^{-10} M) TO THE INCUBATION MEDIUM (TABLE XV A) DOES NOT CHANGE THE PERCENTAGE OF METANEPHRINE FORMED, EITHER IN THE NORMAL OR IN THE THYROIDECTOMIZED GROUP. THE VARIATIONS ARE HIGH IN THE PERCENTAGE AMONG THE INDIVIDUAL RESULTS FOR BOTH GROUPS ; HOWEVER, THE ABSOLUTE AMOUNT OF METANEPHRINE IN THE TISSUE DOES NOT REFLECT SO HIGH VARIATIONS.

WHEN THE CONCENTRATIONS OF THYROXINE IN THE MEDIUM IS TEN TIMES HIGHER (0.20×10^{-10} M) (TABLE XV B), THERE IS A GREATER AMOUNT OF ^3H -METANEPHRINE IN THE THYROIDECTOMIZED THAN IN THE NORMAL ANIMALS. THIS GREATER AMOUNT IS RELATIVE AS WELL AS ABSOLUTE. AGAIN, THE VARIATIONS APPEAR LARGER IN THE RELATIVE AMOUNT OF ^3H -METANEPHRINE FORMED THAN IN THE CONCENTRATION PER GRAM OF TISSUE.

IN TABLE XV C, WHERE THE CONCENTRATION OF THYROXINE IN THE MEDIUM IS 100 TIMES HIGHER, THERE IS A SLIGHT INCREASE IN THE RELATIVE AMOUNT OF ^3H -METANEPHRINE COMPARED TO THAT IN THE PREVIOUS TWO TABLES, FOR BOTH GROUPS. IN THE NORMAL GROUP, THIS CORRESPONDS

TABLE XV
 THE EFFECT OF THYROXINE ON THE FORMATION OF ³H-METANEPHRINE BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ³H-OL-ADRENALINE (8.3 x 10⁻⁶ M)
 (SERIES A & C)

EXPERIMENT	RADIOACTIVE MATERIAL				CORRECTION FOR 50% RECOVERY	³ H-METANEPHRINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		³ H-METANEPHRINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN				
1 N *	2,385	108	4.5	5.0	0.14		
2 N	1,905	96	5.0	10.0	0.15		
3 N	4,200	213	5.1	10.2	0.20		
4 N	3,510	160	4.6	9.2	0.18		
5 N	4,185	178	4.3	8.6	0.16		
1 T0 **	4,445	111	4.5	9.0	0.15		
2 T0	5,250	275	5.2	10.4	0.20		
3 T0	4,950	273	5.5	11.0	0.26		
1 T4 ***	1,000	46	4.6	9.2	0.11		
2 T4	4,905	255	5.2	10.4	0.18		
3 T4	2,670	154	5.8	11.6	0.15		
4 T4	2,790	168	6.0	12.0	0.22		

* N : TISSUE FROM NORMAL RATS ** T0 : TISSUE FROM THYROIDECTOMIZED RATS *** T4 : TISSUE FROM THYROXINE-TREATED RATS.

TABLE XV A
 THE EFFECT OF ADDED THYROXINE (0.02×10^{-10} M) ON THE FORMATION OF ^3H -METANEPHRINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)
 (SERIES C A)

EXPERIMENT #	RADIOACTIVE MATERIAL			CORRECTION FOR 50% RECOVERY %	^3H -METANEPHRINE PER GRAM OF TISSUE 10^{11} MOLES/G
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -METANEPHRINE EXTRACTED COUNTS/MIN		
	COUNTS/MIN	PERCENT OF TOTAL %			
1 N	860		38	8.8	0.15
2 N	930		46	9.8	0.15
3 N	860		52	12.0	0.17
4 N	720		54	15.0	0.20
5 N	1,265		72	11.4	0.21
1 TD**	1,640		36	10.8	0.30
2 TD	1,695		70	8.1	0.20
3 TD	1,210		76	12.6	0.26
4 TD	1,075		54	10.0	0.19
5 TD	1,270		56	8.8	0.21

* N : TISSUE FROM NORMAL RATS ** TD : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E X V B
 THE EFFECT OF ADDED THYROXINE (0.20×10^{-10} M) ON THE FORMATION OF ^3H -METANEPHRINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (0.3×10^{-6} M)
 (SERIES C B)

EXPERIMENT	RADIOACTIVE MATERIAL				CORRECTION FOR 50% RECOVERY	^3H -METANEPHRINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -METANEPHRINE EXTRACTED	PERCENT OF TOTAL		
	COUNTS/MIN					
1 N *	1,160		54	4.7	9.4	0.23
2 N	1,020		66	6.5	13.0	0.26
3 N	1,090		48	4.4	8.8	0.19
4 N	950		54	5.7	11.4	0.22
1 T0 **	1,750		114	6.5	13.0	0.52
2 T0	1,100		72	6.5	13.0	0.39
3 T0	1,380		86	6.2	12.4	0.43
4 T0	1,310		80	6.1	12.2	0.45
5 T0	1,220		62	5.1	10.2	0.36

* N 1 TISSUE FROM NORMAL RATS ** T0 : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XV C
 THE EFFECT OF ADDED THYROXINE (2.00×10^{-10} M) ON THE FORMATION OF ^3H -METANEPHRINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -OL-ADRENALINE (8.3×10^{-8} M)

(SERIES C C)

EXPERIMENT	RADIOACTIVE MATERIAL			CORRECTION FOR 50% RECOVERY	^3H -METANEPHRINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT	^3H -METANEPHRINE EXTRACTED	PERCENT OF TOTAL		
1 N *	970	56	5.8	11.6	0.24
2 N	1,030	74	7.2	14.4	0.30
3 N	1,170	64	5.5	11.0	0.32
4 N	1,200	56	4.7	9.4	0.24
5 N	880	64	7.3	14.6	0.35
1 Td **	1,150	90	7.8	15.6	0.33
2 Td	980	70	7.2	14.4	0.30
4 Td	950	64	6.7	13.4	0.31
5 Td	900	62	6.9	13.8	0.50
6 Td	1,230	54	4.4	8.8	0.32

* N : TISSUE FROM NORMAL RATS ** Td : TISSUE FROM THYROIDECTOMIZED RATS.

TO A GREATER CONCENTRATION OF METANEPHRINE IN THE TISSUE, WHILE IN THE THYROIDECTOMIZED GROUP THE ABSOLUTE AMOUNT OF METANEPHRINE IS DECREASED COMPARED TO THAT WITH A CONCENTRATION OF THYROXINE 10 TIMES LOWER.

STATISTICAL ANALYSIS OF THE RESULTS :

THE STATISTICAL ANALYSIS OF THESE DATA ARE PRESENTED IN TABLE XV D. THYROXINE DOES NOT SEEM TO AFFECT METANEPHRINE FORMATION IN VIVO, (P VALUES HIGHER THAN 0.01 FOR BOTH THYROIDECTOMIZED VERSUS THYROXINE-TREATED AND THYROIDECTOMIZED VERSUS NORMAL GROUP). THE CONCENTRATION OF THYROXINE OF 0.02×10^{-10} M IS NOT SUFFICIENT TO GIVE A SIGNIFICANT INCREASE IN METANEPHRINE FORMATION IN THE THYROIDECTOMIZED COMPARED TO THE NORMAL GROUP (P VALUE HIGHER THAN 0.01). TEN TIMES THAT CONCENTRATION RESULTS IN A SIGNIFICANTLY HIGHER INCREASE IN THE THYROIDECTOMIZED GROUP. INCREASING FURTHER THE CONCENTRATION OF THYROXINE RESULTS IN THE DISAPPEARANCE OF THIS DIFFERENCE. IT SEEMS THAT THE CONCENTRATION OF THYROXINE IS CRITICAL IN THE EFFECT ON METANEPHRINE FORMATION SINCE THE SIGNIFICANT DIFFERENCE IS PRESENT AT ONLY ONE CONCENTRATION OF THYROXINE. AMONG THE NORMAL RATS, THE ADDITION OF THYROXINE TO THE INCUBATION MEDIUM CAUSES AN INCREASE IN THE FORMATION OF ^3H -METANEPHRINE. IN THE THYROIDECTOMIZED GROUP, THERE IS AN OPTIMUM CONCENTRATION OF THYROXINE AT WHICH THE AMOUNT OF ^3H -METANEPHRINE FORMED IS MAXIMUM (0.20×10^{-10} M). IN BOTH GROUPS, THE AMOUNT OF ^3H -METANEPHRINE FORMED CORRESPONDS TO THE AMOUNT OF UPTAKE ; WHEN THE RADIOACTIVE

TABLE XV 0

THE EFFECT OF THYROXINE ON THE FORMATION OF ^3H -METHAMPHETAMINE BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (8.3×10^{-8} M)

GROUP COMPARISON

EXPERIMENTS	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.		T	D.F.	P
		NORMAL	THYROIDECTOMIZED			
C A	$0.00 \times 10^{-10}\text{M}$	0.17 \pm 0.025	0.23 \pm 0.070*	1.818	6	0.2
			0.17 \pm 0.047**	1.373	5	0.3
C A	$0.02 \times 10^{-10}\text{M}$	0.18 \pm 0.089	0.23 \pm 0.045	2.083	8	0.1
C B	$0.20 \times 10^{-10}\text{M}$	0.23 \pm 0.025	0.43 \pm 0.061	6.061	7	0.01
C C	$2.00 \times 10^{-10}\text{M}$	0.29 \pm 0.049	0.31 \pm 0.513	1.273	9	0.3

* GROUP COMPARISON BETWEEN NORMAL AND THYROIDECTOMIZED RATS

** GROUP COMPARISON BETWEEN THYROIDECTOMIZED AND THYROXINE-TREATED RATS.

CONTENT OF THE TISSUE IS INCREASED, SIMILARLY THE CONCENTRATION OF ^3H -METANEPHRINE IS INCREASED AND VICE VERSA.

b) SERIES D : CONCENTRATION OF ^3H -DL-ADRENALINE
 $15.7 \times 10^{-8} \text{ M}$

DOUBLING THE CONCENTRATION OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM DOES NOT SEEM TO AFFECT THE FORMATION OF METANEPHRINE, IN BOTH NORMAL AND THYROIDECTOMIZED GROUPS. AS A MATTER OF FACT, THE PERCENTAGE OF ^3H -METANEPHRINE FORMED (TABLE XVI) IS LOWER THAN THAT OBSERVED AT HALF THE CONCENTRATIONS OF ^3H -ADRENALINE (TABLE XV) THERE IS A SLIGHTLY HIGHER METANEPHRINE PER GRAM OF TISSUE FROM THYROIDECTOMIZED RATS.

THE ADDITION OF THYROXINE ($0.02 \times 10^{-10} \text{ M}$) CAUSES AN INCREASE IN THE RELATIVE AND ABSOLUTE AMOUNT OF ^3H -METANEPHRINE IN THE THYROIDECTOMIZED GROUP (TABLE XVI A). THE NORMAL TISSUES DO NOT APPEAR TO BE AFFECTED BY THAT CONCENTRATION OF THYROXINE. IN BOTH GROUPS, THE VARIATIONS BETWEEN INDIVIDUAL RESULTS ARE HIGH, FOR THE PERCENTAGE VALUES, BUT LOW IN THE ABSOLUTE VALUES. WHEN THE CONCENTRATION OF THYROXINE IS 10 TIMES HIGHER ($0.20 \times 10^{-10} \text{ M}$) (TABLE XVI B) THE VARIATIONS OBSERVED ARE INCREASED, AND THEY DO NOT DISAPPEAR IN THE ABSOLUTE AMOUNTS. THIS IS ESPECIALLY TRUE FOR THE NORMALS. THE AMOUNT OF ^3H -METANEPHRINE IN THE TISSUE DOES NOT APPEAR TO BE AFFECTED BY THIS CONCENTRATION OF THYROXINE OR BY A TEN FOLD INCREASE IN THYROXINE CONCENTRATION (TABLE XVI C).

TABLE XVI
THE EFFECT OF THYROIDECTOMY ON THE FORMATION OF ³H-METANEPHRINE BY INTESTINAL STRIPS
AFTER A 5 MINUTE INCUBATION WITH ³H-OL-ADRENALINE (16.1 x 10⁻⁸ M)

(SERIES D)

EXPERIMENT	RADIOACTIVE MATERIAL			CORRECTION FOR 50% RECOVERY	³ H-METANEPHRINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT	³ H-METANEPHRINE EXTRACTED	PERCENT OF TOTAL		
1 N*	1,330	36	2.7	5.4	0.16
3 N	1,150	76	6.6	13.2	0.37
4 N	1,550	80	5.2	10.4	0.33
1 TD**	2,210	82	3.7	7.4	0.38
2 TD	2,550	102	4.0	8.0	0.47
3 TD	1,740	58	3.3	6.6	0.32
4 TD	1,900	124	6.5	13.0	0.66

* N : TISSUE FROM NORMAL RATS ** TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XVI A
 THE EFFECT OF ADDED THYROXINE (0.02×10^{-10} M) ON THE FORMATION OF ^3H -METANEPHRINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-6} M)
 (SERIES D A)

EXPERIMENT	RADIOACTIVE MATERIAL		CORRECTION FOR 50% RECOVERY	^3H -METANEPHRINE PER GRAM OF TISSUE
	TOTAL PRESENT IN PERCHLORIC EXTRACT	^3H -METANEPHRINE EXTRACTED		
	COUNTS/MIN	COUNTS/MIN		
1 N *	1,520	68	9.0	0.27
2 N	1,220	74	12.2	0.26
3 N	2,740	164	12.0	0.26
4 N	2,330	104	9.0	0.22
5 N	3,050	148	9.8	0.25
6 N	3,750	132	7.0	0.18
1 TD **	3,930	216	11.0	0.42
2 TD	6,280	356	11.4	0.57
3 TD	4,260	276	13.0	0.57

* N : TISSUE FROM NORMAL RATS ** TD : TISSUE FROM THYROIDECTOMIZED RATS.

TABLE XVI B
 THE EFFECT OF ADDED THYROXINE (0.20×10^{-10} H) ON THE FORMATION OF ^3H -METANEPHRINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} H)
 (SERIES D B)

EXPERIMENT #	RADIOACTIVE MATERIAL				CORRECTION FOR 50% RECOVERY	^3H -METANEPHRINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -METANEPHRINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN		COUNTS/MIN				
1 N *	2,320	108	4.7	9.4	0.24		
2 N	4,610	216	4.7	9.4	0.33		
3 N	1,800	132	7.3	14.6	0.54		
4 N	2,790	144	5.2	10.4	0.32		
1 TD **	3,750	166	4.5	9.0	0.39		
2 TD	4,320	200	4.4	8.8	0.38		
3 TD	3,570	192	5.4	10.8	0.53		
4 TD	2,900	146	5.1	10.2	0.37		

* N : TISSUE FROM NORMAL RATS ** TD : TISSUE FROM THYROIDECTOMIZED RATS.

T A B L E X V I C
 THE EFFECT OF ADDED THYROXINE (2.00×10^{-10} M) ON THE FORMATION OF ^3H -METANEPRHINE
 BY INTESTINAL STRIPS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^8 K)

(SERIES U C)

EXPERIMENT #	RADIOACTIVE MATERIAL				CORRECTION FOR 50 % RECOVERY	^3H -METANEPRHINE PER GRAM OF TISSUE	
	TOTAL PRESENT IN PERCHLORIC EXTRACT		^3H -METANEPRHINE EXTRACTED				PERCENT OF TOTAL
	COUNTS/MIN	COUNTS/MIN	COUNTS/MIN	%			
1 N *	2,760	124	4.6	9.2	0.33		
2 N	1,800	142	7.9	15.8	0.46		
3 N	2,070	118	5.7	11.4	0.34		
4 N	2,550	142	5.6	11.2	0.37		
1 Td **	3,570	194	5.4	10.8	0.49		
2 Td	3,790	246	6.5	13.0	0.47		
3 Td	3,410	158	4.6	9.2	0.61		
4 Td	2,940	158	6.4	10.8	0.45		

* N : TISSUE FROM NORMAL RATS ** Td : TISSUE FROM THYROIDECTOMIZED RATS.

STATISTICAL ANALYSIS OF THE RESULTS :

IN TABLE XVI D, THE STATISTICAL ANALYSIS OF THE DATA IS PRESENTED. IN THE ABSENCE OF EXOGENOUS THYROXINE, THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN NORMAL AND THYROIDECTOMIZED GROUPS. THE ADDITION OF THYROXINE (0.02×10^{-10} M) HOWEVER, CAUSES A SIGNIFICANT INCREASE IN METANEPHRINE FORMATION IN THE THYROIDECTOMIZED COMPARED TO THE NORMAL GROUP, (P VALUES LOWER THAN 0.01). FURTHER INCREASE IN THYROXINE CONCENTRATION ABOLISHES THE SIGNIFICANT DIFFERENCE, P VALUE GREATER THAN 0.01.

IN THE NORMAL GROUP, THE ADDITION OF THYROXINE TO THE MEDIUM, CAUSES FIRST A DECREASE THEN AN INCREASE IN METANEPHRINE CONCENTRATION. IN THE THYROIDECTOMIZED GROUP, THE ADDITION OF THYROXINE INCREASES OR DECREASES THE ^3H -METANEPHRINE IN THE TISSUE DEPENDING UPON THE CONCENTRATION OF THYROXINE IN THE MEDIUM. THERE IS NO DEFINITE PATTERN AS IN THE CASE OF NORMAL GROUP.

ANALYSIS OF VARIANCE

THIS STATE OF AFFAIRS IS REFLECTED IN NEXT TABLE XVII, WHERE AN ANALYSIS OF VARIANCE WITH INTERACTION HAS BEEN GIVEN. THE DATA OBTAINED FROM THYROIDECTOMIZED AND NORMAL TISSUES, WHEN THYROXINE WAS ABSENT FROM THE MEDIUM, AND FOR TWO CONCENTRATIONS OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM HAS BEEN USED TO CALCULATE THE EFFECT OF ENDOGENOUS THYROXINE. THERE IS NO EFFECT, IN VIVO, OF EITHER THYROXINE ALONE, OR ADRENALINE ALONE OR ADRENALINE AND THYROXINE TOGETHER (P VALUES GREATER THAN 0.01).

T A B L E X V I D

THE EFFECT OF THYROXINE ON THE FORMATION OF ^3H -METAMEPHRINE BY INTESTINAL STRIPS
 AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE (16.1×10^{-8} M)

GROUP COMPARISON

EXPERIMENTS	THYROXINE CONCENTRATION IN THE MEDIUM	MEAN \pm S. D.		T	DEGREE OF FREEDOM	P
		NORMAL	THYROIDECTOMIZED			
D	0.00×10^{-10} M	0.29 ± 0.11	0.46 ± 0.15	1.706	5	< 0.2
D A	0.02×10^{-10} M	0.23 ± 0.038	0.52 ± 0.088	4.255	5	< 0.01
D B	0.20×10^{-10} M	0.36 ± 0.13	0.41 ± 0.079	0.652	6	> 0.50
D C	2.00×10^{-10} M	0.38 ± 0.059	0.51 ± 0.072	2.784	6	< 0.05

THE EFFECT OF EXOGENOUS THYROXINE, ADRENALINE OR THYROXINE AND ADRENALINE TOGETHER HAS BEEN TESTED ON NORMAL AND THYROIDECTOMIZED ANIMALS. IN THE NORMAL GROUP, THYROXINE ALONE HAS AN EFFECT ON THE FORMATION OF METANEPHRINE. ADRENALINE CONCENTRATION ALONE HAS AN EFFECT ON METANEPHRINE FORMATION, FURTHERMORE THE EFFECT OF ADRENALINE ALONE IS MUCH MORE MARKED THAN THAT OF THYROXINE ALONE (F VALUES). THE NATURE OF THE EFFECT DEPENDS UPON THE CONCENTRATION OF THE HORMONES. WHEN THE CONCENTRATION OF ADRENALINE IS HIGHER, THE FORMATION OF METANEPHRINE IS INCREASED. THE EFFECT OF THYROXINE APPEARS MORE COMPLEX (TABLE XV D AND XVI D). THE TWO HORMONES DO NOT INTERACT AT ALL IN THEIR EFFECT ON METANEPHRINE FORMATION (P VALUE GREATER THAN 0.01). THIS MEANS THAT THE INDIVIDUAL EFFECT OF THYROXINE CONCENTRATION AND ADRENALINE CONCENTRATION WILL BE PRESENT FOR ALL THE CONCENTRATIONS OF HORMONES STUDIED. BECAUSE OF THE GREATER VALUE OF F IN THE CASE OF ADRENALINE, IT WOULD SEEM THAT THIS EFFECT WILL BE MORE AND MORE IMPORTANT AS ADRENALINE CONCENTRATION IS INCREASED, FOR A GIVEN CONCENTRATION OF THYROXINE.

IN THE THYROIDECTOMIZED GROUP, THYROXINE CONCENTRATION ALONE DOES NOT AFFECT ³H-METANEPHRINE FORMATION (P VALUE GREATER THAN 0.01). ADRENALINE CONCENTRATION, HOWEVER, DOES AFFECT METANEPHRINE FORMATION AND THIS EFFECT IS EVEN HIGHER THAN THAT OBTAINED WITH NORMAL RATS (F VALUE IS GREATER). THE EFFECT OF ADRENALINE CONCENTRATION IS TO INCREASE METANEPHRINE FORMATION AS THE CONCENTRATION OF ADRENALINE INCREASES (TABLE XV AND XVI D). THYROXINE AND ADRENALINE CONCENTRATIONS AFFECT

TOGETHER THE FORMATION OF METANEPHRINE. THAT IS TO SAY, AT A GIVEN CONCENTRATION OF ADRENALINE THE PRESENCE OF THYROXINE WILL MODIFY THE EFFECT OF ADRENALINE. WHETHER IT WILL INCREASE OR DECREASE THE EFFECT OF ADRENALINE APPEARS TO DEPEND UPON THE CONCENTRATION STUDIED. AT A GIVEN CONCENTRATION OF THYROXINE, THE EFFECT OF ADRENALINE WILL BE MODIFIED, THIS IS SHOWN BY THE FACT THAT AT A CONCENTRATION OF THYROXINE OF 0.20×10^{-10} M, DOUBLING THE CONCENTRATION OF EXOGENOUS ^3H -METANEPHRINE, DECREASES THE AMOUNT OF ^3H -METANEPHRINE FORMED (TABLE XV D AND XVI D).

TABLE XVII

THE EFFECT OF THYROXINE AND ADRENALINE ON THE FORMATION
OF ³H-METANEPHRINE BY INTESTINAL STRIPS
AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE

ANALYSIS OF VARIANCE WITH INTERACTION

EFFECT OF ENDOGENOUS THYROXINE : SERIES C AND D

SOURCE	D ⁰ F	F	P
THYROXINE	1 + 8	3.983	> 0.05
ADRENALINE	1 + 8 3	8.399	< 0.05
THYROXINE x ADRENALINE	1 + 8	1.355	> 0.05

EFFECT OF ADDED THYROXINE : SERIES C ABC AND D ABC

- NORMAL RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 28	8.205	< 0.01
ADRENALINE	1 + 28	20.513	< 0.01
THYROXINE x ADRENALINE	3 + 28	0.846	> 0.05

- THYROIDECTOMIZED RATS -

SOURCE	D ⁰ F	F	P
THYROXINE	3 + 25	2.375	> 0.05
ADRENALINE	1 + 25	37.500	< 0.01
THYROXINE x ADRENALINE	3 + 25	7.143	< 0.01

III. DISCUSSION

FOR MANY YEARS, THE POTENTIATION OF CATECHOLAMINE EFFECTS IN HYPERTHYROIDISM HAS BEEN OBSERVED. THE ATTEMPTS TO REPRODUCE EXPERIMENTALLY IN THE ANIMALS THE PHYSIOLOGICAL EFFECTS OBSERVED IN THE PATIENTS HAVE BEEN SUCCESSFUL WHERE INCREASED OXYGEN CONSUMPTION, TACHYCARDIA, HYPERGLYCEMIA, INCREASED FREE FATTY ACIDS RELEASE ARE CONCERNED. HOWEVER, THE BIOCHEMICAL ATTEMPTS TO EXPLAIN THE MECHANISM BY WHICH THIS POTENTIATION TAKES PLACE ARE SOMEWHAT CONTRADICTORY. THE DIRECT EFFECT OF EXOGENOUS CATECHOLAMINES ON TISSUE FROM HYPERTHYROID OR THYROID HORMONE-TREATED ANIMALS HAVE BEEN REPORTED INCREASED FOR HEART TISSUE (63, 65), INCREASED OR UNCHANGED FOR THE FREE FATTY ACID RELEASE (72, 76, 156). IN THE CASE OF THE SMOOTH MUSCLE FROM SMALL INTESTINE SOME AUTHORS HAVE REPORTED AN INCREASED EFFECT OF EXOGENOUS ADRENALINE OR NORADRENALINE (52, 81), WHILE OTHERS HAVE REPORTED UNCHANGED EFFECTS (67, 68). THE INCREASE IN EFFECT HAS BEEN REPORTED TO BE SEEN IN AN AUGMENTED DURATION OF THE EFFECT RATHER THAN IN A SENSITIZATION OF THE TISSUE TO A LOWER DOSE OF CATECHOLAMINE (70). NO ATTEMPT HAS BEEN MADE TO STUDY THE MECHANISM BY WHICH THE POTENTIATION OF CATECHOLAMINE EFFECT IN HYPERTHYROID SMALL INTESTINE TAKES PLACE. IN THE HEART OF HYPOTHYROID ANIMAL, AN INCREASE IN ADRENALINE UPTAKE (102), HAS BEEN OBSERVED, WHILE A DECREASE IN NORADRENALINE UPTAKE HAS BEEN OBSERVED IN WHOLE HEARTS (101, 102) OR IN HEART SLICES (160),

FROM HYPERTHYROID ANIMALS. IT WAS THOUGHT THAT A SIMILAR MECHANISM MAY BE INVOLVED IN THE SMALL INTESTINE AND THIS STUDY WAS UNDERTAKEN IN ORDER TO ATTEMPT THE ELUCIDATION OF THIS POINT. ALTHOUGH ADDITION OF THYROXINE IN VITRO, HAS NOT BEEN ALWAYS FOUND TO MIMIC THE POTENTIATING EFFECT OF THYROID HORMONES IN VIVO, ON THE SMALL INTESTINE, SEVERAL REPORTS HAVE BEEN PRESENTED, WITH AN EFFECT OF ADDED THYROXINE ON THE POTENTIATION OF EXOGENOUS ADRENALINE (84, 87). THESE REPORTS SEEMED TO FURNISH ENOUGH GROUND FOR A FURTHER INVESTIGATION OF THE SUBJECT. FROM THE LITERATURE REVIEWED, THE CONCENTRATION OF THYROXINE APPEARED TO BE AN IMPORTANT FACTOR. FOR THIS REASON THE DIFFERENCE IN THE UPTAKE OF ADRENALINE BY INTESTINAL STRIPS FROM NORMAL AND THYROIDECTOMIZED RATS WAS STUDIED AS A FUNCTION OF VARIOUS CONCENTRATIONS OF ADDED THYROXINE.

A) EFFECT OF THYROXINE ON ^3H -ADRENALINE UPTAKE

1) UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS OF NORMAL RATS AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE

THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS OF NORMAL RATS, INCREASES WHEN THE CONCENTRATION OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM IS INCREASED. HOWEVER, THE AMOUNT OF RADIOACTIVE MATERIAL CONCENTRATED BY THE TISSUE DECREASED AS THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM IS INCREASED. THE UPTAKE OF RADIOACTIVE MATERIAL, BY ISOLATED TISSUE HAS BEEN MEASURED BY SEVERAL AUTHORS AT A TIME MUCH LONGER THAN THE ONE USED IN THIS STUDIES. THE NORADRENALINE UPTAKE WAS FOUND BY TITUS AND DENGLE (100), IN SLICES

OF CORTEX TO APPROACH A CONCENTRATION IN THE TISSUE FOUR TIMES THAT IN THE MEDIUM FOR A PERIOD OF INCUBATION OF 40 TO 60 MINUTES. THE CONCENTRATION OF ³H-NORADRENALINE USED BY THESE AUTHORS WAS ABOUT 16 TIMES HIGHER THAN THE HIGHEST CONCENTRATION USED IN THIS WORK. SINCE INCREASING THE CONCENTRATION OF ³H-ADRENALINE APPEARS TO CAUSE A DECREASE IN THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUE, SMALL INTESTINE APPEARS TO BEHAVE IN A DIFFERENT MANNER FROM THE CORTEX. IN THE ATRIA, NORADRENALINE UPTAKE WAS FOUND TO BE 17 % AFTER A 75 MINUTE INCUBATION (160). SINCE THE RATIO IS GIVEN FOR THE TOTAL RADIOACTIVITY IN THE MEDIUM, AND THAT THE VOLUME OF THE INCUBATION MEDIUM AVERAGED 75 ML, IT CAN BE SEEN THAT THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUE WAS MUCH HIGHER THAN THAT IN THE MEDIUM. DENGLER ET AL (154) OBSERVED THAT INCREASING THE CONCENTRATION OF ³H-NORADRENALINE IN THE INCUBATION MEDIUM RESULTED IN A DECREASE IN THE CONCENTRATION OF RADIOACTIVE MATERIAL IN CORTEX SLICES. AT HIGH CONCENTRATIONS OF ³H-NORADRENALINE IN THE MEDIUM, THE TISSUE CONCENTRATION WAS EQUAL TO THAT IN THE MEDIUM (25 µG/ML). THIS APPEARS TO REFLECT A DIFFERENCE IN MEMBRANE SINCE IN SUBCELLULAR CORTEX PARTICLES, MIRKIN ET AL (170) FOUND THE UPTAKE TO BE DIRECTLY PROPORTIONAL TO THE MEDIUM CONCENTRATION. IN THE SAME WORK, DENGLER ET AL OBSERVED THAT LIVER AND MUSCLE DID NOT CONCENTRATE ³H-NORADRENALINE AT A CONCENTRATION SUPERIOR TO THAT IN THE MEDIUM. IN BOTH TISSUES, THE CONCENTRATION OF RADIOACTIVE MATERIAL NEVER EXCEEDED THAT OF THE

MEDIUM. SIMILAR OBSERVATIONS HAVE BEEN MADE, IN VIVO, ON THE RATIO OF TISSUE ADRENALINE OR NORADRENALINE TO PLASMA ADRENALINE OR NORADRENALINE, AFTER INJECTION OF THE AMINE. AT LEVELS WHICH ARE HIGHER THAN PHYSIOLOGICAL LEVEL, THE CONCENTRATION OF RADIOACTIVE MATERIAL IN THE TISSUE EXHIBITS A TENDENCY TO EQUAL THAT IN THE PLASMA (100, 151, 154). ALTHOUGH THE DOSES OF ³H-ADRENALINE USED IN THESE EXPERIMENTS ARE LOWER THAN THOSE USED BY THE AUTHORS, THE SAME DECREASE IN RADIOACTIVE MATERIAL CONCENTRATION OF THE TISSUE IS OBSERVED AS ³H-ADRENALINE CONCENTRATION IS INCREASED. THE LOWEST DOSE OF ³H-ADRENALINE USED IS SUBLIMINAR FOR PHYSIOLOGICAL ACTION. THE HIGHEST DOSE USED IS STILL IN THE PHYSIOLOGICAL RANGE, HOWEVER. THE DIFFERENCE OBSERVED IN THIS CASE, MAY REFLECT THE DIFFERENCE IN FUNCTION BETWEEN HEART OR CORTX AND SMALL INTESTINE. IT IS INTERESTING TO NOTE IN THIS RESPECT, THAT THE LIVER WHICH IS AN ORGAN OF DETOXICATION DOES NOT CONCENTRATE EITHER ADRENALINE OR NORADRENALINE AT A LEVEL SUPERIOR TO THAT OF THE INCUBATION MEDIUM. IN ANY CASE AS SHOWN BY PREVIOUS EXPERIMENTS (154), THE RATE OF UPTAKE OF CA BY TISSUES DOES NOT REACH ITS MAXIMUM UP TILL 20 MINUTE OF INCUBATION. MOREOVER, THE RATE OF UPTAKE FOR THE FIRST TWENTY MINUTES IS DIRECTLY PROPORTIONAL TO TIME. THIS MEANS THAT IN THE SMALL INTESTINE, A 20 MINUTE INCUBATION MAY HAVE RESULTED IN A CONCENTRATION OF RADIOACTIVE MATERIAL APPROACHING THAT IN THE MEDIUM.

2) UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS
FROM THYROXINE-TREATED OR THYROIDECTOMIZED RATS AFTER A 5 MINUTE
INCUBATION WITH ³H-ADRENALINE

A) THYROXINE-TREATED RATS

FOR THE SINGLE CONCENTRATION OF ³H-ADRENALINE ASSAYED IN THE INCUBATION MEDIUM (8.3×10^{-8} M), THE UPTAKE OF RADIOACTIVE MATERIAL BY THE SMALL INTESTINE FROM THYROXINE-TREATED RATS IS IDENTICAL TO THAT FROM NORMAL RATS. A DAILY INJECTION FOR SIX DAYS, OF THYROXINE (P. 97) DOES NOT APPEAR TO AFFECT THE UPTAKE OF RADIOACTIVE MATERIAL BY THE SMALL INTESTINE. HOWEVER, IN VIEW OF WURTHMANN ET AL. FINDINGS (102), THE ABSENCE OF THYROXINE EFFECT ON THE UPTAKE OF RADIOACTIVE MATERIAL MAY BE ONLY APPARENT. THESE AUTHORS FOUND THAT FOLLOWING A SINGLE INJECTION OF ³H-ADRENALINE INTO RATS, 30 SECONDS AFTER THE INJECTION THE RADIOACTIVITY CONTENT OF THE HEART WAS HIGHER AMONG THE THYROXINE-TREATED THAN AMONG THE NORMAL GROUP. THE RELEASE OF ³H-ADRENALINE, HOWEVER, WAS MORE RAPID IN THE HYPERTHYROID ANIMALS SO THAT 5 MINUTES LATER, THE RADIOACTIVE CONTENT OF THE HEART FROM HYPERTHYROID ANIMALS WAS BELOW THAT OF THE HEART FROM NORMAL ANIMALS. IN THIS EXPERIMENT HOWEVER, A SINGLE INJECTION OF ³H-ADRENALINE WAS GIVEN, WHILE IN THE IN VITRO EXPERIMENTS, THE TISSUE WAS IN CONTACT WITH ³H-ADRENALINE FOR THE WHOLE PERIOD OF INCUBATION. FROM THE STUDIES OF DENGLER ET AL (154), WITH NORADRENALINE, IT WOULD APPEAR THAT THE SLICES OF TISSUE PICK UP THE AMINE DURING AT LEAST 20 MINUTES OF INCUBATION. THE TIME COURSE FOR

THE UPTAKE REACHES A PLATEAU AT 25 MINUTES. THE SIMILARITY BETWEEN SMALL INTESTINE FROM THYROXINE-TREATED AND NORMAL ANIMALS SEEMS TO REFLECT A REAL LACK OF DIFFERENCE.

b) THYROIDECTOMIZED ANIMALS

IN THE INTESTINE OF THYROIDECTOMIZED ANIMALS, THE UPTAKE OF RADIOACTIVE MATERIAL AFTER A 5 MINUTE INCUBATION INCREASES WHEN THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM IS INCREASED. SMALL INTESTINE FROM THYROIDECTOMIZED RATS, CONCENTRATES RADIOACTIVE MATERIAL AS A FUNCTION OF ^3H -ADRENALINE CONCENTRATION IN THE MEDIUM; WHEN THE CONCENTRATION OF ^3H -ADRENALINE IS INCREASED THE RADIOACTIVITY CONTENT OF THE TISSUE IS ALSO INCREASED. FOR THE THREE CONCENTRATION OF ADRENALINE ASSAYED, THE TISSUE FROM THYROIDECTOMIZED RATS CONCENTRATES RADIOACTIVITY SLIGHTLY MORE THAN THAT FROM NORMAL ANIMALS. SINCE IN THE FORMER CASE INCREASING ^3H -ADRENALINE CONCENTRATION IN THE MEDIUM PRODUCES AN INCREASED RADIOACTIVE MATERIAL CONCENTRATION IN THE TISSUE, WHILE IN THE LATTER ^3H -ADRENALINE CONCENTRATION IN THE MEDIUM HAS AN OPPOSITE EFFECT, THE DIFFERENCE BETWEEN THE TWO TISSUES IS MORE MARKED AT THE HIGHEST CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM (16.1×10^{-8} M). THE TISSUES FROM THYROIDECTOMIZED RATS WERE THINNER THAN THOSE FROM NORMAL RATS AND THIS MAY PARTLY EXPLAIN THE HIGHER UPTAKE IN THE SMALL INTESTINE FROM THYROIDECTOMIZED ANIMALS. HOWEVER, ANOTHER FACTOR, THE ABSENCE OF THYROXINE MUST ALSO ACCOUNT FOR THIS INCREASE. IN THE NORMAL RAT, INCREASING THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM RESULTED IN A DECREASE IN

TISSUE CONCENTRATION OF THE RADIOACTIVITY. A REVERSE EFFECT IS OBSERVED IN THE CASE OF THYROIDECTOMIZED RATS. IF ONLY THE DIFFERENCE IN THE THICKNESS OF THE TISSUES WAS RESPONSIBLE FOR THE HIGHER UPTAKE, ONE WOULD NOT EXPECT A REVERSAL OF THE EFFECT BUT RATHER A MORE MARKED EFFECT. LEIN AND LOWEN (197) HAVE SHOWN THAT THE DIAPHRAGM FROM SMALLER RATS PICKS UP THYROXINE IN GREATER AMOUNT THAN THAT PICKED UP BY ADULT RATS DIAPHRAGM. THE DIFFERENCE IN THE UPTAKE WAS HIGHER BUT THE EFFECT OF VARIOUS CONCENTRATIONS OF THYROXINE WAS SIMILAR IN BOTH CASES. IT APPEARS THAT THE SAME REASONING COULD BE APPLIED TO THE UPTAKE OF ^3H -ADRENALINE BY THE SMALL INTESTINE.

IN THE ANALYSIS OF VARIANCE WITH INTERACTION THYROXINE ALONE, ADRENALINE ALONE AND THE ASSOCIATION OF THYROXINE PLUS ADRENALINE HAVE BEEN SHOWN TO AFFECT THE UPTAKE OF RADIOACTIVE MATERIAL BY SMALL INTESTINE (TABLE X, PAGE 106). THE COMPLETE ABSENCE OF THYROXINE RESULTS IN A HIGHER UPTAKE OF RADIOACTIVE MATERIAL BY SMALL INTESTINE, AT A GIVEN CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM. INCREASING THE AMOUNT OF ^3H -ADRENALINE IN THE MEDIUM WILL PRODUCE AN INCREASE IN THE AMOUNT OF RADIOACTIVE MATERIAL BY THE TISSUES IN THE ABSENCE OF THYROXINE. SINCE THYROXINE AND ADRENALINE TOGETHER HAVE AN EFFECT, THE EFFECT OF INCREASING ^3H -ADRENALINE IN THE MEDIUM MAY BE DIFFERENT DEPENDING UPON THE CONCENTRATION OF ^3H -ADRENALINE USED. IT IS POSSIBLE TO POSTULATE SUCH AN HYPOTHESIS, SINCE IN THE COMPLETE ABSENCE OF

THYROXINE (THYROIDECTOMIZED RATS) THE TISSUE CONCENTRATION OF RADIOACTIVE MATERIAL INCREASES AS ^3H -ADRENALINE CONCENTRATION IN THE MEDIUM IS INCREASED. IN THE NORMAL ANIMAL INCREASING THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM CAUSES A DECREASE IN THE RADIOACTIVITY CONCENTRATION OF THE TISSUE. THE PRESENCE OF ENDOGENOUS THYROXINE SEEMS TO ANTAGONIZE THE EFFECT OF ^3H -ADRENALINE. HOWEVER, THYROXINE CANNOT REVERSE THE EFFECT OF ^3H -ADRENALINE CONCENTRATION ON THE UPTAKE. THERE WILL ALWAYS BE AN ABSOLUTE INCREASE IN THE UPTAKE OF RADIOACTIVE MATERIAL BY TISSUE FROM NORMAL ANIMALS, EVEN IF THE RELATIVE CONCENTRATION OF RADIOACTIVE MATERIAL DECREASES. THE CHANGE IN THE DIRECTION OF UPTAKE WILL BE BROUGHT ABOUT BY ^3H -ADRENALINE CONCENTRATION BUT NOT BY THE PRESENCE OF ENDOGENOUS THYROXINE.

3) THE EFFECT OF EXOGENOUS THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS FROM NORMAL AND THYROIDECTOMIZED AFTER A 5 MINUTE INCUBATION WITH ^3H -ADRENALINE

NORMAL RATS :

THE EFFECT OF ADDED THYROXINE ON THE UPTAKE OF RADIOACTIVE MATERIAL BY THE SMALL INTESTINE FROM NORMAL RATS DEPENDS UPON THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM. FOR A GIVEN CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM, THE EFFECT DEPENDS UPON THE CONCENTRATION OF THYROXINE ADDED TO THE MEDIUM.

AT THE LOWEST CONCENTRATION OF ^3H -ADRENALINE ASSAYED (1.16×10^{-8} M) THE PRESENCE OF THYROXINE RESULTS IN A DECREASED

UPTAKE, ONLY FOR A CONCENTRATION OF 2.0×10^{-10} M. SMALLER AMOUNT OF THYROXINE DECREASES SOMEWHAT THE UPTAKE BUT NOT SO SIGNIFICANTLY. WHEN THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM IS INCREASED 5 TIMES (8.3×10^{-8} M), THAT CONCENTRATION OF THYROXINE WHICH INHIBITED THE UPTAKE PREVIOUSLY IS NOW CAUSING AN INCREASED UPTAKE. THERE IS ALSO AN INCREASED UPTAKE, BUT SMALLER, FOR THE CONCENTRATION OF THYROXINE 10 TIMES LOWER (0.20×10^{-10} M). THE LOWER CONCENTRATION OF THYROXINE ASSAYED STILL HAS NO EFFECT ON THE UPTAKE (0.02×10^{-10} M) WHEN THE CONCENTRATION OF ^3H -ADRENALINE IS TEN TIMES HIGHER, THYROXINE HAS NO EFFECT ON THE UPTAKE, EXCEPT AT A CONCENTRATION OF 0.02×10^{-10} M.

FOR THE THREE CONCENTRATIONS OF ^3H -ADRENALINE ASSAYED, THE CONCENTRATION OF THYROXINE OF 0.02×10^{-10} M APPEARS TO BE CRITICAL. IT ALWAYS CAUSED A DECREASE IN THE UPTAKE, ALTHOUGH THIS IS NOT SO MARKED AT THE CONCENTRATION OF ^3H -ADRENALINE OF 8.3×10^{-8} M.

THE IMPORTANCE OF THE RELATIVE CONCENTRATIONS OF THE TWO HORMONES IN THE MEDIUM IS POINTED OUT WHEN THE ANALYSIS OF VARIANCE IS PERFORMED. ALTHOUGH ^3H -ADRENALINE ALONE HAS A VERY MARKED EFFECT ON THE UPTAKE, THYROXINE IN THE PRESENCE OF ADRENALINE EXERTS ALSO AN EFFECT ON THE UPTAKE. SINCE THYROXINE ALONE HAS NO EFFECT AT A GIVEN CONCENTRATION OF ADRENALINE IN THE MEDIUM, THE CONJUGATED EFFECT OF ADRENALINE AND THYROXINE WILL ALWAYS BE IN THE SAME DIRECTION. BECAUSE OF THE LARGE EFFECT OF ADRENALINE ALONE, AT A GIVEN CONCENTRATION OF THYROXINE, THE EFFECT OF ADRENALINE ALONE WILL

BE ALWAYS MORE MARKED THAN THAT OF ADRENALINE AND THYROXINE TOGETHER. THIS IS SHOWN IN TABLE VIId(PAGE 90), TABLE VIId(PAGE 97) AND TABLE IX D(PAGE 103). INCREASING THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM RESULTS ALWAYS IN AN INCREASE IN THE UPTAKE OF RADIOACTIVE MATERIAL BY THE TISSUE.

THYROIDECTOMIZED ANIMALS

IN THE THYROIDECTOMIZED ANIMALS, THE UPTAKE OF ^3H -ADRENALINE DEPENDS ALSO ON BOTH THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM AND ON THAT OF THYROXINE IN THE MEDIUM.

FOR THE LOWEST CONCENTRATION OF ^3H -ADRENALINE ASSAYED (1.16×10^{-8} M), THE ADDITION OF A SMALL AMOUNT OF THYROXINE TO THE MEDIUM (0.02×10^{-10} M) CAUSES A SLIGHT INCREASE IN THE UPTAKE BY THE TISSUE. INCREASING FURTHER THE CONCENTRATION OF THYROXINE RESULTS IN THE DECREASE OF THE UPTAKE. WHEN THE CONCENTRATION OF ^3H -ADRENALINE IS 5 TIMES HIGHER, A CONCENTRATION OF THYROXINE TEN TIMES HIGHER IS ALSO REQUIRED TO GIVE A MAXIMUM UPTAKE. FURTHER INCREASE IN THYROXINE CONCENTRATION CAUSES A SLIGHT DECREASE IN THE UPTAKE, BUT THYROXINE IS STILL EFFICIENT. WHEN ^3H -ADRENALINE CONCENTRATION IS INCREASED TENFOLD, THYROXINE FOR ALL CONCENTRATIONS INHIBITS THE UPTAKE. FURTHERMORE AT HIGH CONCENTRATION OF THYROXINE (2.0×10^{-10} M) INTERFERENCE WITH THE UPTAKE IS SHOWN BY THE LARGE VARIATIONS OBSERVED. THIS LAST FACTOR IS SHOWN IN THE ANALYSIS OF VARIANCE (TABLE X). WHILE THYROXINE ALONE HAS NO EFFECT IN THE

UPTAKE OF RADIOACTIVE MATERIAL BY THE TISSUE, IT INTERFERES WITH ADRENALINE. ALTHOUGH THE EFFECT OF ADRENALINE ALONE IS MUCH HIGHER THAN THAT OF BOTH HORMONES TOGETHER, IT DOES NOT PREVENT IT. AT A GIVEN CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM, THYROXINE WILL NOT PREVENT THE EFFECT OF ADRENALINE FOR ANY CONCENTRATIONS OF THYROXINE ASSAYED. THE INCREASING CONCENTRATION OF THYROXINE DOES NOT PREVENT THE UPTAKE OF ADRENALINE. BUT IT MAY DECREASE IT. HOWEVER, SINCE ADRENALINE ALONE HAS A MUCH LARGER EFFECT, THYROXINE AT ALL CONCENTRATIONS WILL NOT PREVENT THIS EFFECT. ADRENALINE CONCENTRATIONS, ON THE OTHER HAND INCREASES THE UPTAKE OF RADIOACTIVE MATERIAL BY SMALL INTESTINE. AT A GIVEN CONCENTRATION OF THYROXINE, INCREASING THE CONCENTRATION OF EXOGENOUS ADRENALINE WILL RESULT IN AN INCREASED UPTAKE. THIS INCREASE IN UPTAKE MAY EXPLAIN WHY VARIOUS AUTHORS OBSERVED, IN THE SMALL INTESTINE AN INCREASE DURATION RATHER THAN A LOWER THRESHOLD IN THE PRESENCE OF THYROXINE (70, 87).

FOR ALL THE CONCENTRATIONS OF ^3H -ADRENALINE OR THYROXINE ASSAYED, THERE IS A GREATER UPTAKE IN TISSUE FROM THYROIDECTOMIZED ANIMALS THAN IN THOSE FROM NORMAL CREB. THE HIGHER UPTAKE IS ALWAYS SIGNIFICANT AT A CONCENTRATION OF THYROXINE IN THE MEDIUM EQUAL TO 0.02×10^{-10} M, FOR THE THREE CONCENTRATIONS OF THYROXINE ASSAYED. THIS SHOWS THAT THE SMALL INTESTINE OF THYROIDECTOMIZED RATS IS VERY SENSITIVE TO THYROXINE. A SMALL AMOUNT OF THYROXINE HAS A LARGE EFFECT

ON THE UPTAKE OF ADRENALINE BY THE TISSUE. THIS RESULT IS IN CONTRADICTION WITH THIBAUT'S FINDINGS, THAT THYROXINE DOES NOT AFFECT ADRENALINE ACTION AT A CONCENTRATION OF THYROXINE IN THE MEDIUM BELOW 10^{-12} (70). THE DOSE OF ADRENALINE USED BY THIS AUTHOR WAS IDENTICAL TO THE HIGHEST ONE USED HERE. WHEN THE CONCENTRATIONS OF THYROXINE IN THE MEDIUM ARE INCREASED, ALTHOUGH THERE IS STILL AN INCREASE IN THE UPTAKE FOR THE THYROIDECTOMIZED GROUP COMPARED TO THE NORMAL ONE, THIS DIFFERENCE IS SIGNIFICANT ONLY IN ONE CASE, AT A CONCENTRATION OF ADRENALINE OF 16.1×10^{-8} M FOR THE CONCENTRATION OF THYROXINE OF 0.02×10^{-10} M.

AS SHOWN IN THE ANALYSIS OF VARIANCE, BOTH TISSUES FROM THYROIDECTOMIZED OR NORMAL ANIMALS, ARE AFFECTED BY THE CONCENTRATION OF ADRENALINE ALONE BUT NOT BY THAT OF THYROXINE ALONE. BOTH HORMONES TOGETHER, HOWEVER, AFFECT THE UPTAKE. AT HIGH CONCENTRATIONS OF THYROXINE, THE INTERACTION BETWEEN BOTH HORMONES MAY BE MORE IMPORTANT. WHILE IN THE NORMAL ANIMAL, THE ENDOGENOUS THYROXINE IS ALREADY PRESENT, IN THYROIDECTOMIZED ANIMAL IT IS NOT THE CASE. THE ADDITION OF A SMALL DOSE OF THYROXINE RESULTS IN A LARGE CHANGE FOR THE THYROIDECTOMIZED ANIMAL, IT WILL ONLY LEAD TO SMALL CHANGES IN THE NORMAL ANIMAL. HOWEVER, WHEN THE CONCENTRATION OF THYROXINE IS INCREASED, THERE IS A TENDENCY FOR BOTH TISSUES TO ATTAIN AN IDENTICAL LEVEL AND THIS WOULD EXPLAIN WHY AT HIGH THYROXINE CONCENTRATION IN THE MEDIUM, THE UPTAKE IN BOTH GROUPS IS NOT SIGNIFICANTLY DIFFERENT.

AT THIS STAGE OF THE PROCEEDINGS, THE DIFFERENCE OBSERVED MAY BE DUE TO THE DIFFERENCE IN THE THICKNESS OF THE TISSUES.

IT HAS BEEN CLAIMED BY SEVERAL WORKERS (81, 83, 162) THAT THE EFFECT IN VITRO OF THYROXINE ON ADRENALINE POTENTIATION WAS DUE TO THE CHELATING POWER OF THYROXINE (AS ALL AMINOACIDS THYROXINE FORMS A COMPLEX WITH COPPER (159)). BY BINDING THE COPPER PRESENT IN THE MEDIUM (DUE TO IMPURITIES IN THE WATER AND IN THE REAGENTS USED TO PREPARE THE MEDIUM) THYROXINE WOULD PREVENT ITS CATALYTIC ACTION ON ADRENALINE OXIDATION. A GREATER AMOUNT OF ADRENALINE WOULD THEN BE AVAILABLE FOR THE TISSUE. IN THE CASE HERE, THE USE OF ALBUMIN HAS BEEN CHOSEN, SO THAT THE COPPER PRESENT IN THE SOLUTION WOULD BE CHELATED BY THIS PROTEIN. IT THUS APPEARS THAT THIS PROPERTY OF THYROXINE HAS BEEN ELIMINATED. THERE IS STILL HOWEVER, AN EFFECT OF THYROXINE. WHETHER THE EFFECT OF THYROXINE ON THE RADIOACTIVE CONTENT OF THE TISSUE IS DUE TO AN ACTUAL INCREASE IN ³H-ADRENALINE OR TO AN INCREASE IN THE METABOLITES CAN BE ELUCIDATED BY IDENTIFYING THE RADIOACTIVE MATERIAL.

B) EFFECT OF THYROXINE ON ³H-ADRENALINE BINDING

THE FACT THAT LARGE QUANTITIES OF ADRENALINE ARE FOUND IN THE TISSUES, AFTER THE PHYSIOLOGICAL EFFECTS OF THE INJECTIONS HAD DISAPPEARED SINCE A LONG TIME, INDUCED MANY AUTHORS TO ASSUME THAT THE ADRENALINE FOUND WAS NOT FREE BUT BOUND TO SOME " BINDING SITES " (98, 105, 151). FURTHER EXPERIMENTS ON THE IDENTIFICATION OF RADIOACTIVE MATERIAL IN TISSUES OF ANIMALS INJECTED ³H- OR ¹⁴C-ADRENALINE

HAS SHOWN THAT AN IMPORTANT PERCENTAGE OF THE RADIOACTIVE MATERIAL REPRESENTED UNCHANGED ADRENALINE. (161, 163). THE FACT THAT NO EFFECT WAS PRESENT LEAD THESE AUTHORS TO ASSUME THAT THE ADRENALINE WAS BOUND. MOREOVER, IT APPEARED THAT THE BINDING OF ADRENALINE REPRESENTED A RAPID INACTIVATION OF THE AMINE (161). THIS EFFECT HAS BEEN SHOWN IN THE HEART, ATRIA OF CATS, RATS AND ALSO IN THE WHOLE HOUSE (101, 188, 160). IN VIEW OF THESE FINDINGS IT WAS THOUGHT THAT PART OF THE ³H-ADRENALINE RECOVERED IN THE INTESTINAL TISSUE AFTER A 5 MINUTE INCUBATION WAS PROBABLY BOUND ³H-ADRENALINE. THE INHIBITORY EFFECT OF ADRENALINE ON THE SMOOTH MUSCLE OF THE SMALL INTESTINE IS RAPID AND SHORT IN DURATION (160). A RELAXATION PERIOD IS EXHIBITED BY THIS TISSUE AS BY ALL THE TISSUES ON WHICH NERVOUS TRANSMITTERS HAVE AN ACTION (165). IT SEEMS THEN, THAT ALTHOUGH THE TISSUE WAS IN CONTACT WITH ³H-ADRENALINE FOR FIVE MINUTES, THE EFFECT OF ADRENALINE COULD BE ONLY INTERMITTENT. FOR THESE TWO REASONS, THE ³H-ADRENALINE IDENTIFIED WAS CONSIDERED AS BOUND ADRENALINE. THE RINSING OF THE TISSUES AFTER INCUBATION PERMITTED TO POSTULATE THAT THE EXTRACELLULAR OR NOT FIRMLY BOUND ADRENALINE WOULD HAVE BEEN REMOVED BY THIS PROCESS.

1) BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS FROM NORMAL RATS AFTER A 5 MINUTE INCUBATION WITH ³H-ADRENALINE

THE ³H-ADRENALINE BOUND BY THE SMALL INTESTINE FROM NORMAL RAT REPRESENTS A LARGE PERCENTAGE OF THE TOTAL RADIOACTIVE CONTENT OF THE TISSUE, WHEN THE CONCENTRATION OF ³H-ADRENALINE IN THE MEDIUM IS 8.3×10^{-8} M (TABLE XII, PAGE 111). WHEN THE CONCENTRATION

OF ^3H -ADRENALINE IN THE MEDIUM IS DOUBLED, THE PERCENTAGE OF BOUND ^3H -ADRENALINE IS ABOUT HALF. THE ABSOLUTE AMOUNT OF ^3H -ADRENALINE IN BOTH CASES, HOWEVER IS SIMILAR, BECAUSE THE UPTAKE WAS LARGER AT A HIGHER CONCENTRATION OF ^3H -ADRENALINE. THIS FINDING APPEARS TO IMPLY THAT THE SMALL INTESTINE FROM NORMAL RATS DOES NOT HAVE UNLIMITED BINDING SITES. THE SATURATION OF THIS SITES SEEMSTO OCCUR VERY RAPIDLY, SINCE BY DOUBLING THE CONCENTRATION OF A SMALL CONCENTRATION OF ^3H -ADRENALINE, THE CONCENTRATION OF ^3H -ADRENALINE IN THE TISSUE IS IDENTICAL. IN THEIR IN VIVO EXPERIMENTS, WURTMANN ET AL (102), FOUND THAT IN THE HEART OF NORMAL RATS, ^3H -ADRENALINE REPRESENTED 67 % OF THE TOTAL RADIOACTIVITY CONTENT. IVERSEN AND WHITBY (100), IN THE WHOLE MOUSE, FOUND THAT AFTER A SINGLE INJECTION OF ^3H -ADRENALINE, ^3H -ADRENALINE REPRESENTED ABOUT 40-45 % OF THE TOTAL RADIOACTIVITY CONTENT. THE PERCENTAGE OF UNCHANGED ^3H -ADRENALINE DECREASED WHEN THE INJECTED DOSE WAS INCREASED. THE CHANGE IN PERCENTAGE WAS HOWEVER, MUCH SMALLER THAN THAT OCCURRING IN THIS EXPERIMENT. IT IS HOWEVER DIFFICULT TO COMPARE QUANTITATIVELY THE DOSES OF INJECTED ^3H -ADRENALINE TO THOSE USED IN AN INCUBATION MEDIUM. A QUALITATIVE COMPARISON SEEMS MORE APPROPRIATE. IN CAT CEREBRAL CORTEX SLICES INCUBATED FOR 60 MIN WITH ^3H -NORADRENALINE, DENGLER ET AL FOUND THAT ABOUT 75 % OF THE RADIOACTIVITY COULD BE ACCOUNTED FOR BY UNCHANGED NORADRENALINE (154). SIMILAR RESULTS WERE OBTAINED FOR THE HEARTS. BRODIE ET AL. (155) HAD PREVIOUSLY REPORTED THAT SLICES OF BRAIN LIVER AND SKELETAL MUSCLE INCUBATED FOR 60 MINUTES WITH

³H-ADRENALINE SHOWED THAT ³H-ADRENALINE WAS FOUND IN THE TISSUES. HOWEVER, AS THESE AUTHORS IDENTIFIED DIRECTLY THE AMINE, WITHOUT MEASURING THE RADIOACTIVE CONTENT OF THE TISSUES, THE PERCENTAGE OF ³H-ADRENALINE IS NOT GIVEN AS A FUNCTION OF THE TOTAL RADIOACTIVITY IN THE TISSUE. WILSON ET AL. (156) MORE RECENTLY SHOWED THAT AFTER ONE HOUR INCUBATION WITH ³H-ADRENALINE, CAT CORTX SLICES CONCENTRATED ³H-ADRENALINE FROM THE INCUBATION MEDIUM. HOWEVER, THESE AUTHORS AS THE PREVIOUSLY CITED ONES DID NOT MEASURE THE TOTAL RADIOACTIVITY CONTENT OF THE TISSUE PRIOR TO ³H-ADRENALINE IDENTIFICATION. THE SMALL INTESTINE IN THIS REGARD, APPEARS TO BEHAVE LIKE THE HEART OR CORTX, SINCE THE PERCENTAGE OF ³H-ADRENALINE BOUND IS A LARGE PART OF THE TOTAL RADIOACTIVITY CONTENT.

2) BINDING OF ³H-ADRENALINE BY INTESTINAL STRIPS FROM THYROIDECTOMIZED OR THYROXINE-TREATED ANIMALS, AFTER A 5 MINUTE INCUBATION WITH ³H-ADRENALINE

THE PRETREATMENT OF ANIMALS WITH THYROXINE DOES NOT APPEAR TO HAVE ANY EFFECT ON THE BINDING OF ³H-ADRENALINE FOR THE CONCENTRATION OF ³H-ADRENALINE USED IN THE MEDIUM. THYROIDECTOMY DOES NOT APPEAR TO HAVE AN EFFECT EITHER ON ³H-ADRENALINE BINDING WHEN THE CONCENTRATION OF ³H-ADRENALINE IN THE MEDIUM WAS 8.3×10^{-8} M. WHEN THE CONCENTRATION OF ³H-ADRENALINE IN THE MEDIUM WAS DOUBLED, THERE IS A SIGNIFICANTLY HIGHER BINDING OF ³H-ADRENALINE BY TISSUE FROM THYROIDECTOMIZED ANIMALS.

IN THE ANALYSIS OF VARIANCE, THE EFFECT OF THE ABSENCE OF THYROXINE ON THE BINDING OF ^3H -ADRENALINE BY THE TISSUE IS SHOWN (TABLE XIV, PAGE 136). THE ABSENCE OF THYROXINE HAS AN EFFECT ON THE BINDING OF ^3H -ADRENALINE BY THE SMALL INTESTINE. THIS EFFECT IS LARGE AND IS SHOWN BY AN INCREASED BINDING IN THE ABSENCE OF ENDOGENOUS THYROXINE. THESE RESULTS ARE IN THE SAME LINE THAN THOSE OF DENGLER AND TITUS (104), WHERE A DECREASE IN THE RADIOACTIVITY CONTENT OF HYPERTHYROID HEART WAS OBSERVED.

3) THE EFFECT OF EXOGENOUS THYROXINE ON THE BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS FROM NORMAL AND THYROIDECTOMIZED ANIMALS AFTER A 5 MINUTE INCUBATION WITH ^3H -ADRENALINE

IN THE TISSUE FROM NORMAL RAT, FOR ALL THE CONCENTRATIONS OF ^3H -ADRENALINE STUDIED, THE BINDING OF ^3H -ADRENALINE IS PROPORTIONAL TO THE UPTAKE. THYROXINE AFFECTS ^3H -ADRENALINE BINDING IN THE SAME DIRECTION AS THE UPTAKE, THE CONCENTRATION OF $0.02 \times 10^{-10}\text{M}$ THYROXINE, WHEN THE CONCENTRATION OF ^3H -ADRENALINE IS $8.3 \times 10^{-6}\text{M}$ INHIBITS THE BINDING OF ^3H -ADRENALINE. THE PRESENCE OF THYROXINE IN THE MEDIUM DOES NOT APPEAR TO AFFECT THE RELATIVE AMOUNT OF ^3H -ADRENALINE BOUND. THE CONCENTRATION OF THYROXINE HOWEVER IS IMPORTANT IN THE BINDING OF ^3H -ADRENALINE BY SMALL INTESTINE (TABLE XIV, PAGE 126). FROM THE INDIVIDUAL EXPERIMENTS, THE NATURE OF THE EFFECT APPEARS TO BE AN INHIBITORY EFFECT. THIS IS CONSISTENT WITH PREVIOUS FINDINGS IN VIVO, WHEN THE AMOUNT OF ^3H -ADRENALINE WAS FOUND LOWER IN HYPERTHYROID ANIMALS (102).

IN THE THYROIDECTOMIZED GROUP, THE ADDITION OF THYROXINE TO THE MEDIUM DECREASES THE RELATIVE AMOUNT OF ^3H -ADRENALINE BOUND FOR THE TWO CONCENTRATIONS OF ^3H -ADRENALINE ASSAYED. HIGHER CONCENTRATION OF THYROXINE DECREASES RELATIVELY MORE THE BINDING OF ^3H -ADRENALINE AT A GIVEN CONCENTRATION OF ^3H -ADRENALINE.

THERE IS A SIGNIFICANTLY HIGHER BINDING OF ^3H -ADRENALINE BY TISSUES FROM THYROIDECTOMIZED ANIMALS COMPARED TO NORMAL ONES. THE SIGNIFICANT DIFFERENCE IS CONCOMITANT WITH THAT OBSERVED IN THE UPTAKE. THIS WOULD BE IN AGREEMENT WITH THE THEORY THAT THE RAPID INACTIVATION OF ADRENALINE TAKES PLACE BY BINDING OF THE EXOGENOUS AMINE TO THE TISSUES. THYROXINE BY INHIBITING THE BINDING OF ^3H -ADRENALINE COULD INCREASE THE CONCENTRATION OF FREE AMINE IN THE TISSUE AND THUS PREVENTS FURTHER ENTRANCE OF THE EXOGENOUS AMINE INTO THE TISSUE. THIS WOULD EXPLAIN WHY THERE IS IN SOME CASES A DECREASING UPTAKE AS A FUNCTION OF THYROXINE CONCENTRATION IN THE MEDIUM. ON THE OTHER HAND, THYROXINE BY PREVENTING THE BINDING OF ^3H -ADRENALINE COULD INCREASE THE LEVEL OF FREE AMINE IN THE TISSUE, WHICH THUS WOULD BE BROUGHT DOWN BY ENZYMIC DEGRADATION OF THE FREE AMINE. SINCE CATECHOL-O-METHYL TRANSFERASE APPEARS TO BE THE ENZYME MAINLY INVOLVED IN THE METABOLISM OF "FREE" ADRENALINE (2), IT WAS THOUGHT THAT IT WAS OF SOME INTEREST TO MEASURE THE ^3H -METANEPHRINE CONTENT OF THE TISSUE. IN THE LIGHT OF D'IBRIO AND NAVRIDES RESULTS (129, 131), IT WAS ALSO INTERESTING TO STUDY THE POSSIBLE EFFECT OF THYROXINE ON THE FORMATION OF METANEPHRINE.

C) EFFECT OF THYROXINE ON ³H-METANEPHRINE FORMATION

1) THE FORMATION OF ³H-METANEPHRINE BY INTESTINAL STRIPS FROM NORMAL RATS AFTER A 5 MINUTE INCUBATION WITH ³H-ADRENALINE

AFTER A 5 MINUTE INCUBATION WITH ³H-ADRENALINE, THE AMOUNT OF ³H-METANEPHRINE FORMED IN THE SMALL INTESTINE FROM NORMAL RATS ACCOUNTS FOR ABOUT 10 % OF THE TOTAL RADIOACTIVITY CONTENT OF THE TISSUE (TABLE XV) WHETHER THE AMOUNT OF ³H-ADRENALINE IN THE MEDIUM IS 0,3 OR 16.1 x 10⁻⁸ M. BECAUSE THE UPTAKE OF RADIOACTIVE MATERIAL IS HIGHER IN THE LATTER CONCENTRATION OF ³H-ADRENALINE IN THE MEDIUM, THE ABSOLUTE AMOUNT OF ³H-METANEPHRINE IS ALSO HIGHER. SO FOR THE TWO CONCENTRATIONS OF ³H-ADRENALINE USED, CATECHOL-O-METHYL TRANSFERASE IS NOT SATURATED. IVERSEN AND WHITBY HAVE FOUND THAT IN THE WHOLE MOUSE, THE INJECTION OF ³H-ADRENALINE RESULTED AFTER 30 MINUTES IN THE PRESENCE OF 16 % O-METHYLATED METABOLITES. WHEN THAT DOSE OF ADRENALINE WAS 13 TIMES HIGHER, THE PERCENTAGE OF O-METHYLATED COMPOUND WAS HIGHER (20 %) (99). HOWEVER, THE TOTAL RADIOACTIVITY CONTENT OF THE MOUSE IS NOT GIVEN, SO THAT THE ACTUAL AMOUNT PRESENT IN THE MOUSE IS NOT KNOWN. WHAT SEEMS IMPORTANT IS THE FACT THAT THE ENZYME IS NOT SATURATED AT DOSES OF ³H-ADRENALINE LARGER THAN PHYSIOLOGICAL DOSES. THIRTY MINUTES AFTER THE BEGINNING OF A SLOW INFUSION OF ³H-ADRENALINE INTO CATS AXELROD ET AL. (143) OBSERVED THAT EQUAL AMOUNT OF ³H-ADRENALINE AND ³H-METANEPHRINE WERE PRESENT IN THE SMALL INTESTINE. SINCE THE ANIMAL WAS SACRIFICED IMMEDIATELY AT THE END OF THE INFUSION, THE

EXPERIMENTAL CONDITIONS APPEAR TO MIMIC THAT OF THESE EXPERIMENTS.

2) THE FORMATION OF ³H-METANEPHRINE BY INTESTINAL STRIPS FROM THYROIDECTOMIZED AND THYROXINE-TREATED RATS AFTER A 5 MINUTE INCUBATION WITH ³H-DL-ADRENALINE

THYROXINE-PRETREATMENT OF RATS, DOES NOT APPEAR TO HAVE ANY EFFECT ON THE FORMATION OF ³H-METANEPHRINE IN THE SMALL INTESTINE. THE PERCENTAGE OF ³H-METANEPHRINE FORMED BY SMALL INTESTINE FROM THYROXINE-TREATED RATS IS SIMILAR TO THAT FOUND IN THE NORMAL RATS. MOREOVER, THE ABSOLUTE AMOUNT OF ³H-METANEPHRINE FORMED IS ALSO IDENTICAL. IT WAS SUGGESTED BY D'IBRIO AND LEDUC, THAT IN THYROTOXICOSIS, THE LEVEL OF COMT ACTIVITY WAS MARKEDLY DECREASED BECAUSE, IN THIS CASE A DECREASE IN PROTEIN SYNTHESIS IS OBSERVED (123). THE EFFECT OF THYROXINE INJECTIONS WOULD BE A REFLECTION OF A GENERAL EFFECT OF THYROXINE ON PROTEIN SYNTHESIS. WHEN SMALLER DOSES OF THYROXINE ARE INJECTED AND NO THYROTOXIC STATE IS ATTAINED, THE LEVEL OF COMT HAS BEEN FOUND DECREASED OR UNCHANGED IN HEART OR LIVER (102, 123). ON THE OTHER HAND, D'IBRIO AND MAYRIDES DID NOT FIND ANY EFFECT OF THYROXINE OR THYROXINE ANALOGUES IN VITRO ON THE PARTIALLY PURIFIED LIVER COMT (129, 131). THESE AUTHORS SUGGESTED THAT THYROXINE EFFECT IN VIVO COULD BE DUE TO A DEPLETION BY THYROXINE OF VITAMIN B 12 (166). AN INDIRECT EFFECT OF THYROXINE ON THE METHIONINE SOURCE OF METHYL GROUP FOR TRANS-METHYLATION COULD BE RESPONSIBLE FOR THE DECREASE OF METANEPHRINE OR NORMETANEPHRINE IN VIVO. RECENTLY, BARRIEUX, PROULX AND D'IBRIO COULD NOT FIND ANY EFFECT OF THE VITAMIN B 12 ON THE LIVER OR HEART COMT

IN NORMAL OR IODINATED-CASEIN-FED RATS (123).

IN THE THYROIDECTOMIZED RATS, THE FORMATION OF ^3H -METANEPHRINE IS NOT SIGNIFICANTLY HIGHER THAN THAT FROM THE NORMAL RATS, IN THE SMALL INTESTINE, FOR THE TWO CONCENTRATIONS OF ^3H -ADRENALINE USED IN THE INCUBATION MEDIUM. THE ABSENCE OF THYROXINE DOES NOT HAVE ANY EFFECT ON THE FORMATION OF METANEPHRINE IN THE SMALL INTESTINE. IT SHOULD BE TAKEN INTO ACCOUNT THAT THE CONJUGATED ^3H -METANEPHRINE HAS NOT BEEN IDENTIFIED. A DIFFERENCE BETWEEN THE TWO GROUPS COULD THUS BE UNAPPARENT. HOWEVER, IN THE LIGHT OF THYROXINE METABOLISM WHERE CONJUGATED THYROXINE IS SPLIT IN THE SMALL INTESTINE INTO FREE THYROXINE AND GLUCURONIC ACID (167), IT CAN BE ASSUMED THAT THE GLUCURONIDE CONJUGATE OF ^3H -METANEPHRINE DOES NOT CONTRIBUTE FOR A LARGE PART TO THE TOTAL METABOLITES OF ^3H -ADRENALINE, IN THESE EXPERIMENTAL CONDITIONS.

THE OXIDATIVE DEAMINATED METABOLITES OF ^3H -METANEPHRINE COULD CONTRIBUTE A LARGE PERCENTAGE IN ADRENALINE METABOLITES. THEY WOULD ALL NECESSITATE THE ACTIVE PARTICIPATION OF MAO. THEIR PERCENTAGE COULD BE DEDUCTED FROM THE DIFFERENCE BETWEEN TOTAL UPTAKE AND THE SUM OF BOUND ^3H -ADRENALINE AND ^3H -METANEPHRINE FORMED. AT TWICE THE CONCENTRATION OF ^3H -ADRENALINE IN THE INCUBATION MEDIUM, IT APPEARS THAT THERE IS A LARGE INCREASE IN MAO METABOLITES. THE ABSENCE OF THYROXINE DOES NOT APPEAR TO AFFECT THE MAO ACTIVITY OF SMALL INTESTINAL STRIPS AT A GIVEN CONCENTRATION OF ^3H -ADRENALINE. THIS IS IN CONTRADICTION WITH WURTMAN'S RESULTS. IN THEIR IN VIVO

EXPERIMENTS, THESE AUTHORS FOUND A LARGE INCREASE IN THE MAO METABOLITES IN HEART OF THYROIDECTOMIZED ANIMALS COMPARED TO NORMAL (102). HOWEVER HEART MAO ACTIVITY IS SMALL IN THE NORMAL ANIMAL, WHILE IT APPEARS TO BE THE MAIN ENZYME IN THE SMALL INTESTINE (168). ON THE OTHER HAND, UTLEY FOUND THAT LIVER MAO WAS INCREASED IN THYROIDECTOMIZED FEMALE RATS, WHILE TREATMENT OF THESE ANIMALS WITH T3 REDUCED THAT INCREASE (171). IT SEEMS THAT IF THE INTESTINAL MAO ACTIVITY IS LARGE, AT THE DOSE OF ^3H -ADRENALINE USED IN THE MEDIUM, THE ENZYME IS NOT SATURATED (HIGHER ACTIVITY WHEN HIGHER SUBSTRATE) AND THUS A DIFFERENCE IN THE ACTIVITY WHEN THYROXINE IS PRESENT MAY VERY WELL NOT BE APPARENT.

3) THE EFFECT OF EXOGENOUS THYROXINE ON THE FORMATION OF ^3H -METANEPHRINE BY INTESTINAL STRIPS FROM NORMAL AND THYROIDECTOMIZED RATS AFTER A 5 MINUTE INCUBATION WITH ^3H -ADRENALINE

NORMAL RATE

IN THE NORMAL RAT THYROXINE ADDITION TO THE INCUBATION MEDIUM APPEARS TO AFFECT THE FORMATION OF ^3H -METANEPHRINE BY THE SMALL INTESTINE.

AT THE LOWEST CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM (8.3×10^{-8} M), THE ADDITION OF THYROXINE RESULTED IN A SLIGHT INCREASE IN THE PERCENTAGE OF METANEPHRINE FORMED. THIS INCREASE IS ALSO PRESENT WHEN THE RESULTS ARE EXPRESSED AS THE ABSOLUTE AMOUNT OF ^3H -METANEPHRINE PER GRAM OF TISSUE (TABLE XV D). BY DOUBLING THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM (16.1×10^{-8} M) THE EFFECT OF THYROXINE ON THE FORMATION OF ^3H -METANEPHRINE IS STILL PRESENT. HOWEVER, WHEN THE RESULTS ARE EXPRESSED AS ABSOLUTE VALUES THERE IS A SLIGHT DECREASE

IN METANEPHRINE FORMATION AT A CONCENTRATION OF THYROXINE IN THE MEDIUM OF 0.02×10^{-10} M. THIS SLIGHT DECREASE APPEARS TO REFLECT A DECREASE IN UPTAKE RATHER THAN A DECREASE IN COMT ACTIVITY, SINCE THE PERCENTAGE OF ^3H -METANEPHRINE FORMED WAS EQUAL TO THAT IN THE ABSENCE OF THYROXINE IN THE MEDIUM. THE INCREASE IN METANEPHRINE FORMATION AT THE HIGHEST CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM, IN THE PRESENCE OF THYROXINE PARALLELS THE INCREASE IN THE UPTAKE. THE STATISTICAL ANALYSIS (TABLE XVII, PAGE 144) SHOWS THAT THYROXINE ALONE HAS AN EFFECT ON THE FORMATION OF METANEPHRINE BY INTESTINAL STRIPS, IN THE NORMAL RATS.

AT A CONSTANT CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM, INCREASING THE CONCENTRATION OF THYROXINE WILL RESULT IN AN INCREASE IN ^3H -METANEPHRINE FORMED. THIS EFFECT APPEARS NOT TO BE DUE TO A DIRECT EFFECT OF THYROXINE ON COMT ACTIVITY, BUT RATHER TO A HIGHER UPTAKE OF ^3H -ADRENALINE BY THE TISSUE, RESULTING IN A LARGER CONCENTRATION OF SUBSTRATE AVAILABLE FOR THE ENZYME. THIS FINDING APPEARS TO CONFIRM D'ICRISIO AND MAVRIDES' HYPOTHESIS (166), THAT THE LACK OF DIRECT EFFECT OF THYROXINE, IN VITRO, ON LIVER COMT SHOWED THAT THIS ENZYME WAS NOT DIRECTLY AFFECTED BY THYROXINE, IN VIVO. THIS ALSO SHOWS, THAT INTERPOLATION OF IN VITRO TO IN VIVO EXPERIMENTS REQUIRES GREAT CAUTION, SINCE IN VITRO, THYROXINE APPEARS TO INCREASE THE FORMATION OF METANEPHRINE, WHILE IN VIVO THIS HORMONE DECREASES PROTEIN SYNTHESIS (AT LARGE DOSES) AND THUS ACTUALLY DECREASES METANEPHRINE FORMATION.

ADRENALINE ALONE DOES HAVE AN EFFECT ON METANEPHRINE

FORMATION IN THE SMALL INTESTINE FROM NORMAL RATS. AND HERE AGAIN THIS EFFECT SEEMS TO BE RELATED TO A LARGER AMOUNT OF SUBSTRATE AVAILABLE RATHER THAN TO A SUBSTRATE-ACTIVATION OF THE ENZYME. THIS HYPOTHESIS IS BASED UPON OUR OWN FINDINGS THAT INCREASING ^3H -ADRENALINE IN THE MEDIUM RESULTS IN AN INCREASED UPTAKE, IT IS ALSO SUPPORTED BY DI IORIO AND MAVRIDES' IN VITRO EXPERIMENTS ON LIVER COMT (129, 131). SINCE HOWEVER, COMT FROM DIFFERENT ORGANS HAS BEEN SHOWN TO EXHIBIT A DIFFERENT REACTION TO THYROXINE (102), THE SUPPORT GIVEN BY DI IORIO AND MAVRIDES' FINDINGS IS SOMEWHAT LIMITED.

THE DIFFERENCE BETWEEN UPTAKE AND (ADRENALINE PLUS METANEPHRINE), REFLECTING MAO ACTIVITY, CHANGES IN THE PRESENCE OF THYROXINE. THYROXINE HAS SOME EFFECT ON OXIDATIVE DEAMINATION OF THE AMINE, THE PERCENTAGE OF UNIDENTIFIED METABOLITES INCREASES WITH THE FIRST ADDITION OF THYROXINE. INCREASING FURTHER THE CONCENTRATION OF THYROXINE DOES NOT RESULT IN FURTHER INCREASE IN THE DEAMINATION. SMALL INTESTINE FROM NORMAL RAT SEEMS TO BE VERY SENSITIVE TO THE ADDITION OF A SMALL DOSE OF THYROXINE, WHERE MAO ACTIVITY IS CONCERNED. INTERACTION BETWEEN THYROXINE AND ADRENALINE DOES NOT AFFECT THE FORMATION OF ^3H -METANEPHRINE BY THE SMALL INTESTINE.

THYROIDECTOMIZED ANIMALS

IN SMALL INTESTINE FROM THYROIDECTOMIZED RATS, ^3H -METANEPHRINE FORMATION SEEMS UNCHANGED OR INCREASED BY THE

ADDITION OF THYROXINE TO THE MEDIUM. THE PERCENTAGE OF ^3H -METANEPHRINE FORMED AT THE LOWEST CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM IS UNCHANGED BY ADDITION OF THYROXINE TO THE MEDIUM. HOWEVER, SINCE ^3H -ADRENALINE UPTAKE INCREASES WITH THE ADDITION OF THYROXINE, THE CONCENTRATION OF ^3H -METANEPHRINE BY GRAM OF TISSUE IS ALSO INCREASED.

WHEN THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM IS TWICE INCREASED, THERE IS AN INCREASE IN THE METANEPHRINE FORMED AT THE TWO EXTREME CONCENTRATIONS OF THYROXINE ASSAYED (TABLE XVI D, PAGE 141). THERE IS A SLIGHT DECREASE IN ^3H -METANEPHRINE FORMATION IN THE INTERMEDIATE CONCENTRATION OF THYROXINE ASSAYED. THE FORMATION OF ^3H -METANEPHRINE DOES NOT REFLECT THE INCREASE IN THE UPTAKE. IN THE SMALL INTESTINE FROM THYROIDECTOMIZED RATS, THYROXINE APPEARS TO AFFECT COMT ACTIVITY IN A MANNER DIFFERENT OF THAT IN THE NORMAL RATS. THIS IS SHOWN (TABLE XVII, PAGE 144) BY THE FACT THAT IN THE THYROIDECTOMIZED ANIMALS, THYROXINE ALONE DOES NOT AFFECT METANEPHRINE FORMATION WHILE IT DOES AFFECT IT IN CONJUNCTION WITH ADRENALINE. ADRENALINE CONCENTRATION DOES AFFECT THE FORMATION OF ^3H -METANEPHRINE BY THE SMALL INTESTINE FROM THYROIDECTOMIZED ANIMALS. IT IS INTERESTING TO NOTE THAT BOTH IN THE UPTAKE AND IN THE FORMATION OF ^3H -METANEPHRINE, IN THE THYROIDECTOMIZED RATS, THYROXINE ALONE HAS NO EFFECT, BUT DOES HAVE ONE IN RELATION WITH ADRENALINE. IT WOULD APPEAR THAT THE EFFECT OF THYROXINE TAKES PLACE AT TWO STAGES. AT LOW CONCENTRATIONS OF THYROXINE, THYROXINE ALONE DOES NOT HAVE ANY EFFECT (THYROIDECTOMIZED RATS).

AT HIGHER CONCENTRATION THYROXINE ALONE DOES HAVE AN EFFECT WHILE NO INTERACTION OCCURS (NORMAL RATS). IN THE THYROIDECTOMIZED RATS, ADDITION OF THYROXINE TO THE MEDIUM, FOR THE TWO CONCENTRATIONS OF ³H-ADRENALINE ASSAYED RESULTED IN AN INCREASE IN THE MAO METABOLITES FORMED.

NORMAL AND THYROIDECTOMIZED RATS

THERE IS AN INCREASE IN ³H-METANEPHRINE FORMED BY THE SMALL INTESTINE FROM THYROIDECTOMIZED WHEN COMPARED TO NORMAL RATS. THIS INCREASE IS FOUND TO BE SIGNIFICANT FOR BOTH CONCENTRATIONS OF ³H-DL-ADRENALINE ASSAYED, AT A DIFFERENT CONCENTRATION OF THYROXINE. THE AMOUNT OF THYROXINE, NECESSARY TO BRING ABOUT A SIGNIFICANT DIFFERENCE IS DECREASED TENFOLD, WHEN THE CONCENTRATION OF ³H-DL-ADRENALINE IS INCREASED TWICE. FOR THE UPTAKE AND BINDING, THE CRITICAL CONCENTRATION OF THYROXINE WAS 0.02×10^{-10} M, WHATEVER THE CONCENTRATION OF EXOGENOUS ADRENALINE WERE. IN THE CASE OF METANEPHRINE FORMATION, THIS CONCENTRATION IS CRITICAL ONLY AT A HIGH CONCENTRATION OF EXOGENOUS ADRENALINE. AT A LOW CONCENTRATION OF ADRENALINE, A HIGHER CONCENTRATION OF THYROXINE IS NECESSARY FOR THE FORMATION OF METANEPHRINE TO BE AFFECTED. IT APPEARS, THUS, THAT THE UPTAKE AND THE BINDING OF EXOGENOUS ADRENALINE ARE AFFECTED BY SMALLER DOSES OF THYROXINE THAN THE ENZYME. THYROXINE IS KNOWN TO PENETRATE VERY SLOWLY INTO THE CELL (169), AND THIS DIFFERENCE IN SENSITIVITY MAY REFLECT :

- 1) THAT THE PENETRATION OF ³H-ADRENALINE IS FACILITATED BY THYROXINE,
- 2) THAT IF THYROXINE ACTS AS AN ACTIVATOR OF BINDING AND AN INHIBITOR

OF THE ENZYME, EITHER THE BINDING SITES ARE MORE READILY AVAILABLE THAN THE ACTIVE SITES OF THE ENZYME TO THYROXINE OR, THAT THE AFFINITY OF THE FORMER IS GREATER THAN THAT OF THE LATTER FOR THYROXINE.

THE FACT THAT THYROXINE DOES HAVE AN EFFECT ON METANEPHRINE FORMATION SHOWS THAT THYROXINE DOES NOT ACT ONLY BY CHELATING COPPER (81, 83). THE INCREASED FORMATION OF ³H-METANEPHRINE IN THE PRESENCE OF THYROXINE APPEARS TO BE IN CONTRADICTION WITH THE IN VIVO FINDINGS THAT IN HYPERTHYROID ANIMALS, THE LEVEL OF COMT IN BOTH HEART AND LIVER IS DECREASED OR UNCHANGED COMPARED TO NORMAL ANIMALS. IT APPEARS ALSO TO BE IN CONTRADICTION WITH THE IN VITRO ASSAY OF THYROXINE ON COMT ACTIVITY (166). HOWEVER, THE CONTRADICTION IS ONLY APPARENT SINCE THE EFFECT OF THYROXINE IN THE FORMATION OF METANEPHRINE SEEMS MAINLY DUE TO AN INDIRECT EFFECT THROUGH INCREASED SUBSTRATE AVAILABILITY FOR THE ENZYME.

SUMMARY AND CONCLUSION

THE UPTAKE OF RADIOACTIVE MATERIAL BY INTESTINAL STRIPS FROM NORMAL, THYROXINE-TREATED AND THYROIDECTOMIZED RATS, AFTER A 5 MINUTE INCUBATION WITH ^3H -DL-ADRENALINE HAS BEEN STUDIED. INTESTINE FROM THYROXINE-TREATED AND NORMAL RATS EXHIBITED IDENTICAL UPTAKE OF RADIOACTIVE MATERIAL, WHILE A LARGER UPTAKE WAS OBSERVED IN THE SMALL INTESTINE FROM THYROIDECTOMIZED RATS. FOR THE THREE CONCENTRATIONS OF ^3H -ADRENALINE ASSAYED IN THE MEDIUM, INTESTINE FROM THYROIDECTOMIZED RATS EXHIBITED ALWAYS A LARGER UPTAKE WHEN COMPARED TO NORMAL RATS. FOR BOTH GROUPS, A HIGHER UPTAKE WAS SEEN WHEN THE CONCENTRATION OF ^3H -ADRENALINE IN THE MEDIUM WAS INCREASED. THE ADDITION OF EXOGENOUS THYROXINE TO THE MEDIUM RESULTED IN A CHANGE IN THE UPTAKE AT A CONSTANT CONCENTRATION OF ^3H -ADRENALINE FOR BOTH NORMAL AND THYROIDECTOMIZED GROUPS. THE EFFECT OF THYROXINE FOR BOTH GROUPS WAS FOUND TO DEPEND UPON THE CONCENTRATIONS OF THYROXINE AND OF ^3H -ADRENALINE ASSAYED. THYROXINE ALONE WAS FOUND WITHOUT EFFECT, WHILE ADRENALINE ALONE WAS VERY EFFICIENT. BOTH HORMONES TOGETHER AFFECT THE UPTAKE BY INTESTINAL STRIPS FROM NORMAL OR THYROIDECTOMIZED RATS.

THE BINDING OF ^3H -ADRENALINE BY INTESTINE FROM NORMAL AND THYROXINE-TREATED RATS WAS FOUND SIMILAR. THYROIDECTOMY RESULTED IN A HIGHER BINDING OF ^3H -ADRENALINE BY INTESTINAL STRIPS WHEN COMPARED TO THE NORMAL GROUP. THE ADDITION OF THYROXINE TO THE

INCUBATION MEDIUM RESULTED IN A LOWER BINDING OF ^3H -ADRENALINE IN BOTH NORMAL AND THYROIDECTOMIZED GROUPS.

THE FORMATION OF ^3H -METANEPHRINE BY INTESTINAL STRIPS WAS NOT DIRECTLY AFFECTED BY THYROXINE, EITHER ENDOGENOUS OR EXOGENOUS. THE INCREASED AMOUNT OF ^3H -METANEPHRINE FORMED IN THE PRESENCE OF THYROXINE, APPEARS TO BE INDIRECTLY DUE TO A LARGER UPTAKE. ON THE OTHER HAND THE UNIDENTIFIED METABOLITES WERE AFFECTED BY THE ADDITION OF EXOGENOUS THYROXINE.

THE RESULTS WERE DISCUSSED IN THE LIGHT OF PREVIOUS WORK. THE SMALL INTESTINE FROM RATS APPEARS TO BEHAVE IN A MANNER SIMILAR TO THE OTHER SMOOTH MUSCLE STUDIED WHERE THE UPTAKE AND BINDING OF ^3H -ADRENALINE ARE CONCERNED. ON THE OTHER HAND, IT WOULD SEEM THAT THE ACTIVITY OF MAO IN THE SMALL INTESTINE IS INCREASED IN THE PRESENCE OF THYROXINE. SINCE THE EVIDENCE ON MAO IS INDIRECT, IT WOULD SEEM INTERESTING TO IDENTIFY THE "UNKNOWN MATERIAL" TO SEE WHETHER IT CORRESPONDS TO DEAMINATED METABOLITES OF ADRENALINE OR TO CONJUGATED METABOLITES. BECAUSE MAO HAS BEEN POSTULATED TO DEGRADE GATECHOLAMINES WHICH HAVE BEEN BOUND IN THE CELL, IT WOULD BE OF INTEREST TO STUDY A TIME-COURSE OF ADRENALINE UPTAKE, BINDING AND METABOLISM AS A FUNCTION OF ADRENALINE AND THYROXINE CONCENTRATIONS. FROM THE WORK PRESENTED HERE, THE RELATIVE CONCENTRATIONS OF BOTH THYROXINE AND ADRENALINE SEEM TO PLAY AN IMPORTANT ROLE IN THE UPTAKE, THE BINDING AND METABOLISM OF ADRENALINE IN THE INTESTINE OF THE RAT.

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