

THE STUDY OF PHAGOCYTOSIS

BY
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INTRODUCTION

THE WORD PHAGOCYTOSIS HAS A COLORFUL ETYMOLOGY, IT COMES FROM TWO GREEK WORDS - PHAGEIN, TO EAT; AND KYTOS, CELL, THEREFORE AN EATING CELL. THESE CELLS HAVE ALSO BEEN FIGURATIVELY REFERRED TO AS "THE POLICEMAN OF THE BLOOD."

PHAGOCYTOSIS WAS FIRST DEMONSTRATED BY ELIE METCHNIKOFF, A RUSSIAN BIOLOGIST, IN THE LATE NINETEENTH CENTURY. SINCE HIS TIME MANY THEORIES HAVE BEEN FORWARDED ON THIS WONDERFUL PHENOMENA. FOR A NUMBER OF YEARS AFTER ITS DISCOVERY IT ENJOYED AN ERA OF GREAT IMPORTANCE. HOWEVER, WITH THE DISCOVERY OF OUR MODERN ANTIBODY-ANTIGEN REACTION THEORY IN 1935 BY HEILDERBERGER ITS IMPORTANCE HAS DROPPED TO PRACTICALLY NIL.

IN THE PAST 23 YEARS THEREFORE PHAGOCYTOSIS HAS BEEN A MUCH ABUSED SUBJECT AND WRONGLY SO. IT SHOULD ACTUALLY, I BELIEVE, BE ONE OF THE MOST IMPORTANT PHASES OF IMMUNOLOGY. AS A RESULT OF THIS NEGLECT WE KNOW HARDLY ANYTHING ABOUT THE ACTION, THE CAUSE, OR EVEN THE RESULT OF THIS FASCINATING MECHANISM. I WOULD LIKE TO POINT OUT THAT WITHOUT PHAGOCYTOSIS, ANTIBODIES WOULD BE PRACTICALLY WORTHLESS. THIS IS DUE TO THE SIMPLE FACT THAT ANTIBODIES DO NOT, FOR THE MOST PART, KILL BACTERIA BUT MERELY TEMPORARILY INACTIVATE THEM

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1. HOERR, N.L. AND A. OBAL, NEW GOULD MEDICAL DICTIONARY, PP.896
 2. IBID., PP.725
 3. FROM NOTES TAKEN IN PATHOGENIC MICROBIOLOGY.

OR AGGLUTINATE THEM SO THE PHAGOCYTE MIGHT DO THEIR JOB EASIER. I BELIEVE THAT SOMEDAY AFTER ENOUGH KNOWLEDGE IS GAINED IN THIS FIELD, PHAGOCYTOSIS MAY BE CONTROLLED, PERHAPS EVEN TO THE DEGREE TO MAKE ANTIBODIES UNNECESSARY. DURING THE COURSE OF THIS THESIS I HAVE OFTEN ASKED MYSELF THIS QUESTION; WHY WOULD IT NOT BE POSSIBLE TO DEVELOP SOME TYPE OF AGENT THAT WOULD "SPUR ON" OR ACTIVATE PHAGOCYTES TO TREMENDOUSLY INCREASE THEIR EFFICIENCY. OR, INSTEAD OF "PREPARING" THE INVADING ORGANISMS, AS ANTIBODIES DO, WHY SHOULD NOT THE PROBLEM BE ATTACKED AT ITS BASE, AT THE PHAGOCYTE ITSELF. SUCH A DEVELOPMENT WOULD SURELY BE OF MONUMENTAL SIGNIFICANCE TO THE FIELD OF IMMUNIZATION.

THESE ARE MERELY THEORETICAL IDEAS, HOWEVER IN THIS THESIS I WILL ATTEMPT TO ANSWER AND PROVE THE FOLLOWING FUNDAMENTAL QUESTIONS ABOUT PHAGOCYTOSIS. 1) WILL PHAGOCYTOSIS WORK OUTSIDE THE BODY? 2) IS IT AS EFFECTIVE AS PHAGOCYTOSIS INSIDE THE BODY? 3) WILL PHAGOCYTOSIS WORK IN PHYSIOLOGICAL SALINE SOLUTION AND IS BLOOD SERUM AN IMPORTANT FACTOR IN THE OPERATION? 4) WHAT IS THE ROLE OF OPSONINS (ANTIBODIES) IN PHAGOCYTOSIS?

CHAPTER I

THE TERMINOLOGY AND CLASSIFICATION OF THE PHAGOCYTES

THE TERM PHAGOCYTE, ALTHOUGH USED SPECIFICALLY IS ACTUALLY QUITE A NEBULOUS ONE. THE STANDARD CLASSIFICATION IS NOT UNIVERSALLY ACCEPTED AND IN THE CASE OF SOME OF THE CELLS THEIR PHAGOCYTTIC PROPERTIES ARE NOT EVEN KNOWN.

ALL OF THE PHAGOCYTES OF THE BODY HOWEVER CAN BE PLACED UNDER TWO VERY GENERAL HEADINGS, THE LEUCOCYTES OF THE BLOOD AND THE RETICULO-ENDOTHELIAL SYSTEM. THE CELLS OF THE LATTER ARE BY FAR THE MOST NUMEROUS PHAGOCYTES OF THE BODY. THESE CELLS HOWEVER DO NOT LEND THEMSELVES EASILY TO STUDY BECAUSE OF THEIR LOCATIONS IN THE BODY AND THE NECESSITY OF HAVING TO REMOVE ENTIRE TISSUE BLOCKS TO OBTAIN THEM. THEREFORE IN ALL THE EXPERIMENTS OF THIS THESIS I HAVE UTILIZED THE PHAGOCYTES OF THE BLOOD.

THE LEUCOCYTES OF THE BLOOD MAY BE CLASSIFIED EASILY. THERE ARE FIVE TYPES OF THESE CELLS WHICH SHALL BE CONSIDERED INDEPENDENTLY. THESE FIVE TYPES FALL INTO TWO MAIN CATEGORIES, THE AGRANULOCYTES AND THE GRANULOCYTES.

I AGRANULOCYTES - THIS NAME WOULD IMPLY THAT THE CELLS CONTAIN

1. STILES, KARL A., HANDBOOK OF MICROSCOPIC CHARACTERISTICS OF TISSUES AND ORGANS. PP.38

NO GRANULES HOWEVER THIS IS NOT TRUE. THE CELLS DO CONTAIN GRANULES, BUT THEY ARE NOT VISIBLE UNDER ORDINARY STAINING METHODS AND ARE TOO FINE TO BE SEEN WITH THE ORDINARY LIGHT MICROSCOPE. THE CELLS IN THIS CATEGORY MAY ALSO BE REFERRED TO AS MONONUCLEOCYTES FOR THEY CONTAIN ONLY ONE SPHERICAL NUCLEUS. TWO OF THE FIVE TYPES FALL INTO THIS DIVISION, NAMELY THE LYMPHOCYTES AND THE MONOCYTES.

1) LYMPHOCYTES - THESE CELLS HAVE A LARGE RANGE IN SIZE THEY VARY FROM 7 TO 12 MICRONS. THE NUCLEUS IS VERY LARGE, SPHERICAL BUT WITH SOMETIMES A SLIGHT INDENTATION, AND COMPOSES MOST OF THE CELL. THE CYTOPLASM IS MERELY A THIN RING AROUND THE NUCLEUS. THESE CELLS COMPRISE ABOUT 25% OF THE LEUCOCYTES IN THE BLOOD OR ABOUT 2,000 CELLS PER CUBIC MILLIMETER. THE FUNCTION OF THESE CELLS IS NOT WELL UNDERSTOOD, IF THEY ARE PHAGOCYtic AT ALL IT IS ONLY TO A VERY SLIGHT DEGREE.

2) MONOCYTES (SEE FIG. 1) - THE APPEARANCE OF THESE CELLS IS VERY SIMILAR TO THE LYMPHOCYTE, HOWEVER THEY ARE ALWAYS LARGER RANGING BETWEEN 12 AND 15 MICRONS. THEY HAVE A RELATIVELY LARGER AMOUNT OF CYTOPLASM THAN DOES THE LYMPHOCYTES, BUT THE NUCLEUS STILL OCCUPIES THE GREATER PART OF THE CELLS. THE MONOCYTES ARE 3 TO 8% OF THE LEUCOCYTES OR ABOUT 400 CELLS PER CUBIC MILLIMETER. THESE ARE VERY IMPORTANT IN THE DISCUSSION OF

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1. IBID., PP.42
 2. LOC. CIT.
 3. LOC. CIT.
 4. LOC. CIT.

PHAGOCYTES BECAUSE THEY ARE READILY MOTILE AND VERY PHAGOCYTTIC.

THE MONOCYTES POSE A SLIGHT PROBLEM IN THEIR CLASSIFICATION. THE GENERAL SYSTEM OF THE CLASSIFICATION OF THE PHAGOCYTES IS BASED ON THE ORIGIN OF THE CELL. THE ORIGIN OF THE MONOCYTES HAS BEEN THE SUBJECT OF CONSIDERABLE CONTROVERSY. ALEXANDER MAXIMOW OF THE UNIVERSITY OF CHICAGO BELIEVES THAT THE MONOCYTES ARISE FROM LYMPHOCYTES AND STATES THAT IN SOME SITUATION, E.G., IN THE SPLEEN, ALL THE TRANSITIONAL STAGES FROM THE LYMPHOCYTE TO MONOCYTE MAY BE SEEN. THEREFORE, ACCORDING TO MAXIMOW, THE MONOCYTES SHOULD BE CLASSIFIED UNDER THE LEUCOCYTE. LEWIS HAS SHOWN, ON THE CONTRARY, THAT IN HANGING DROP CULTURES MACROPHAGES (SEE UNDER RETICULO-ENDOTHELIAL SYSTEM) MAY BE TRANSFORMED INTO MONOCYTES OR VICE VERSA. THIS SCHOOL OF THOUGHT, WHICH WILL BE EXPLAINED LATER, SEEMS TO INDICATE THAT THE MONOCYTES ARE DERIVED FROM HISTIOCYTES AND THEREFORE SHOULD BE CLASSIFIED UNDER THE RETICULO-ENDOTHELIAL SYSTEM. REGARDLESS OF THEIR CLASSIFICATION THEY ARE VERY IMPORTANT PHAGOCYTES AND MUCH MORE WILL BE SAID ABOUT THEM IN THIS THESIS.

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1. MAXIMOW, ALEXANDER A. AND WILLIAM BLOOM, A TEXTBOOK OF HISTOLOGY, PP.100
 2. BEST, C.H. AND N. B. TAYLOR, THE PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE, PP.81

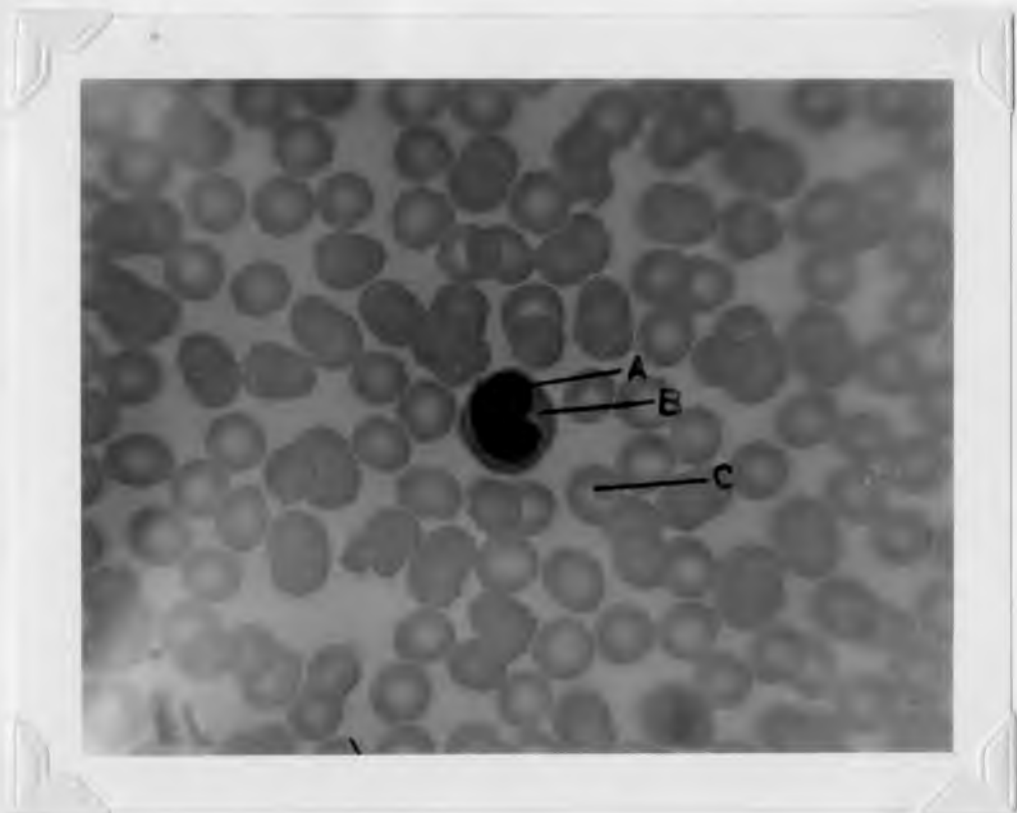


FIGURE 1 - MONOCYTE

KEY: A) NUCLEUS
B) CYTOPLASM
C) ERYTHROCYTES

11 GRANULOCYTES - THESE CELLS ARE DIVIDED INTO THREE GROUPS ACCORDING TO THE STAINING PROPERTIES OF THEIR CYTOPLASMIC GRANULES. THESE STAINING PROPERTIES ARE SPECIFIC TO MAN AND CANNOT NECESSARILY BE APPLIED IN THE SAME MANNER TO LABORATORY ANIMALS. COMMON TO ALL THE CELLS IN THIS GROUP IS THE FACT THAT THEY HAVE A BI, TRI, OR MUTILOBED NUCLEUS AND HENCE ANOTHER NAME COMMONLY USED TO REFER TO THIS GROUP OF CELLS IS THE POLYMORPHONUCLEARCYTES OR SIMPLY POLYMORPHOCYTES.

1) NEUTROPHILS (SEE FIGURE 2) - THESE STAIN WITH NEUTRAL DYES, THAT IS, A MIXTURE OF ACID AND BASIC DYES AND GIVE A FAINT LILAC COLOR TO THE CYTOPLASMIC GRANULES. THE NEUTROPHILS GENERALLY RANGE FROM 9 TO 12 MICRONS¹. THEIR NUCLEUS IS HIGHLY POLYMORPHOUS, APPEARING AS AN ELONGATED, BENT, OR TWISTED BODY WITH THREE TO FIVE LOBES CONNECTED BY THIN CHROMATIC THREADS. THE NEUTROPHILS ARE THE MOST COMMON LEUCOCYTES IN THE BLOOD RANGING FROM 60 TO 75%² OF THE TOTAL NUMBER OF LEUCOCYTES WHICH IS ABOUT 5,600 PER CUBIC MILLIMETER. IN THIS THESIS THE NEUTROPHIL IS BY FAR THE MOST IMPORTANT CELL. IT IS EXTREMELY PHAGOCYTTIC AND POSSESSES GOOD POWERS OF MOTILITY AND AMEBOID ACTION.

2) EOSINOPHIL - THESE STAIN WITH ACID DYES, E.G., EOSIN, FROM WHICH THE CELL DERIVES ITS NAME, TO GIVE A RED COLOR TO THE COARSE GRANULES OF THE CYTOPLASM. THE EOSINOPHIL IS THE LARGEST OF THE GRANULOCYTES RANGING FROM 10 TO 14 MICRONS³. A BILOBED NUCLEUS IS CHARACTERISTIC OF THIS TYPE, THE LOBES ARE CONNECTED BY A THIN CHROMATIC

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1. STILES, KARL A., OP.CIT., PP.42
 2. LOC. CIT.
 3. LOC .CIT.

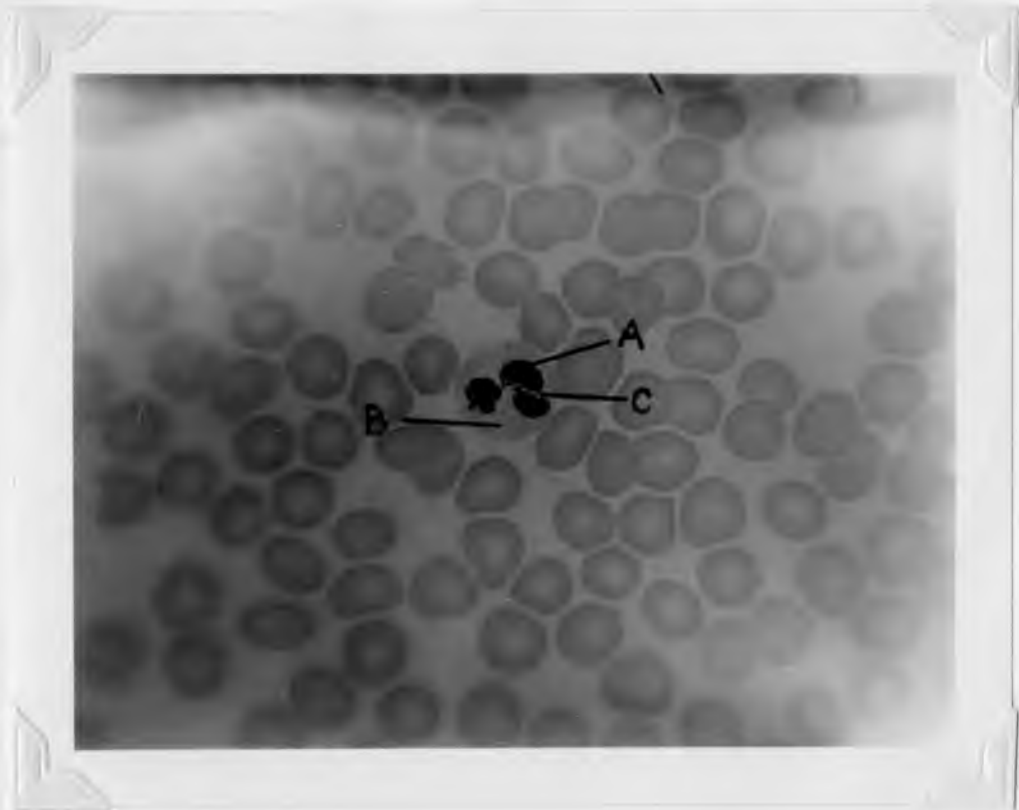


FIGURE 2 NEUTROPHIL

KEY: A) LOBES OF NUCLEUS
B) CYTOPLASM
C) CHROMATIN THREAD

THREAD. THEY ARE NOT NUMEROUS IN THE BLOOD AND COMPRISE ONLY ABOUT 2 TO ¹5% OF THE LEUCOCYTES. THEIR PHAGOCYTTIC PROPERTIES, IF ANY, ARE VERY SLIGHT. 3) BASOPHIL - HERE AGAIN, FOR THIS STUDY, WE HAVE AN UNIMPORTANT LEUCOCYTE. THE BASOPHILS ARE ONLY PRESENT TO 0.5% OF THE LEUCOCYTES, OR LESS.² THE GRANULES IN THIS CELL STAIN DEEP BLUE WITH BASIC DYES. THEIR FUNCTION IS UNKNOWN, THEY HAVE NO PHAGOCYTTIC POWERS AND ARE THOUGHT, BY SOME, TO BE A DEGENERATE FORM OF NEUTROPHIL.

THIS COMPLETES THE CLASSIFICATION OF THOSE PHAGOCYTES, AND OTHER RELATED CELLS, WHICH FALL UNDER THE GENERAL HEAD-ING OF LEUCOCYTES.

RETICULO-ENDOTHELIAL SYSTEM

THIS TERM WAS COINED BY ASCHOFF,³ AND WAS ORIGINALLY MEANT TO DESIGNATE ONLY TWO SPECIES OF CELLS WITHIN THE SYSTEM AS IT IS KNOWN TODAY. SINCE HIS TIME, HOWEVER, OTHER CELLS HAVE BEEN DISCOVERED SIMILAR TO THESE AND ADDED TO THE GROUP WHICH THIS TERM NOW IMPLIES. THESE CELLS ARE ALSO COMMONLY REFERRED TO AS HISTIOCYTES. MACROPHAGE IS ANOTHER TERM WHICH IS USED AS A SYNONYM AND IS CONTRASTED BY THE WORD MICROPHAGE WHICH REFERS TO THE PHAGOCYTTIC CELLS OF THE BLOOD. WHICHEVER IS PREFERRED, IT IS STILL AGREED ON BY ALL THAT IT WOULD BE VERY DIFFICULT TO COIN A WORD WHICH WOULD EMBRACE ALL THE VARIOUS TYPES OF CELLS IN THIS SYSTEM AND

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1. LOC. CIT.
 2. LOC. CIT.
 3. MAXIMOW, ALEXANDER A. AND WILLIAM BLOOM, A TEXTBOOK OF HISTOLOGY, PP.64

YET DISTINGUISH THEM FROM THE OTHER SIMILAR CELLS OF THE BLOOD AND TISSUE. IN ONE CHARACTERISTIC, HOWEVER, THE HISTIOCYTE DIFFERS FROM ALL THE OTHER CELLS OF THE BODY. THIS IS THE FACT THAT THEY WILL STAIN SUPRAVITALLY WITH NON-TOXIC NEUTRAL DYES LIKE PYRROL BLUE, TRYPAN BLUE AND LITHIUM CARMINE. THE SUPRAVITAL STAINING TECHNIQUE IS NOT AN ACTUAL CHEMICAL STAINING OF THE CELL AT ALL. IT IS MERELY AN ULTRAMICROSCOPIC PHAGOCYTOSIS OF THE STAIN PARTICLES BY THE CELL, AND BECAUSE OF THE LARGE ACCUMULATION OF THE STAIN IN THE CYTOPLASM THEY BECOME VISIBLE UNDER THE MICROSCOPE. OTHER SCIENTISTS DECIDED TO CALL THESE CELLS PYRROL CELL BECAUSE OF THIS SPECIAL STAINING ABILITY WITH PYRROL BLUE. THIS TERM IS UNDOUBTEDLY THE MOST SIGNIFICANT OF THE GROUP AND UNFORTUNATELY IT IS THE ONE WHICH IS THE LEAST UNIVERZALLY ACCEPTED. IT CAN BE READILY SEEN HOW EASILY A NEW STUDENT COULD BECOME CONFUSED BY THIS CLASSIFICATION AND NOMENCLATURE.

HOWEVER DISSIMILAR THESE CELLS MAY BE IN MORPHOLOGY OR IN THEIR LOCATION IN THE BODY THEY ARE ALL HIGHLY PHAGOCYTIC AND VERY USEFUL TO THE BODY.

THE HISTIOCYTES MAY BE DIVIDED, FOR CONVENIENCE OF DESCRIPTION, INTO TWO GROUPS - FIXED AND WANDERING.

I. BEST, C.H. AND N.B. TAYLOR, THE PHYSIOLOGICAL BASIS OF MEDICAL PRACTICE PP.80

1 FIXED HISTIOCYTES - THIS GROUP IS AGAIN BROKEN INTO FOUR SUBGROUPS. 1) CELLS OF THE COMMON CONNECTIVE TISSUE - THESE ARE FOUND IN LOOSE CONNECTIVE TISSUE, AND THE SEROUS MEMBRANES. THEIR MORPHOLOGY IS QUITE VARIOUS, THEY MAY BE ROUND, OVAL OR FLAT AND SOME EVEN HAVE LONG PROCESSES WHICH RESEMBLE THE AXONS AND DENDRITES OF NEURONS. THEY LIE AMONG THE FIBROBLASTIC ELEMENTS OF THE TISSUE AND AT ANY TIME, AS A RESULT OF SOME STIMULUS, THEY MAY BECOME FREE AND WANDER THROUGH THE TISSUES, HENCE THEIR COMMON NAME "RESTING WANDERING CELLS"¹. AFTER THE STIMULUS HAS BEEN REMOVED THEY WILL AGAIN COME TO REST SOMEWHERE IN THE TISSUE. 2) RETICULAR CELLS - THESE ARE FOUND IN THE RETICULUM OF THE SPLEEN, LYMPH GLANDS AND BONE MARROW, USUALLY BEING ATTACHED TO THE FIBERS OF THE RETICULAR STROMA. THEY ARE VERY LARGE CELLS WHICH ARE JOINED TO ONE ANOTHER BY MEANS OF LONG BRANCHING PROCESSES. 3) ENDOTHELIAL CELLS -- THESE CELLS ARE DEFINITELY SQUAMOUS IN APPEARANCE. THEY LINE THE BLOOD VESSELS AND SINUSES OF THE SPLEEN, BONE MARROW, ADRENAL CORTEX AND PITUITARY GLAND. IN THIS GROUP ARE ALSO THE INTERESTING KUPFFER CELLS OF THE LIVER (SEE FIGURE 3).² THESE CELLS CAN ACTUALLY HANG INTO THE LUMEN OF A CAPILLARY BEING HELD ONLY BY THIN PROTOPLASMIC PROCESSES TO THE WALLS. THEY ARE VERY VALUABLE TO THE BODY FOR IT IS ALMOST IMPOSSIBLE FOR ANY FOREIGN SUBSTANCE TO PASS THEM.

1. LOC. CIT.

2. BURTON - OPITZ, RUSSEL, A TEXTBOOK OF PHYSIOLOGY,
PP.207

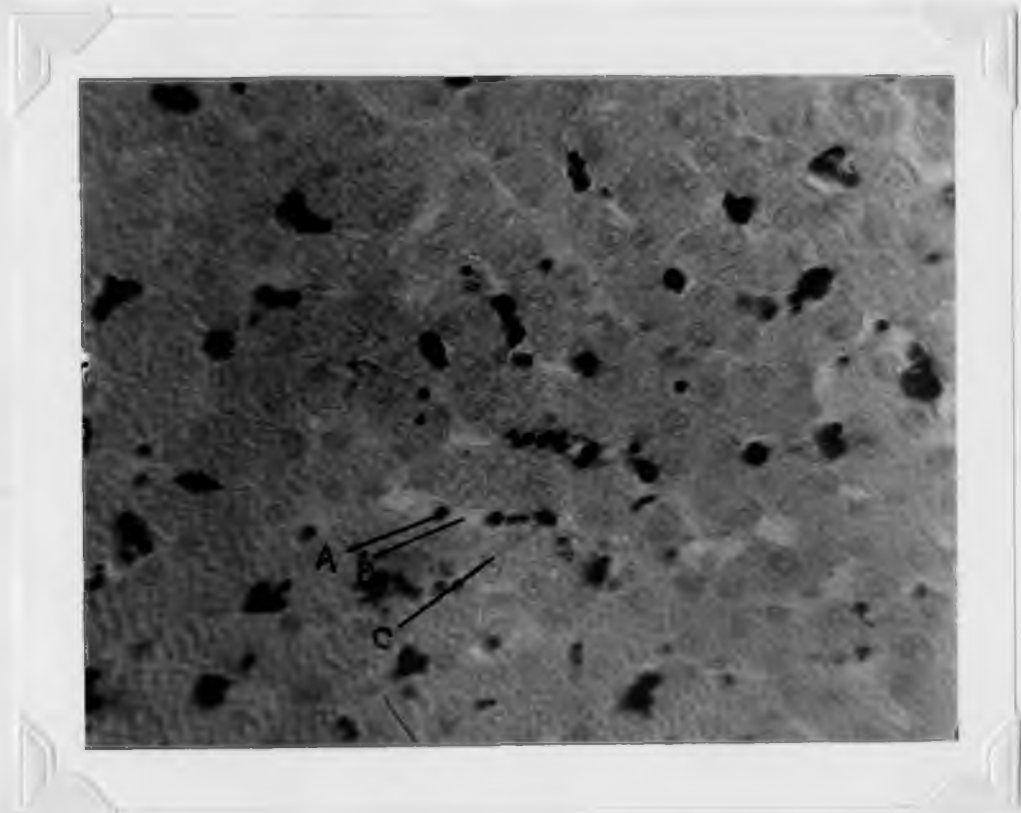


FIGURE 3: KUPFFER CELLS OF THE LIVER
THIS LIVER SLIDE CAME FROM AN ANIMAL
INJECTED WITH INDIA INK WHILE STILL
ALIVE. THE DARK BLACK IS THE PHAGO-
CYTOSSED INDIA INK.

**KEY: A) KUPFFER CELLS
B) CAPILLARIES
C) LIVER CELLS**

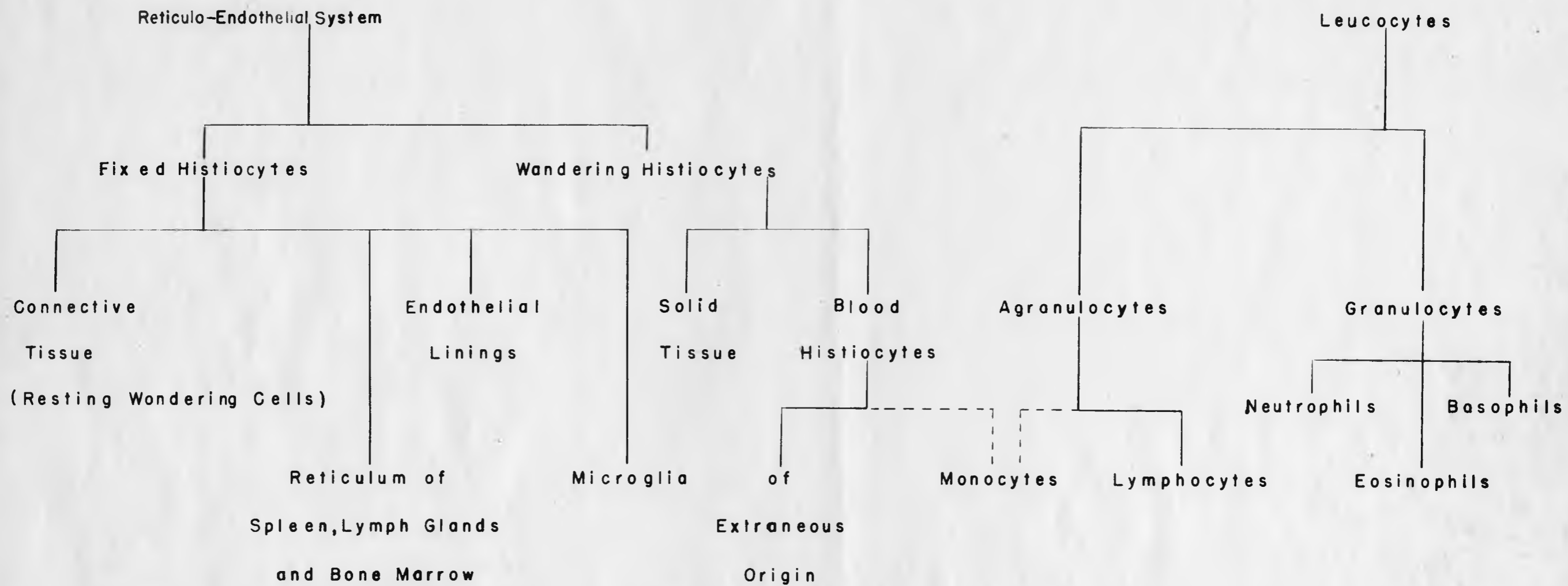
4) MICROGLIA - THESE CELLS ARE THE PROTECTORS OF THE CENTRAL NERVOUS SYSTEM. THEY ARE FOUND SCATTERED THROUGH ITS ENTIRETY IN GREAT QUANTITIES. THE CELL ITSELF IS PRACTICALLY ALL NUCLEUS WITH A FEW CYTOPLASMIC PROJECTIONS WHICH ARE TWISTED IN VARIOUS WAYS AND ARE COVERED WITH A CONSIDERABLE NUMBER OF TINY SPINES. MICROGLIA ARE SOMETIMES REFERRED TO AS HORTESA'S CELLS.

11 WANDERING HISTIOCYTES - THIS GROUP IS SUBDIVIDED INTO TWO SUBGROUPS. 1) SOLID TISSUE HISTIOCYTES - THESE CELLS ARE VERY SIMILAR TO THE LYMPHOCYTES OF THE BLOOD, HAVING A LARGE NUCLEUS WITH A SMALL AMOUNT OF BASOPHILE CYTOPLASM. THEY WANDER AT RANDOM THROUGH THE CONNECTIVE TISSUE AND ONLY VERY SELDOM COME TO REST. 2) BLOOD HISTIOCYTES - THESE ARE CELLS THAT WANDER IN THE BLOOD BUT ARE OF EXTRANEIOUS ORIGIN. THIS IS RARE AND COMES ABOUT ONLY UNDER INTENSE PATHOLOGICAL STIMULI. IN LEUKEMIA, FOR INSTANCE, THE ORDINARY CONNECTIVE TISSUE MACROPHAGES MAY BE FOUND IN LARGE NUMBERS IN THE BLOOD STREAM. THESE ARISE CHIEFLY FROM THE SPLEEN AND BONE MARROW. THEY ARE FOUND IN LARGE NUMBERS IN THE VENOUS SYSTEM BUT RARELY REACH THE ARTERIAL SIDE BECAUSE THEY ARE FILTERED OUT BY THE CAPILLARIES OF THE LUNG. THEIR VERY LARGE SIZE, ABOUT 30 MICRONS, IS RESPONSIBLE FOR THIS. AS WAS NOTED BEFORE THERE IS A CONTROVERSY ABOUT THE CLASSIFICATION OF THE MONOCYTES (SEE UNDER, 1 AGRANULOCYTES). MAXIMOW'S EVIDENCE SEEMS TO

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1. MAXIMOW, ALEXANDER A. AND WILLIAM BLOOM, A TEXTBOOK OF HISTOLOGY PP. 186
 2. BEST AND TAYLOR, OP. CIT. PP.81

PROVE THEY SHOULD BE CLASSIFIED UNDER THE LEUCOCYTES AND ON THE CONTRARY LEWIS HAS GOOD EVIDENCE THAT THEY ARE HISTIOCYTES. I PERSONALLY CAN SEE NO CONTRADICTION IN THE SUPPOSITION THAT THEY COULD ORIGINATE THEM FROM BOTH GROUPS OR THAT THE DERIVED CELLS ARE SO SIMILAR THAT IT IS IMPOSSIBLE, WITH OUR PRESENT KNOWLEDGE TO DISTINGUISH THEM. THIS IDEA ALSO FOLLOWS THE USUAL TREND IN ARRIVING AT SCIENTIFIC TRUTH, I.E., WHERE THERE IS TWO COMPLETELY CONTRADICTORY VIEWS ON THE SAME PROBLEM THE ACTUAL TRUTH IS USUALLY A COMBINATION OF BOTH OR A COMPROMISE BETWEEN THEM.

THIS CONCLUDES THE STUDY OF THE CLASSIFICATION OF THE PHAGOCYTES. THE FOLLOWING CHART GRAPHICALLY SHOWS THE DIFFERENT RELATIONSHIPS.



Graph I-CLASSIFICATION OF THE PHAGOCYTES

The dashed lines leading to the monocytes illustrate the controversy over its origin between the reticulo-endothelial system and the leucocytes

CHAPTER II

THE PHYSIOLOGY OF THE PHAGOCYTES

AS FAR AS I CAN DETERMINE THE FOLLOWING IS THE BEST ORGANIZATION OF THE DIFFERENT ASPECTS OF THE PHYSIOLOGY OF THE PHAGOCYTES FOR PURPOSES OF EASY STUDY.

MOTILITY

THE MOTILITY OF A PHAGOCYTE IS VERY IMPORTANT TO THEIR PHAGOCYTTIC PROPERTIES AND THUS HAS A BEARING ON THEIR USEFULNESS TO THE BODY. A MOLECULAR MOVEMENT OF THE CYTOPLASM HAS BEEN OBSERVED IN ALL THE LEUCOCYTES, BUT WITH THE EXCEPTION OF THE NEUTROPHIL AND MONOCYTE, IT IS NOT SUFFICIENTLY STRONG ENOUGH TO CAUSE MOTION. THE EOSINOPHILS AND BASOPHILS ARE NOT MARKEDLY MOTILE AT ALL. THE LYMPHOCYTES ARE ONLY CAPABLE OF A CERTAIN SLOW PROGRESSION, AS A RESULT OF SPASMODIC MOVEMENTS OF THE CELL NUCLEUS. THE NEUTROPHILS AND MONOCYTES, ON THE OTHER HAND, ARE VERY MOTILE. THEY EXHIBIT A MOVEMENT OF THEIR CYTOPLASM WHICH IS VERY SIMILAR TO THAT DISPLAYED BY THE "AMEBA". PROLONGATIONS OF THE CYTOPLASM, WHICH ARE COMMONLY CALLED PSEUDOPODIA, ARE SENT OUT IN VARYING DIRECTIONS ABOUT THE CELL AND ARE THEN CONTRACTED AGAIN INTO THE CELL BODY. IT SEEMS, IN THIS TYPE OF MOVEMENT, THAT THE NUCLEUS ACTS AS A PIVOT FOR IT USUALLY REMAINS VERY CLOSE TO THE SAME SPOT. OCCASIONALLY A PSEUDOPODIUM WILL

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1. BURTON-OPITZ, RUSSEL, A TEXTBOOK OF PHYSIOLOGY, PP.202 FIRST OBSERVED BY WHARTON JONES IN 1846, AND PROVED FOR THE HUMAN LEUCOCYTE BY DAVAINE IN 1850.
 2. BEST AND TAYLOR, OP.CIT., PP.73
 3. LOC. CIT.

BECOME ATTACHED TO A SURFACE AND THEN THE REMAINING MASS OF THE CELL IS CONTRACTED INTO IT. THIS PROPERTY OF LEUCOCYTES TO BECOME ATTACHED TO A SURFACE IS ATTRIBUTABLE TO ITS POWER OF SECRETING A MUCOUS SUBSTANCE.¹ IN THIS MANNER THESE MOTILE LEUCOCYTES CAN AT A RATE TRAVEL 30 TO 35 MICRONS PER MINUTE AT BODY TEMPERATURE.² WHEN LEUCOCYTES ARE FREELY MOVING WITH THE CIRCULATION OF THE BLOOD THERE IS OF COURSE NO ACTUAL MOTILITY AND THE CELLS APPEAR AS A GLOBULAR SUBSTANCE WHICH INDICATES A STATE OF CONTRACTION.

DIAPEDESIS

BY THIS ACTION OF DIAPEDESIS MYRIADS OF LEUCOCYTES MAY PASS OUT OF THE VASCULAR SYSTEM IN A REMARKEDLY SHORT TIME. THIS TERM, DIAPEDESIS, WAS ORIGINALLY COINED TO REFER TO ANY CELLULAR ELEMENTS IN THE BLOOD PASSING OUT THROUGH THE WALLS OF THE BLOOD VESSEL. COHNHEIM, HOWEVER, PROVED THAT THIS IS A DISTINCT CHARACTERISTIC OF LEUCOCYTES ONLY.³ THE AMEBOID PROPERTIES OF THE LEUCOCYTE IS RESPONSIBLE FOR THIS PHENOMENA. A THIN PSEUDOPODIUM IS FIRST EXTENDED OUT THROUGH A PERFORATION IN THE VESSEL WALL, AFTER WHICH THE MAIN MASS OF THE CELL IS SLOWLY DRAWN THROUGH THE OPENING WITH THE ENTIRE CELL OUTSIDE OF THE VESSEL. GREAT NUMBERS OF THESE CELLS MIGRATE OUT OF THE VASCULAR SYSTEM WHENEVER THERE IS TISSUE DAMAGE OR INFECTION. THIS MIGRATION IS GREATLY FACILITATED BY CERTAIN CHANGES IN THE FLOW OF THE BLOOD. THESE ARE:⁴ 1) THE CAPILLARY

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1. BURTON-OPITZ, RUSSEL, OP. CIT., PP.203
 2. BEST AND TAYLOR, OP. CIT., PP.73
 3. BURTON-OPITZ, RUSSEL, OP. CIT. PP.206
 4. LOC. CIT.

WALLS BECOME RELAXED IN THE REGION OF THE INFECTION THUS SUPPLYING A LARGER BLOOD BED. 2) THIS LARGER BLOOD BED IN TURN CAUSES A LOCAL RISE IN TEMPERATURE WHICH ACTIVATES THE LEUCOCYTES. 3) THIS INCREASED SIZE OF THE CAPILLARIES OF COURSE DECREASES THE SPEED OF THE BLOOD GOING THRU THEM. THIS ENABLED THE LEUCOCYTES TO BECOME ATTACHED TO THE WALLS MORE EASILY AND THUS THE RATE OF DIAPEDESIS IS INCREASED.

CHEMOTAXIS

THIS IS THE TERM WHICH APPLIES TO AN UNKNOWN "FORCE" WHICH DRAWS THE LEUCOCYTES OUT OF THEIR STOREHOUSES INTO THE BLOOD STREAM AND THEN DRAWS THEM OUT OF THE VASCULAR SYSTEM TO THE EXACT POINT OF INJURY. IT WAS FORMERLY THOUGHT THAT THE BACTERIAL TOXINS CAUSED THIS BUT HAS SINCE BEEN PROVEN THAT THE NUCLEIC ACIDS OF THE BODY CELLS AND OF THE LEUCOCYTES THEMSELVES ARE RESPONSIBLE. AN INJECTION OF THESE NUCLEIC ACIDS WILL DEFINITELY CAUSE THIS REACTION. BESIDES THIS, SUBSTANCES INTRODUCED INTO THE BODY WHICH DO NOT PRODUCE TOXINS WILL STILL CAUSE THE ATTRACTION OF MANY ADDITIONAL LEUCOCYTES, E.G., PARTICLES OF CINNEBAR OR CARBON. IN THIS CASE THE NUCLEIC ACIDS ARE RELEASED FROM CELLS WHICH ARE INJURED IN THE INJECTION ITSELF. JUST EXACTLY HOW THESE NUCLEIC ACIDS CAN CAUSE THIS HOWEVER STILL REMAINS A PROBLEM TO BE SOLVED. IT HAS ALSO BEEN SUGGESTED THAT THE ATTRACTION IS ELECTRICAL IN NATURE RATHER THAN CHEMICAL AND THAT A

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1. BEST AND TAYLOR, OP. CIT., PP.74
 2. LOC. CIT.

POTENTIAL EXISTS BETWEEN HEALTHY AND DISEASED OR DESTROYED TISSUE. THE LEUCOCYTES, IN THIS CASE, WOULD EXHIBIT WHAT IS KNOWN AS GALVANOTROPISM. MENKEN DISCOVERED A SUBSTANCE, LEUKOTAXINE, WHICH IS A NITROGENOUS CRYSTAL AND HE BELIEVES IS RESPONSIBLE FOR THE INCREASED CAPILLARY PERMEABILITY AND THE MIGRATION OF THE LEUCOCYTES THROUGH THE WALLS. HE HAS ISOLATED THIS COMPOUND FROM INFLAMED TISSUE. ALL IN ALL, CHEMOTAXIS IS A VERY INTERESTING PHENOMENA AND THE UNCOVERING OF ALL ITS SECRETS WILL UNDOUBTEDLY BE VERY VALUABLE IN THE PROPHYLAXIS OF DISEASE.

PHAGOCYTOSIS

THE PRECEEDING TOPICS HAVE LAID A GOOD FOUNDATION AND NOW THE DISCUSSION OF PHASOCYTOSIS ITSELF SHALL BE LOOKED AT. IT IS EVIDENT THAT THIS IS BY FAR THE MOST IMPORTANT FUNCTION OF THE LEUCOCYTES AND THE OTHERS ARE MERELY ALLIED FUNCTIONS WHICH ENABLE THE LEUCOCYTE TO PHASOCYTOSE MORE EFFICIENTLY. A BACTERIA, UPON ENTERING THE BODY WILL IMMEDIATELY BEGIN TO GROW (REPRODUCE); THIS IS ACCOMPANIED BY THE DESTRUCTION OF BODY TISSUE WHICH WILL IN TURN RELEASE ITS NUCLEIC ACIDS TO CAUSE THE CHEMOTAXIS OF THE LEUCOCYTES. IF THE BACTERIA IS A NONPATHOGEN IT WILL BE DEVoured BY THE PHAGOCYTES AND NO ILL EFFECTS WILL BE FELT BY THE BODY. NOT ONLY BACTERIA BUT ALSO ANY FOREIGN MATERIAL, WHETHER IT BE A SPLINTER OR A SURGICAL SUTURE, IS REMOVED IN THIS WAY. IF THE BACTERIA IS A HISHLY VIRULENT PATHOSEN HOWEVER, THE PHAGOCYTES WILL HAVE A LITTLE MORE DIFFICULTY. THIS DIFFICULTY MAY BE DUE TO ONE OF

TWO FACTORS OR BOTH. FIRST, THE PATHOGEN MAY SECRETE A HIGHLY DEADLY TOXIN WHICH IN SOME WAY KILLS OR INACTIVATES THE PHAGOCYTE. SECONDLY, THE BACTERIA MAY HAVE A SLIME LAYER. THIS IS AN OUTER SHELL ON THE PATHOGEN WHICH IS COMPOSED OF PROTEINS OR HIGHLY POLYMERIZED POLYSACCHARIDES AND MAKES THE BACTERIA RESISTANT TO PHAGOCYTOSIS. THE BODY, HOWEVER, HAS GOOD DEFENSE MECHANISMS AGAINST THESE FACTORS. TOXINS BEING PRESENT CAUSE THE FORMATION OF THE ANTITOXINS IN THE BODY WHICH CAN "NEUTRALIZE" THESE TOXINS AND RENDER THEM HARMLESS. WHEN THIS IS DONE THE PHAGOCYTES CAN DO THEIR WORK WITHOUT DANGER. THE SLIME LAYER, WHICH WAS MENTIONED EARLIER, CAN BE VISUALIZED AS A LAYER OF GREASE WHICH MAKES THE BACTERIA SLIPPERY AND THEREFORE CANNOT BE SUCCESSFULLY ENGULFED BY THE PHAGOCYTE. IN THIS CASE THE BODY PRODUCES AN ANTIBODY CALLED OPSONIN, WHICH SHALL BE COMPLETELY EXPLAINED LATER. THIS ANTIBODY IS CAPABLE OF COATING THE SLIME LAYER OR REACTING WITH IT SO THE PATHOGEN MAY BE ENGULFED. IT IS INTERESTING TO NOTE THAT THESE ANTIBODIES, INCLUDING ANTITOXINS, DO NOT ACTUALLY KILL THE BACTERIA, BUT MERELY RENDER THEM HELPLESS AGAINST THE INVADING PHAGOCYTES. AFTER THESE STEPS HAVE BEEN TAKEN MANY MONOCYTES AND MEMBERS OF THE RETICULO-ENDOTHELIAL SYSTEM UNDERGO TRANSFORMATION TO FORM A SHIELD WHICH ISOLATES THE DISEASED TISSUE AND THE PATHOGENS FROM THE HEALTHY TISSUE. THE CIRCUMSCRIBING WALL AND ITS CONTENTS ARE WHAT IS KNOWN AS AN ABSCESS.² THE CONTENTS WE CALL PUS, IS DEAD LEUCOCYTES (BUS CELLS), THE BACTERIAL GROWTH, LIQUIFIED

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1. SMITH, DAVID T. AND NORMAN F. CONANT, ZINSEER BACTERIOLOGY, PP.20
 2. BEST AND TAYLOR, OP.CIT. PP.73

TISSUE CELLS, PLASMA, AND A FEW ERYTHROCYTES. AGAIN THE PHAGOCYTES GO TO WORK AND, AIDED BY A PROTEIN DIGESTING ENZYME, ERODE AWAY THE OVERLYING STRUCTURES, WHETHER IT BE CONNECTIVE TISSUE, MUCOSA, OR SKIN. IN THIS WAY THE CONTENTS OF THE ABSCESS ARE DISCHARGED TO THE EXTERIOR. AS IT MAY SEEM, PHAGOCYTOSIS IS CHIEFLY USED AS A DEFENSE MECHANISM, HOWEVER, IT HAS MANY OTHER USEFUL PURPOSES. THE REMOVAL OF DEAD TISSUE, A BLOOD CLOT, OR DEVITALIZED BONE IS ALSO ACCOMPLISHED IN THIS MANNER. THE DISAPPEARANCE OF NO LONGER USEFUL ORGANS LIKE THE TAIL AND GILLS OF A METAMORPHOSING TAD-POLE OR THE CREEPING MUSCLES OF INSECT LARVAE IS EFFECTED BY SIMILAR PHAGOCYTES.

OPSONINS

AT AN EARLY DATE IT WAS THOUGHT THAT PHAGOCYTES COULD NOT ENGULF BACTERIA UNLESS THEY WERE DEAD. SINCE THAT TIME HOWEVER IT HAS BEEN DISCOVERED THAT THE BACTERIA WERE NOT DEAD BUT MERELY INACTIVATED BY SOME CONSTITUENT OF THE BLOOD SERUM. IT WAS NOTICED THAT THE PHAGOCYTTIC PROCESS OF A PERSON WHO HAD PREVIOUSLY ENCOUNTERED A DISEASE AND WAS HAVING IT FOR THE SECOND TIME DIFFERED FROM THE PROCESS OF AN INDIVIDUAL WHO WAS HAVING THE DISEASE FOR THE FIRST TIME. THE FORMER PERSON IS REFERRED TO AS BEING "IMMUNE"² TO THE DISEASE. IN THIS IMMUNE PERSON THE BACTERIA WERE PHAGOCYTOSED MUCH FASTER AND EASIER, AND IN SOME CASES THE BACTERIA WERE

1. BURTON-OPITZ, RUSSEL, A TEXTBOOK OF PHYSIOLOGY, PP.204
 2. /SMITH AND CONANT, OP. CIT. PP.120

FOUND IN LARGE CLUMPS WHICH FACILITATED THE ENGLUFGING. IT WAS THEN FOUND THAT AN ANTIBODY WAS FORMED UPON CONTACT OF THE DISEASE AND REMAINED IN THE BODY FOR QUITE SOME TIME. THIS ANTIBODY, OPSONIN, SERVES AS AN INTERMEDIATE AGENT TO INACTIVATE THE BACTERIA ALLOWING THE PHAGOCYTE TO ENGLUF IT. THE BODY CAN ONLY PRODUCE THE BEST RESULTS WHEN THE OPSONINS AND THE NUMBER AND KIND OF LEUCOCYTES ARE PROPERLY BALANCED.

ALLIED FUNCTIONS OF THE PHAGOCYTES OF THE BLOOD

BESIDES THE JOB OF REMOVING EXTRANEIOUS AND USELESS MATERIAL FROM THE BODY, THE PHAGOCYTES ALSO HAVE VERY USEFUL PURPOSES IN SOME METABOLIC PROCESSES. THE MOST IMPORTANT OF THESE IS THEIR POWER OF TAKING UP NUTRITIVE MATERIALS AND CARRYING IT TO DIFFERENT PARTS OF THE BODY E.G., THE LYMPHOCYTES ARE SAID TO ABSORB FAT GLOBULES AND CONVEY THEM TO THE LYMPH CHANNELS. THEY ARE ALSO SUPPOSED TO AID IN THE ABSORPTION OF THE PEPTONES AND TO HELP IN MAINTAINING A PROPER PROTIEIN CONTENT OF THE BLOOD. BOTH OF THESE ABOVE FUNCTIONS ARE ATTRIBUTABLE TO THEIR PHAGOCYTIC PROPERTIES.

PHYSIOLOGY OF THE RETICULO-ENDOTHELIAL SYSTEM

AS IT HAS ALREADY BEEN STATED, THE CHIEF FUNCTION OF THE RETICULO-ENDOTHELIAL SYSTEM IS THEIR ABILITY TO PHAGOCYTOSE. THIS DOES NOT DIFFER APPRECIABLY FROM THE PHAGOCYTOSIS PROCESS OF THE NEUTROPHIL, HOWEVER, THERE ARE A FEW ADDITIONAL

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1. BURTON-OPITZ, RUSSEL, OP.CIT. PP.206
 2. IBID PP. 201
 3. LOC. CIT.

APPLICATIONS OF IT IN THIS CASE. THE ACTION OF THE HISTIOCYTES IS MUCH LESS MOTILE AND MORE LOCALIZED THAN THAT OF THE NEUTROPHILS. THE HISTIOCYTES WITH THE AID OF THE LYMPHOCYTES ARE RESPONSIBLE FOR PRATICALLY ALL OF THE REPAIR PROCESS WHICH FOLLOWS ANY TISSUE DAMAGE. SOME OF THESE CELLS NAMED THE SO-CALLED DUST CELLS OF THE LUNGS ARE RESPONSIBLE FOR THE REMOVAL OF ANY EXTRANEIOUS MATTER WHICH MAY ENTER INTO THE BODY WITH THE INSPIRED AIR. THE KUPFFER CELLS OF THE LIVER HAVE THE ABILITY TO ENGULF THORIUM DIOXIDE WHICH IS RADIOACTIVE AND SHOWS UP OPAQUE ON AN X-RAY. THIS IS VERY VALUABLE FOR THE STUDY OF THE LIVER IN A LIVING SUBJECT. THE SPLEEN, PLACENTA, URETER AND KIDNEY PELVIS, THE VESSELS OF THE EXTREMITIES OR OF THE BRAIN, AND THE CEREBRAL VENTRICLES CAN ALSO BE STUDIED IN LIVE SUBJECTS UTILIZING THIS SAME PRINCIPLE. THE OMENTUM IS VERY IMPORTANT DUE TO THE MANY HISTIOCYTES IT CONTAINS. IT HAS THE ABILITY TO FORM ADHESIONS WHICH SERVES TO SEAL PERFORATIONS OF THE GASTRO-INTESTINAL TRACT AND ALSO IT CAN ISOLATE INFECTED REGIONS IN THE ABDOMINAL CAVITY. THE FORMATION OF BILE PIGMENT AND THE DESTRUCTION OF USED UP ERTHROCYTES IN THE SPLEEN ARE OTHER WELL ESTABLISHED FUNCTIONS OF THE HISTIOCYTES. THE MACROPHASES OF THE LYMPH GLANDS ARE USEFUL IN THAT THEY CAN DEVOUR AND DIGEST NEUTROPHILS WHICH HAVE BECOME PACKED WITH BACTERIA AND ARE THEREFORE RENDERED USELESS FOR ANY FURTHER PHAGOCYTOSIS.

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1. BEST AND TAYLOR, OP. CIT. PP.81
 2. LOC. CIT.
 3. LOC. CIT.
 4. IBID. PP.82
 5. LOC. CIT.
 6. LOC. CIT.

CHAPTER III

EXPERIMENTS IN PHAGOCYTOSIS

EXPERIMENT I "OBTAINING A PURE CULTURE OF LEUCOCYTES"

PROBLEM: IT IS A WELL KNOWN FACT THAT LEUCOCYTES ARE RELATIVELY RARE IN WHOLE BLOOD. FOR EVERY LEUCOCYTE THERE ARE APPROXIMATELY 600 ERTHROCYTES. THIS EXPERIMENT DEALT WITH THE PROBLEM OF TRYING TO OBTAIN A "PURE CULTURE", OR AT LEAST A RELATIVELY HIGH PERCENTAGE OF LEUCOCYTES IN WHOLE BLOOD. THIS WOULD FACILITATE THEIR STUDY VERY MUCH.

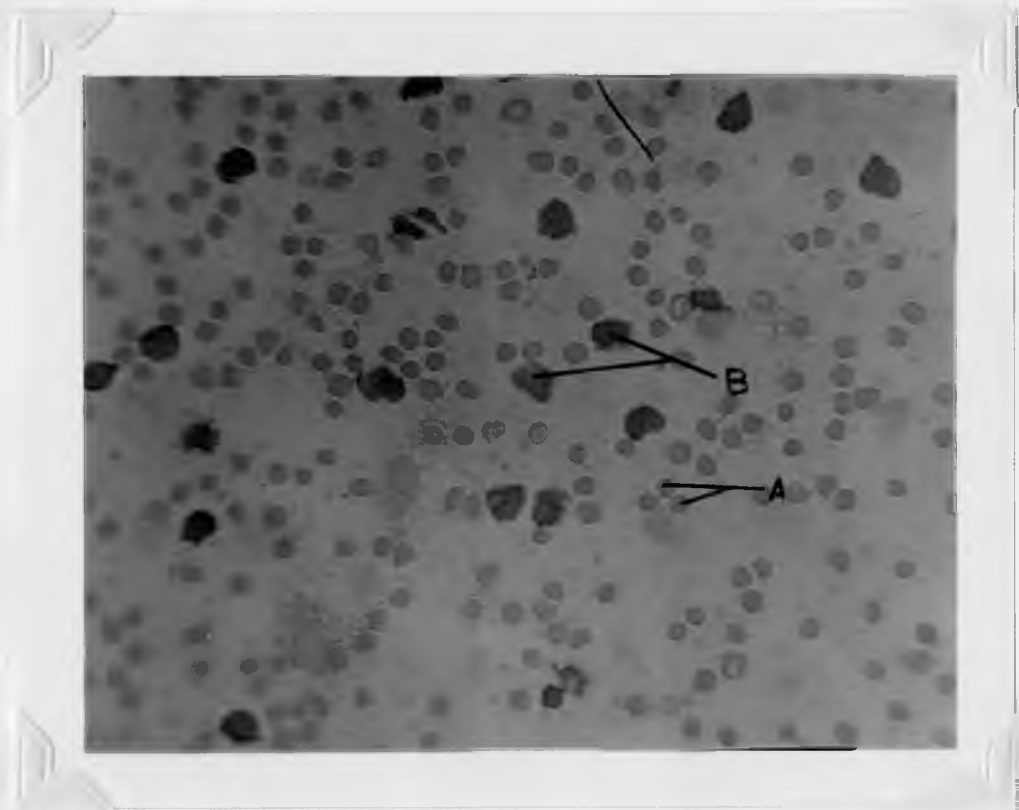
PROCEDURE: THIS PROBLEM WAS APPROACHED FROM TWO DIFFERENT ANGLES, CENTRIFUGALIZATION AND SEDIMENTATION. 1) CENTRIFUGALIZATION - 8CC OF BLOOD WAS EXTRACTED INTRAVENEOUSLY AND ADDED TO 4CC OF SODIUM CITRATE SOLUTION, THIS WAS CENTRIFUGED FOR 5 MINUTES AT MODERATE SPEED. THE SERUM, IN THIS TIME HAD SEPARATED FROM THE CELLS BUT NO DISTINCT LAYERS IN THE CELLS WERE OBSERVABLE. AFTER 15 MINUTES OF CENTRIFUGIN TWO LAYERS OF CELLS WERE FOUND. SLIDES WERE MADE OF BOTH OF THESE LAYERS. THE TOP LAYER WHICH HAD A WHITE CLOUDY APPEARANCE WAS BELIEVED TO BE MAINLY LEUCOCYTES. IT WAS PIPETTED OFF AND ADDED TO RINSER'S SOLUTION FOR FURTHER CENTRIFUGALIZATION, HOWEVER, COAGULATION AT ONCE TOOK PLACE. THIS WAS DUE TO A CHEMICAL REACTION BETWEEN THE CITRATE, WHICH

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1. STILES, KARL A., HANDBOOK OF MICROSCOPIC CHARACTERISTICS OF TISSUES AND ORGANS, PP.42
 2. HOERR, N.L. AND A. OSAL, NEW GOULD MEDICAL DICTIONARY PP.1115

TIES UP THE CALCIUM IN THE BLOOD, AND THE IONS OF RINGER'S SOLUTION. IT WAS FOUND THAT SODIUM DID NOT REPLACE THE CALCIUM AND THEREFORE ISOTONIC SODIUM CHLORIDE SOLUTION COULD BE USED. FURTHER TESTS WERE THEN MADE IN SALINE SOLUTION, BUT THEY DID NOT CHANGE THE RELATIVE PERCENTAGE SIGNIFICANTLY. 2) SEDIMENTATION - THIS IS VIRTUALLY BASED ON THE SAME THEORY AS THE CENTRIFUGALIZATION WAS I.E., THAT LEUCOCYTES ARE LESS DENSE THAN ERYTHROCYTES AND THEY WOULD REMAIN ON TOP WHILE THE ERYTHROCYTES WOULD SETTLE TO THE BOTTOM OF THE CELLULAR MASS. A LARGE PIPETTE WAS FILLED WITH BLOOD AND ALLOWED TO STAND IN THE REFRIGERATOR FOR ONE WEEK. AT THE END OF THIS WEEK THE CELLS WERE DIVIDED VERY NICELY INTO TWO LAYERS. THE LOWER ONE WAS DARK RED AND THE UPPER LAYER WAS WHITE. SLIDES WERE MADE OF THESE LAYERS ALSO.

CONCLUSION:

THE LOWER LAYERS OF BOTH TESTS CONTAINED A VERY HIGH CONCENTRATION OF ERYTHROCYTES AND PRACTICALLY NO LEUCOCYTES. THE UPPER LAYER IN THE CENTRIFUGED BLOOD CONTAINED A RATIO OF ABOUT 20 ERYTHROCYTES TO ONE LEUCOCYTE. THE LEUCOCYTES WERE WELL FORMED AND COULD EASILY BE USED FOR EXPERIMENTATION IN PHAGOCYTOSIS. IN THE UPPER LAYER OF THE SEDIMENTATION HOWEVER, THE LEUCOCYTES WERE POORLY DEFINED AND MANY HAD UNDERGONE LYSIS. THE RATIO WAS A LITTLE BETTER IN THIS CASE, BEING ABOUT 15 TO 1. THESE CELLS WERE OBVIOUSLY USELESS FOR ANY EXPERIMENTATION. (SEE FIGURE 4). THEREFORE, I CONCLUDE, WHILE IT IS POSSIBLE TO OBTAIN A BLOOD SPECIMEN



**FIGURE 4 - LEUCOCYTES WHICH HAVE UNDERGONE
SEDIMENTATION FOR ONE WEEK.**

**THIS FIELD CLEARLY SHOWS THE INCREASED RATIO
OF ERTHROCYTES TO LEUCOCYTES DUE TO SEDIMENTAT-
ION. SLIDE WAS MADE FROM THE UPPER LAYER (WHITE)
AND STAINED WITH WRIGHT'S STAIN.**

**KEY: A) ERTHROCYTES
B) LEUCOCYTES**

WITH AN INCREASED LEUCOCYTE COUNT IT IS VIRTUALLY IM-
POSSIBLE TO PREPARE AN ABSOLUTE "PURE CULTURE" USING THE
ABOVE MEANS.

EXPERIMENT II "PHAGOCYTOSIS OUTSIDE THE BODY"

PROBLEM: THIS PROBLEM IS MERELY TO ASCERTAIN WHETHER THE
PHAGOCYTES OF THE BLOOD WOULD ENVELOPE BACTERIA WITHIN THEIR
NORMAL BLOOD SERUM BUT OUTSIDE OF THE BODY.

PROCEDURE: TWO DIFFERENT ORGANISMS AND TWO METHODS WERE
USED IN THIS EXPERIMENT ALSO. 1) ON A CLEAN GLASS SLIDE WAS
PLACED A DROP OF ISOTONIC SALINE SOLUTION, IT WAS THEN
MIXED WITH A LOOP OF STAPHYLOCOCCUS ALBUS FROM A NUTRIENT
AGAR CULTURE. TO THIS, A DROP OF FRESH BLOOD DIRECTLY FROM
A PIERCED FINGER WAS ADDED. IT WAS AGAIN MIXED AND ALLOWED
TO STAND FOR ONE MINUTE. AT THE END OF THIS MINUTE THE DROP
WAS DRAWN OUT INTO A SMEAR, DRIED, FIXED AND STAINED WITH
MACHIARELLO'S STAIN. THE RESULTS WERE VERY SURPRISING. A
GREAT DEAL OF PHAGOCYTOSIS HAD COME ABOUT AS CAN BE EASILY
SEEN IN FIGURE 5 WHICH WAS TAKEN FROM THIS SLIDE. 2) A
BLOOD SPECIMEN WAS PREPARED BY ADDING 5CC OF FRESH WHOLE
BLOOD TO 0.2CC OF A 20% SOLUTION OF SODIUM CITRATE IN
ISOTONIC SALINE SOLUTION. A BACTERIAL SUSPENSION WAS PRE-
PARED BY MIXING LOOPFULS OF BRUCELLA ABORTUS INTO 2CC OF

1. COMMITTEE ON BACTERIOLOGICAL TECHNIC OF THE SOCIETY OF
AMERICAN BACTERIOLOGISTS, MANUAL OF METHODS FOR PURE
CULTURE STUDY OF BACTERIA.

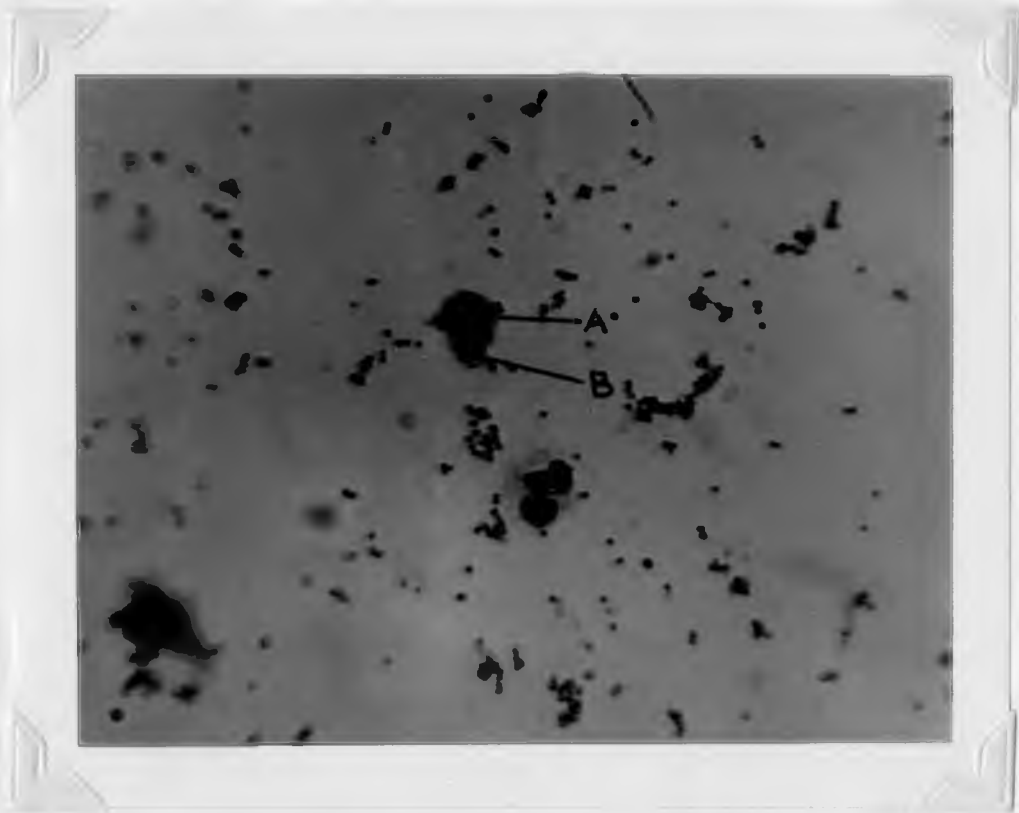


FIGURE 5 - PHAGOCYTOSIS OF STAPHYLOCOCCUS ALBUS OUTSIDE THE BODY.

**KEY: A) PHAGOCYtic MONOCYTE
B) PHAGOCYTOSED STAPHYLOCOCCUS ALBUS ORGANISMS.**

MACCHIAVELLO'S STAIN

ISOTONIC SALINE UNTIL A SLIGHTLY CLOUDY MIXTURE WAS OBTAINED. THEN 0.1CC OF BOTH THE BLOOD SPECIMIN AND THE BACTERIAL SUSPENSION WERE MIXED IN A SEPARATE TUBE AND ALLOWED TO STAND IN THE INCUBATOR (37°C) FOR $\frac{1}{2}$ HOUR. AT THE END OF THIS TIME A SLIDE WAS MADE OF THE MIXTURE. HERE AGAIN VERY GOOD PHAGOCYTOSIS WAS OBSERVED. SEE FIGURE 6.

CONCLUSION: PHAGOCYTOSIS WILL OPERATE VERY EFFECTIVELY OUTSIDE THE BODY. THE PROCESS OF PHAGOCYTOSIS TAKES BUT A VERY SHORT TIME AS WAS EVIDENCED BY PART I OF THIS EXPERIMENT. IT IS STILL NOT DETERMINED WHETHER THE PHAGOCYTE IS COMPLETELY INDEPENDENT I.E., WILL THEY OPERATE WITHOUT THE PRESENCE OF BLOOD SERUM IN SOME OTHER INTRACELLULAR MEDIA LIKE PHYSIOLOGICAL SALINE SOLUTION.

EXPERIMENT III "PHAGOCYTOSIS IN PHYSIOLOGICAL SALINE SOLUTION"

PROBLEM: WILL PHAGOCYTOSIS WORK OUTSIDE THE BODY AND WITH PHYSIOLOGICAL SALINE SOLUTION FOR ITS INTERCELLULAR MEDIA?

PROCEDURE: 10CC OF FRESHLY EXTRACTED CITRATED BLOOD WAS CENTRIFUGED FOR 10 MINUTES. AT THE END OF THIS 10 MINUTES THE SUPERNATANT SERUM WAS REMOVED FROM THE CELLS AND REPLACED WITH THE SAME VOLUME OF PHYSIOLOGICAL SALINE SOLUTION. THIS WAS THOROUGHLY MIXED AND CENTRIFUGED AGAIN. THIS PROCESS WAS REPEATED THREE TIMES. AFTER THE LAST MIXING THE CELLS WERE NOW SUSPENDED IN A SOLUTION WHICH WAS MAINLY PHYSIOLOGICAL



FIGURE 6 - PHAGOCYTOSIS OF BRUCELLA ABORTUS
OUTSIDE THE BODY.

KEY: A) PHAGOCYTES
B) BRUCELLA ABORTUS ORGANISMS

MACCHIAVELLO'S STAIN



FIGURE 7 - PHAGOCYTOSIS WITHIN A PHYSIOLOGICAL SALINE MEDIA

NOTE THE DEFORMITY OF THE NEUTROPHIL

**KEY: A) NEUTROPHIL
B) BRUCELLA ABORTUS ORGANISMS**

MACCHIAVELLO'S STAIN

SALINE SOLUTION WITH A VERY LOW PERCENTAGE OF ACTUAL BLOOD SERUM. THEN 0.1CC OF THIS BLOOD SOLUTION WAS MIXED WITH 0.1CC OF A BACTERIAL SUSPENSION IDENTICAL WITH THE SUSPENSION PREPARED IN EXPERIMENT II. THIS MIXTURE WAS THEN INCUBATED AT 37°C FOR HALF AN HOUR. A SLIDE WAS THEN MADE OF THIS MIXTURE AND STAINED WITH MACCHIAVELLO'S STAIN. A WRIGHT'S STAIN WAS ALSO MADE OF THE BLOOD IN SALINE SOLUTION BEFORE THE ADDITION OF THE BACTERIAL SUSPENSION.

CONCLUSION: ON THE SLIDE OF THE INCUBATED MIXTURE VERY FEW LEUCOCYTES WERE FOUND AND THOSE WERE USUALLY HIGHLY DEFORMED AND PHAGOCYTOSIS WAS VERY SLIGHT, SEE FIGURE 7, THE WRIGHT'S STAIN WAS MADE PRIMARILY TO TEST THE OSMOTIC PRESSURE OF THE ASSUMED ISOTONIC SALINE SOLUTION. AS ONE CAN EASILY SEE FROM FIGURE 8 NEITHER PLASMOLYSIS NOR PLASMOPTOSIS HAS OCCURRED IN ANY DEGREE. THEREFORE, THE SOLUTION WAS TRULY ISOTONIC AND WAS NOT RESPONSIBLE FOR EITHER THE LOSS OR THE DEFORMITY OF THE LEUCOCYTES. ONE CAN ALSO SEE FROM FIGURE 8 WHICH WAS A FIELD SELECTED AT RANDOM, THAT THERE IS NOT ONE SINGLE LEUCOCYTE IN THE ENTIRE FIELD. THIS IS A RARE SIGHT FOR A FIELD OF THIS SIZE. TWO THEORIES WERE FORMULATED TO ACCOUNT FOR THIS. 1) THE LEUCOCYTES OF THE BLOOD, BEING LIGHTER, DID NOT CENTRIFUGE WITH THE ERYTHROCYTES AND WERE REMOVED IN THE PIPPETING. 2) THERE IS SOME CONSTITUENT OF THE BLOOD SERUM WHICH IS NECESSARY FOR A LEUCOCYTES EXISTENCE AND PHAGOCYTTIC PROPERTIES. THE FIRST THEORY, HOWEVER, CONTRADICTS EXPERIMENT I OF THIS THESIS AND THEREFORE I BELIEVE THE LATTER TO BE CORRECT.



FIGURE 8 - BLOOD CELLS SUSPENDED IN SALINE SOLUTION

**NOTE COMPLETE ABSENCE OF LEUCOCYTES IN THE
MASS OF ERYTHROCYTES**

WRIGHT'S STAIN

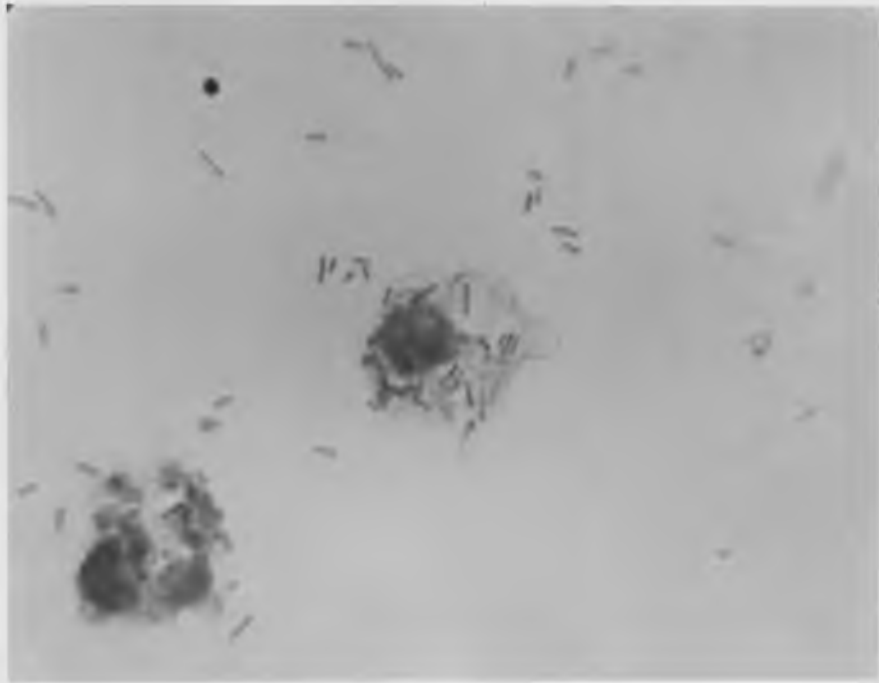
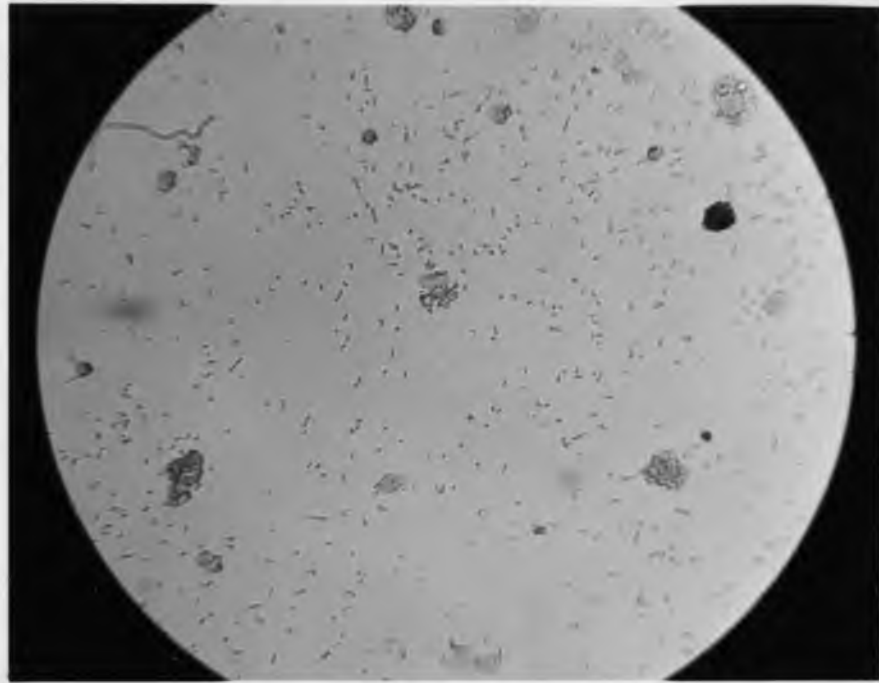


FIGURE 9 - HIGH POWER AND OIL IMMERSION REPRESENTATIONS
OF PHAGOCYTOSIS OF INJECTED E. COLI IN THE
ABDOMINAL CAVITY OF A WHITE MOUSE

MACCHIAVELLO'S STAIN

EXPERIMENT IV "PHAGOCYTOSIS IN VIVO"

PROBLEMS: THIS EXPERIMENT DEMONSTRATES THE PHAGOCYTOSIS OF THE MACROPHAGES AND LEUCOCYTES OPERATING IN THE LIVING BODY AND ALSO TO COMPARE IT WITH THE PHAGOCYTOSIS WHICH WAS PERFORMED OUTSIDE THE BODY.

PROCEDURE: TWO WHITE MICE WERE USED IN THIS EXPERIMENT. ONE OF THE MICE WAS USED AS A CONTROL AND THE OTHER WAS INJECTED INTRAPERITONEALLY WITH 0.5CC OF A 24 HOUR OLD NUTRIENT BRDTH CULTURE OF ESCHERICHIA COLI. TWO HOURS AFTER THE INJECTION, BOTH MICE WERE SACRIFICED BY PUTTING THEM IN AN ETHER CHAMBER. THEIR ABDOMINAL CAVITY WAS OPENED AND A SHEAR WAS MADE OF THE LYMPHATIC FLUID PRESENT IN THE CAVITY.

CONCLUSION: NO GROSS CHANGES IN THE PERITONEUM OR VISCERA OF THE INJECTED MOUSE WERE NOTICABLE. HOWEVER, UPON EXAMINING THE SLIDES, VERY GOOD PHAGOCYTOSIS WAS FOUND. IN THE INJECTED MOUSE, AS MANY AS 50 SINGLE BACTERIA WERE FOUND IN A SINGLE MACROPHAGE. THE PHAGOCYTES FOUND IN THE ABDOMINAL CAVITY WERE BOTH LEUCOCYTES (NEUTROPHILS AND MONOCYTES) AND WANDERING CELLS FROM THE RETICULO-ENDOTHELIAL SYSTEM (MACROPHAGES.) FIGURE 9 CLEARLY SHOWS THE PHAGOCYTOSIS IN THESE CELLS. ON THE SLIDE MADE FROM THE CONTROL MOUSE NOT NEARLY AS MANY PHAGOCYTES WERE FOUND. THIS WAS A GOOD EXAMPLE OF HOW CHEMOTAXIS HAD OPERATED IN THE CASE OF THE INJECTED MOUSE. IN THE CONTROL MOUSE A FEW COCCI WERE FOUND PHAGOCYTOSED, HOWEVER THESE WERE BELIEVED TO BE BACTERIA FROM OTHER EXTERNAL

SOURCES, SEE FIGURE 10. ALL IN ALL THE PHAGOCYTOSIS WHICH HAD COME ABOUT IN THE CAVITY OF THE LIVING ANIMAL EXCEEDED ANY PHAGOCYTOSIS WHICH WAS SYNTHETICALLY INDUCED OUTSIDE THE BODY.

EXPERIMENT V THE VALUE OF OPSONING

PROBLEM: WHAT ACTUAL EFFECT DO THESE ANTIBODIES CALLED OPSONING HAVE ON PHAGOCYTOSIS? HOW DOES PHAGOCYTOSIS, INFLUENCED WITH OPSONING DIFFER FROM THE USUAL PHAGOCYTOSIS PERFORMED IN AND OUTSIDE THE BODY?

PROCEDURE: A WHITE MOUSE WAS ALSO USED IN THIS EXPERIMENT. A VACCINE WAS PREPARED USING A 24 HOUR OLD NUTRIENT BROTH CULTURE OF ESCHERICHIA COLI. THE CULTURE WAS CENTRIFUGED UNTIL THE SUPERNATANT BROTH WAS CLEAR. THIS BROTH WAS PIPETTED OFF AND REPLACED WITH THE SAME VOLUME OF STERILE ISOTONIC SALINE SOLUTION. THIS WAS THEN SHAKEN AND PLACED IN A WATER BATH REGULATED AT 60°C FOR 30 MINUTES. THIS HEAT TREATMENT KILLS THE BACTERIA, BUT DOES NOT CHEMICALLY ALTER THE ENDOTOXINS PRODUCED BY THE ORGANISM. AFTER THE VACCINE WAS COLLED 0.5CC WAS INJECTED INTRAPERITONEALLY INTO THE MOUSE. FIFTEEN DAYS LATER THE MOUSE WAS GIVEN A SECOND INJECTION, BUT THIS TIME IT WAS 0.5CC OF A 24 HOUR OLD NUTRIENT BROTH CULTURE OF LIVE ESCHERICHIA COLI. TWO HOURS AFTER THE SECOND INJECTION THE MOUSE WAS SACRIFICED WITH ETHER AND SLIDES WERE MADE OF THE LYMPHATIC FLUID PRESENT IN THE ABDOMINAL CAVITY.

CONCLUSIONS NOTE THAT WITH THE EXCEPTION OF THE VACCINATION, THIS PROCEDURE WAS IDENTICAL WITH THAT USED IN EXPERIMENT IV. THEREFORE, BOTH THE MICE USED IN THAT EXPERIMENT COULD BE USED AS CONTROLS HERE. AFTER THE VACCINATION THE MOUSE BECAME VERY ILL FOR A PERIOD OF ABOUT 20 HOURS, BUT THEN RECOVERED. THIS WAS DUE TO THE VIOLENT TOXIN-ANTITOXIN REACTION WHICH WAS PRODUCED. AGAIN THERE WERE NO GROSS CHANGES WHICH COULD BE NOTICED IN THE POST-MORTEM. THE SLIDES GAVE VERY EXCELLENT RESULTS, HOWEVER, THE OPERATION OF THE OPSONING COULD BE EASILY SEEN. AS CAN BE SEEN IN FIGURE 9 FROM THE PREVIOUS EXPERIMENT THE BACTERIA ARE SCATTERED AND AN ACTUAL COUNT OF THEM COULD BE MADE. ALSO IN THE OIL IMMERSION REPRESENTATION THE BACTERIA WERE FOUND IN SINGLES IN THE MACROPHAGES. THIS CAN BE SHARPLY CONTRASTED WITH FIGURE 10 OF THIS EXPERIMENT WHICH WAS MADE IN THE IDENTICAL MANNER. THE BACTERIA HERE ARE NOT FOUND IN SINGLES BUT RATHER THEY ARE AGGLUTINATED INTO LARGE CLUMPS DUE TO THE ACTION OF THE OPSONING. IN THE OIL IMMERSION REPRESENTATION IT CAN BE SEEN HOW THE MACROPHAGES ENGULF THE BACTERIA IN THESE LARGE CLUMPS MAKING THE OPERATION VERY MUCH MORE EFFICIENT. IT IS OBVIOUS THEN THAT PHAGOCYTOSIS AIDED WITH THESE OPSONING IS MUCH SUPERIOR TO THE PHAGOCYTOSIS PERFORMED OUTSIDE OF THE BODY AND ALSO TO THAT PERFORMED INSIDE THE BODY WITHOUT THE AID OF THESE ANTIBODIES.

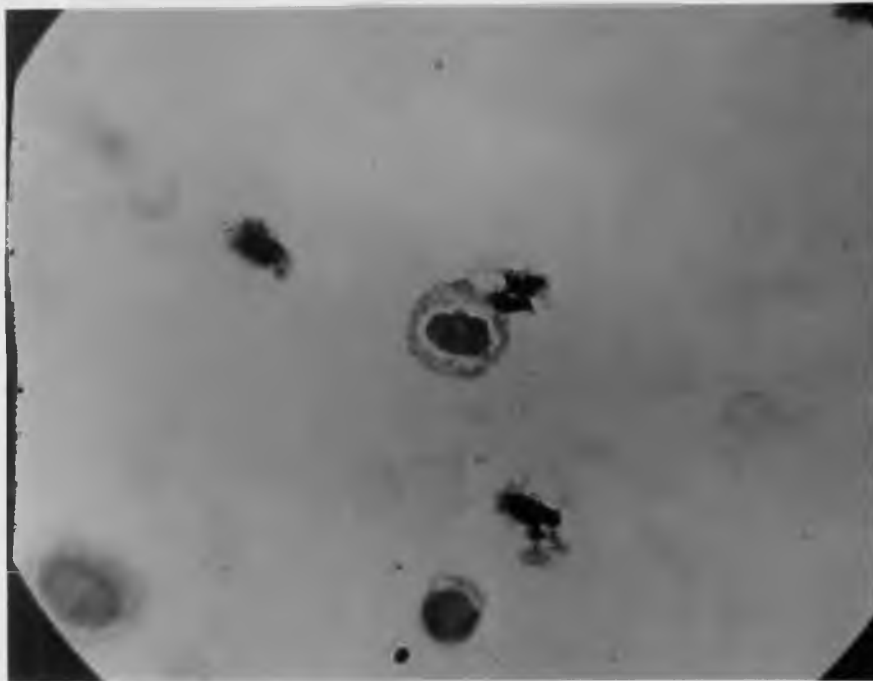
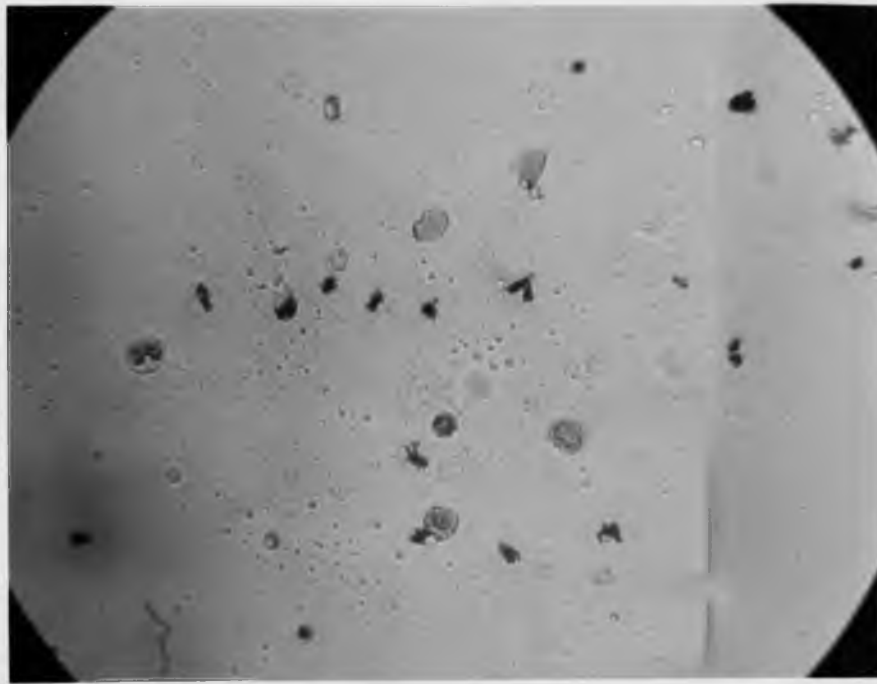


FIGURE 10 - HIGH POWER AND OIL IMMERSION REPRESENTATIONS
OF PHAGOCYTOSIS OF INJECTED E. COLI IN THE
ABDOMINAL CAVITY OF A PREVIOUSLY IMMUNIZED
WHITE MOUSE.

MACCHIAVELLO'S STAIN

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