BEHAVIORAL EFFECTS OF DEXTRAN-INDUCED INTRAVASCULAR AGGREGATION OF RED BLOOD CELLS (SLUDGE) IN THE RABBIT

By
KARL MICHAEL BROOKS

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

April, 1966


University of Florida
1853

ACKNOWLEDGMENTS

This work was supported by the Agricultural Experiment Station and was carried out in the laboratories of the Department of Food Technology and Nutrition. The author wishes to express his deep appreciation to Dr. Raymond A. Dennison and to the staff of the Department of Food Technology and Nutrition for their friendly interest and cooperation.

The author also wishes to express his gratitude to Dr. Bradford N. Bunnell for supervising the conduct of the research and to Dr. Wilse B. Webb, Dr. Frederick A. King and Dr. Robert L. King for their advice and counsel as members of the supervisory committee. Special thanks are extended to Dr. Ralph C. Robbins who not only introduced the author to the problem area but who as a member of the supervisory committee served as a continuing source of encouragement.

The provocative criticisms and advice of Dr. Rowland B. French and the enthusiastic assistance of Mr. Howard L. Povey are gratefully acknowledged.

Finally, the author wishes to acknowledge his debt to his wife, Sally, for her very capable assistance in gathering and analyzing the data and for her typing of the manuscript. No criticism is implied in noting that the typing was interrupted by the sudden arrival of a first born baby girl named Julie Elizabeth.

TABLE OF CONTENTS

LIST OF TABLES

APPENDICES

LIST OF FIGURES

Figure | Page

INTRODUCTION

A series of experiments was carried out to investigate the effect of severe intravascular aggregation of red blood cells ("sludge") on behavior. Observations were made on levels of food and water intake, on home-cage activity and exploratory behavior, on response latencies during successive exposures to a shock-escape learning situation, on response latency and running time during successive exposures to a food reinforced maze-runway learning problem and on bar press performance under a DRL (differential reinforcement of low response rates) schedule. The sludging was induced by the infusion of 1 gm/kg body weight of 2000k molecular weight dextran and evaluated by means of blood smears. Separate groups were used to control for the effects of the dextran per se and of the saline solution.

No statistically significant differences attributable to the effects of the dextran-induced sludge were found on any of the behavioral measures.

Background

The phenomenon of intravascular aggregation as a concomitant to illness and injury has been known under a variety of names and guises since the time of Hippocrates (Bigelow, Heimbecker and Harrison, 1949; Fahraeus, 1929; Knisely, 1951; Thygesen, 1942).

It has been demonstrated in a large number of disease states

(Ditzel, 1955; Fahraeus, 1929; Hirschbeck and Woo, 1950; Knisely and Bloch, 1942) and in pregnancy (Fahraeus, 1929; Odell, Aragon and Pottinger, 1947). It has been produced by the traumatic manipulation of tissue such as burning (Brooks, Dragstedt, Warner and Knisely, 1950; Gelin, 1959), crushing (Gelin, 1956; Knisely, Eliot and Bloch, 1945), by hypothermia (Lofstrom, 1959), by feeding a high fat diet (Swank and Cullen, 1953), by the infusion of high molecular weight substances such as thrombin, fibrinogen, gelatin or dextran (Cullen and Swank, 1954; Thorsen and Hint, 1950) and even by ingestion of alcohol (Bloch, 1956; Knisely, 1951).

A variety of processes might contribute to the aggregation under the several conditions listed above (Gelin, 1956; Gelin, 1959; Thygesen, 1942). Gelin (1959) has classified these alternatives as specific (agglutination or coagulation) and non-specific (pseudo-agglutination) factors. The specific processes include, for example, the antigen-antibody reaction and the conversion of fibrinogen into fibrin. The non-specific processes relate to the aggregation generally associated with the increase in the proportion of high molecular weight substances in the blood stream and to the clumping which has been found to occur with the complete interruption of blood flow (Gelin, 1956).

It should be emphasized that the present paper will be concerned with red cell aggregation induced by non-specific processes (pseudo-agglutination), although the pathologic effects of the aggregation may be independent of the process by which the aggregation was induced.

History of Sludge

The earliest reported observations of experimentally produced

intravascular aggregation were those of Joseph Lister (1858) who as early as 1827 (Norris, 1869) studied changes in the blood flowing through the small vessels in the wing of the bat following injury to the tissue. Richard Norris (1862, 1869) not only made extensive observation of experimentally produced red cell aggregation in the web of the frog, but studied the mechanism of aggregation by adding such substances as gum, gelatin and very thick solutions of starch to samples of blood. By adding increasing amounts of these substances he saw the character of the cellular aggregates change from that of mild rouleaux to firm masses of cells in which the outlines of the cells became less and less distinct until the individual identities of the cells were wholly lost.

Norris (1869) also described the process of venous stasis and the development of inflammation through the loss of fluid from the dilated vessels. This effect was usually associated with a condition of homogeneous stasis in which the outlines of the individual red cells could not be recognized.

Red cell aggregation was given a name ("sludge") and widely publicized through the work of Melvin Knisely and his coworkers (Knisely, 1951). Knisely (1936, 1938) had developed a fused quartz rod illuminator with which he was able to make continuous observations of living tissue without truamatizing the tissue itself. He began by studying the vascular system of the normal animal (Knisely, 1940) and eventually applied his techniques to the study of blood flow in pathological states.

He first studied malaria in the monkey. The monkey was experimentally infected with Plasmodium Knowlesi malaria. Blood flow in the internal organs was studied and photographed throughout the course of

the disease. The motion pictures taken of the severe aggregation during Stage III of the Knowlesi malaria were shown publicly (Knisely, Stratman-Thomas and Eliot, 1940). There followed a number of papers and motion pictures dealing with red cell aggregation (which Knisely and his co-workers called "sludge") in the experimentally infected malarial monkey (Knisely, Stratman-Thomas and Eliot, 1941) and in canaries experimentally infected with avian malaria (Lack, 1942). Blood flow and "sludge" were also observed in a large number of human disease states (Knisely and Bloch, 1942).

In 1945, Knisely and his coworkers produced potentially lethal sludging in the monkey by infecting it with malaria and then reversed the condition with atabrine or quinine. Later these same researchers (Knisely, Stratman-Thomas, Eliot and Bloch, 1949, 1951) gave the malaria infected monkeys heparin shortly before the parasites segmented. Under these conditions the severe sludging did not occur although the parasites in the blood multiplied greatly. Those animals which showed severe sludging died regardless of parasite count while the ones receiving the heparin showed high parasite counts but few outward signs of the illness. It was also noted that those animals developing the thick pasty sludge went into "slowly deepening comatose condition ending in a deep coma before death." (Knisely, Bloch, Eliot and Warner, 1947).

In 1947, Knisely and his coworkers published a paper entitled "Sludged Blood" in which they described various types of sludge and reviewed and extended the arguments for the pathologic significance of sludge. Sludge was supposed to have two functions: (1) it was a pathologic indicator; (2) it had of itself a highly significant effect on the course of disease.

Functions of Sludge

Sludge as an indicator of pathology

According to the investigators referred to above, sludge was a general term for aggregates of many different sizes and kinds. There were "basic masses" - aggregates which were sufficiently firm or rigid to resist breakup upon passage through the peripheral vessels; such basics might vary greatly in size. Depending on the severity of the aggregation, some or all of the red cells might be clumped. The several types of sludge described by Knisely et al. had one thing in common. There were always some rigid masses.

A definition of healthy, normal blood was provided as a baseline against which to measure pathology. In all organisms completely healthy blood flow is "streamlined", or laminar, and always so rapid that in vessels of from 60 to 120 microns it is impossible to distinguish the individual red cells. When observed in vitro, healthy red cells have sharply defined boundaries and the cells tend to repel each other within the blood fluid. As this blood flows through the capillaries, there is a minimum of loss of fluid through the capillaries and no detectable hemoconcentration.

Knisely's speculations concerning sludge were an invitation to controversy. First of all, Fahraeus's classic review (1929) had equated red cell aggregation with rouleaux and mild rouleaux with normal good health. Knisely (Knisely, Eliot and Bloch, 1945) stated emphatically that what he was studying was "not rouleaux", but masses of cells within which the individual cell lost its shape and identity. Apparently neither author was familiar with Richard Norris' (1869) demonstration that

rouleaux and the formless masses of cells represented only different degrees of the same phenomenon. Further, had either author been familiar with the papers by Lister (1858) or Norris (1862, 1869), the issue of whether mild sludging was compatible with good health might never have arisen since the earlier writers had pointed out that blood which gave no indication of aggregation in vivo, even during complete stasis, nevertheless readily formed rouleaux when placed on slides. And Knisely and his coworkers made their observations in vivo while Fahraeus used in vitro techniques almost exclusively.

The significance of sludge as an indicator of pathology remained a lively issue. Lutz, Fulton and Akers (1951) reported that they were unable to demonstrate rigid cellular aggregates in the hamster following trauma, burns or neoplasia. Also, a number of investigators, basing their arguments largely on clinical studies, reported that sludge in the conjunctival vessels was a common finding even in the absence of clinical evidence of pathology (Lack, Adolph, Ralston, Leiby, Winsor and Griffith, 1949; Robertson, Wolf and Wolff, 1950), and that sludge was of little diagnostic significance.

Robertson, Wolf and Wolff (1950) reported that they could find no correlation between sludge and clinical symptoms of illness. Further, they stated that sludging even within the individual patient varied greatly from day to day and from blood vessel to blood vessel. To them flow rate seemed to be the critical variable since presence of sludge was found to correlate only with degree of vasodilation. It was therefore hypothesized that the observed sludging was the result rather than the cause of the reduced blood flow rate. To test this hypothesis vasoconstriction was induced in one eye while the other eye served as control.

Following application of neosynephrine solution there was severe vasoconstriction and the disappearance of sludge from the blood stream while the control eye continued to show sludging. Then, when vasodilation was induced by histamine phosphate, sludge appeared where before there had been a complete absence of sludge. It was suggested that blood flow in the conjunctivae is the result of an increase in the volume of the local vascular beds and a decrease in the velocity of blood flow. Moreover, these experimenters were confident that "widespread sludging is compatible with good health and well being".

The temporary sludging that Fowler (1949) saw in conjunctival vessels following cervical sympathetic nervous stimulation or injections of noradrenaline may have been related to flow changes alone especially since small doses of novacaine reversed the effect. However, Lutz (1951) offered the alternative suggestion that this "sludge" may have been merely an illusion produced by rhythmic contractions of the small vessels of the conjunctiva.

All of the above findings were consistent with the implications of Ploman's (1920) demonstration that red cell aggregation could be produced in vivo, at least in man, by pressing the eyeball and inducing stasis within the retinal vessels. The resulting aggregation, or reaggregation, could then be observed in the larger venules and veins by opthalmoscopy. Ploman was a student of Fahraeus (1929); and his experiment contributed to Fahraeus' conviction that rouleaux represented a completely normal condition of the blood.

Laufman (1951) reviewed some of the evidence against Knisely's arguments and conceded the possibility that sludge may be such a sensitive indicator of pathology that the ailment may be undetectable by

ordinary clinical tests even though sludge is present. This was what Knisely and his coworkers believed (Knisely et al., 1947). They had studied 50 normal, healthy medical students and student nurses and found an absence of sludge. Fahraeus (1929) had stated that the healthy horse shows a high red cell sedimentation rate and a strong tendency to red cell aggregation, an observation which he considered as strong evidence for his conclusion that red cell clumping is fully compatible with good health. In an attack on Fahraeus' argument, Knisely, Bloch, Brooks and Warner (1950) made their own observations (they looked into the horse's eye) and found that sludging in the healthy horse is associated with fright and rough handling. Sludge, it seemed, was not a normal condition even in the horse. On the other hand, hundreds of seriously ill patients representing a wide range of diagnoses, including some psychiatric cases, which were studied by Knisely and his coworkers (1947), all showed sludge ("not rouleaux"). Thus, according to these workers, sludge does not occur in health, but it does occur in pathology.

In spite of Knisely's confidence, the question of whether sludge represents a sensitive indicator of pathology remains unresolved. Possibly the resolution of this issue must wait for a more reliable means for measuring the sludge.

The effects of sludge

Sludge and the body. - Knisely and his coworkers (Knisely, Bloch, Eliot and Warner, 1950) also remained convinced that sludge had a highly significant and detrimental effect upon the organism. Sludge, they were sure, could damage the body through a number of mechanisms. The

blood flow rate is reduced. This in turn restricts the transport of oxygen and glucose to the tissues including the vascular endothelium. Stagnant anoxia of the tissues develops. The anoxic walls of the capillaries become increasingly permeable to the plasma proteins and increasing quantities of proteins and blood fluid are lost into the surrounding tissues. Impacted cells and fluid loss reduce blood volume. Clumped red cells are destroyed by the phagocyte cells of the liver and spleen, promoting a condition of anemia.

Histological attempts to implicate tissue anoxia in death following severe sludging failed; there was no indication of the cause of death (Knisely, Stratman-Thomas, Eliot and Bloch, 1951). It was argued, however, that if tissue anoxia were the cause of death, then comparison with the "normal" should yield no new information since "... all, or almost all, of the tissues studied by the student of "normal histology" have died or been killed by processes directly involving anoxia" (Knisely, 1951, p.88).

In the "thick, mucklike sludge" such as Knisely et al. (1945) found in the terminal stage of Knowlesi malaria in the monkey there may be justification for the tissue anoxia hypothesis in spite of lack of histological verification; but for less severe sludge perhaps the hypothesis should sound more like a question. Landis (1928) did show that stagnant anoxia produced by compressing the mesenteric artery and vein produced in about 3 min. an increase in the permeability of the capillary walls sufficient to increase the rate of fluid loss to four times above normal, and to permit the loss of such quantities of plasma proteins that the osmotic pressure of the remaining blood was reduced to one-half of its previous value. It should be noted, however, that

immediately upon renewal of circulation the capillary permeability returned to normal. With less than complete anoxia the effect upon the blood vessel walls appears to be extremely difficult to demonstrate. Also, Norris (1869) observed spontaneous deaggregation and a sudden and apparently complete recovery of normal vascular function after a 3 hour period of complete capillary stasis in the frog. Perfusion studies suggest that the vascular endothelium is actually highly resistant to anoxia since the oxygen tension of perfusion fluids must be reduced almost to zero before the endothelium is affected (Zweifach, 1961). Finally Van Liere and Stickney (1963) after reviewing the literature relating to endothelial hypoxia concluded that hypoxia within the physiological range has little effect if any on capilliary permeability.

Muscle tissue also seems to be highly resistant to oxygen lack. Stainsby and Otis (1964) found that oxygen uptake by resting muscle was not affected by reduction of the blood oxygen tension until a critical low value occurred. Further, the contracting muscle with eight times the oxygen consumption of the resting muscle was found to have a lower oxygen tension than the resting muscle. Changes in capillary density were advanced to explain these results. This explanation is consistent with Krogh's (1919) estimate that the number of open capillaries, which may be less than 100 per mm^3 in the resting muscle, may rise to more than 2500 per mm^3 when the muscle is activated.

Yet it is conceivable that even this reserve capacity of the capillary networks may not be adequate at all times. Venous blood from traumatized tissue has been found to have a high oxygen tension (Blalock, 1943) even though severe sludging occurs at the site of any tissue trauma (Knisely, Eliot and Bloch, 1945; Gelin, 1956). However,

since arteriovenous shunts consistently dilate in response to blockage of capillary beds (Heimbecher and Bigelow, 1950) this increase in oxygen content of the venous blood may be associated with reduced capillary flow.

Sludging and vascular stasis have been shown to be associated with inflammation and tissue trauma (Knisely, Bloch, Brooks and Warner, 1950; Lister, 1858; Morris, 1869). Yet Gessler (1932) found a 10 fold increase in metabolic rate in the inflamed tissue of the rabbit's ear. While such highly localized metabolic changes may be explained, at least in part, by increases in the rate of anaerobic glycolysis (Frunder, 1953), the finding of sustained increases in oxygen consumption of 30 to 60 per cent in severely burned patients during the early postburn period (Cope, Nardi, Quijano, Rovit, Stanbury and Wight, 1953), when the sludging should already have been severe (Brooks, Dragstedt, Warner and Knisely, 1950; Gelin, 1956), seems to present no alternative to the conclusion that sludge may be associated with an increase in O_2 uptake. Even scorbutic guinea pigs show a level of O_2 uptake 20 per cent higher than normals (Evans and Hughes, 1964) in spite of the fact that the condition is associated with severe blood sludge (Robbins, to be published).

However, Lofstrom (1959), studying the effect of sludging on oxygen uptake, induced the sludge with infusion of high molecular dextran and subsequently reversed the effect with low molecular weight dextran. He found that oxygen uptake decreased significantly with the sludging and recovered as blood flow improved. While the obvious conclusion from this study has been widely accepted (Gelin and Zederfeldt, 1961; Long, Sanchez, Varco and Lillehei, 1961), attempts by the present author to replicate these findings have failed (Robbins, 1963-1964).

Lofstrom (1959) was also able to show what appeared to be an oxygen deficit during the rewarming phase of hypothermia. Infusions of low molecular weight dextran reduced sludge and improved flow rate and brought an increase in oxygen uptake. He suggested that some tissues do develop serious oxygen deficits and even acidosis during severe sludge. Such a differential effect should then be difficult to detect through analysis of the peripheral blood because of the delayed removal of the acid metabolites into the blood stream.

Gelin (1956) found visceral damage following a prolonged period of severe sludging and recommended "tissue anoxia" as the causal mechanism. The sludging was produced by fracture, contusions, burns, and by infusions of thrombin or high molecular weight dextran. All treatments resulted in profound sludging. All animals were sacrificed at the end of the third day. Inspection of visceral organs revealed necrotic areas in the livers, degenerative changes in the kidney tubules and hemmorhages in the lungs; and the pathology appeared to be largely independent of the means by which the intravascular aggregation was induced. Apparently the "anoxia" hypothesis was justified both because it followed logically from the observation that during severe sludging blood flow rate is much reduced and because of the nature of the resulting injuries.

Severe sludging is a common finding during and following extracorporeal circulation (Clowes, 1960). Tissue damage such as microinfarctions (much like that described by Gelin, 1956) of the kidney, liver, and myocardium are also a common finding in prolonged total body perfusion. However, such damage has been prevented through the use of low molecular weight dextran, which prevents the aggregation (Finsterbusch, Long, Sellers, Amplatz and Lillehei, 1961).

While the mechanism for the selective tissue damage associated with the sludge has not been fully demonstrated, the evidence seems to be consistent with the view that selective redistribution of the blood flow by sympathetic nervous activity compounds the effects of the sludge to produce localized tissue anoxia and acidosis (Gelin, 1962; Long, Sanchez, Varco and Lillehei, 1961). Gelin (1962) has noted that studies of the metabolic effects of induced red cell aggregation suggest the accumulation of acid materials in the tissues during aggregation with their subsequent removal when flow is improved. This hypothesis is based on the finding of a transient acidosis together with a reduced blood pH and a rise in pCO_2 and lactic acid which follows the increase in blood flow.

Additional evidence indicates, however, that the mechanism underlying the pathologic action of severe intravascular aggregation may be related to abnormal increases in oxygen uptake as well as to reduced oxygen availability. Gilmore and Fozzard (1960) studied liver function following severe thermal trauma to 30 percent of the body, a condition which should have resulted in very severe sludging (Brooks et al., 1950). Under these conditions the arteriovenous O_2 difference rose and there was an increase in oxygen uptake in the liver. It was their conclusion that "... hepatic hypoxia does not contribute to the production of early hepatic injury following severe thermal trauma." On the other hand, Hinshaw, Pories, Harris, Davis and Schwartz (1960) have reported a reduced oxygen tension in liver and kidneys following single injections of high molecular weight dextran in the anesthetized dog. The differences between these two sets of observations could reflect a difference in levels of sludge, and presumably therefore also in blood flow rates.

The evidence appears to be generally favorable to the idea that

sludge promotes a condition of stagnant anoxia and that this effect represents a mediating mechanism for the pathophysiologic consequences of sludge.

At this point all of the critical variables seem to be joined. Stagnant anoxia mediates the effects of sludge. The nervous system mediates behavior. The highly active neural tissue should be sensitive to the effects of anoxia and so it should also be sensitive to the sludge.

Sludge and the nervous system. - Knisely and his coworkers felt that the nervous system was particularly vulnerable to the effects of sludge. Upon finding sludge in the eye of a psychiatric patient they hypothesized that the patient's nervous system had been permanently damaged by capillary plugs of red cells masses; and the assumed mechanism of action of the sludge became evident "when one considers the parallelism between the known effects on normal persons of breathing slowly decreasing concentrations of oxygen (cerebral effects of anoxia), the slightly to greatly increased irritability, the euphoric tendency to laugh uproariously at meaningless trivia, the dull-witted phases, the compulsive behavior at times and the comatose condition as the anoxia approached the lethal stage and similar phases of some of the symptom complexes studied in the hospitals " (Knisely, Bloch, Eliot and Warner, 1950, p. 104).

There is certainly justification for taking a long look at the nervous system, particularly the cerebral cortex, as possibly one of the most sludge-sensitive organs in the entire body. Perhaps all of the blood which perfuses the brain must pass through the capillaries, for there are apparently no arteriovenous shunts in the brain (Forbes, 1954).

While in the phylogenetically older parts of the brain some anastomoses are found (especially in the reticular formation) and may even form extensive networks in pathologic conditions, these represent almost exclusively interarterial connections (Klosovskii, 1963).

These anatomical considerations argue for an intimate relationship between blood sludge and stagnant anoxia. If blood flow through the brain is dependent upon the functional integrity of the capillary, and if severe blood sludge plugs capillaries and induces a condition of stagnant anoxia as Knisely (1951) has insisted, then blood sludge and stagnant anoxia should be equated and the effects of blood sludge should be the effects of stagnant anoxia.

Equating sludge with stagnant anoxia should permit some confident predictions concerning the effects of sludge. It happens, however, that this analogy is less restrictive than expected, for stagnant anoxia may be equated with all other anoxias and therefore all that is known of anoxia may be relevant to the question of the effect of sludge on behavior.

The equivalence of all anoxias was pointed out long ago by Peters and Van Slyke (1931). These investigators studied the effects of oxygen deprivation under a variety of conditions and they reviewed the literature. They expressed their conclusions as follows: "One might expect these different types of anoxias to retard oxidation predominantly in different sets of tissues and produce correspondingly different outstanding symptoms. But the recorded effects of general anoxias of varying origins, including even the histotonic, impress one rather with their similarity when the causative conditions are comparable - severity, rapidity of onset, and duration." (Peters and Van Slyke, 1931, p. 585). With regard to stagnant anoxia specifically, Van Slyke pointed to the

fact that the behavioral symptoms described for cardiac failure where stagnant anoxia may occur with a minimum of confounding - "a sense of exhaustion, dizziness, stupor, syncope, dull headache, memory impaired especially for recent events, hallucinations, depression or exaltation, disturbances of the special senses" (Levy, 1920, cited by Peters and Van Slyke, 1931, p. 585) - are essentially the same as those described for anoxia due to altitude (Barcroft, 1920).

Anoxia and behavior. - The brain has an extremely high oxygen requirement. With perhaps 2 per cent of the body's total weight it accounts for 20 per cent of the total oxygen uptake when the body is at rest (Kety, 1955). The brain seems unable to tolerate any measureable decrease in its normal level of oxygen uptake without the development of mental symptoms of cerebral hypoxia. This relationship between brain function and oxygen was summarized by Lassen (1959) who concluded that "In all conditions of semicoma or coma which have been studied - whether due to anesthetics, acute hypoglycemia, apoplexy or any other cause - the reduction in consciousness correlates roughly with the decrease of cerebral oxygen uptake regardless of the cause of the acute cerebral disorder."

A variety of neurological and behavioral consequences have been shown to occur with anoxia. In a study of the effects of acute anoxia, Hurder (1952) exposed rats to varying degrees of hypoxic anoxia and later made cell counts of areas 10, 17 and 24 of the cerebral cortex. He found reductions in cell count in all areas studied, and the relationship between level of anoxia and cell count varied with the location, remaining constant for all levels in area 10, but increasing in areas 17 and 24

with increasing exposure time. Further, he found that maze performance error scores varied directly with cell counts in areas 17 and 24.

Studies on the neurological effects of asphyxia indicate a differential effect upon excitatory and inhibitory systems. In 1939, Van Harreveld and Marmont found that cats whose spinal cords had been asphyxiated for various periods of time showed exaggerated extensor tone (after recovery) which usually lasted for the about three weeks preceding death. It was suggested that the observed effect was due to selective damage to inhibitory mechanisms. Additional support for this hypothesis comes from observations that asphyxiation abolishes reciprocal innervation (Van Harreveld, 1939).

Consistent with this hypothesis is the finding that hypoxia enhances the distinction between simple and choice reaction times. A number of studies (Bauer, 1928) have demonstrated only a slight prolongation of simple reaction time from anoxic hypoxia at levels under 20,000 feet altitude which represents the approximate point of collapse for the unadapted subject. On the other hand, choice reaction times were found to be lengthened at a simulated altitude of about 16,000 feet (Jongbloed, 1935), and both accuracy and speed may be significantly affected at simulated altitudes above 15,000 feet (Tanaka, 1928) and 18,000 feet (McFarland, 1932).

Sensory thresholds also appear to be responsive to anoxia. Stokes, Chapman and Smith (1948) investigated the effect of anoxia, hypoxia and hypercapnia on cutaneous pain thresholds in man. There was no significant effect from a 10 per cent oxygen mixture, but a 13 and 28 per cent rise in threshold was obtained from 5 and 7.5 per cent mixtures, respectively. In the rat a 7.5 per cent oxygen mixture reduced response to

thermal pain and a 5 per cent mixture eliminated it completely (Bullard and Synder, 1961). A decrement in auditory sensitivity at low frequencies and a slight enhancement at higher frequencies has been found with reduced oxygen intake (Klein, Mendelson and Gallagher, 1961).

The extreme dependence on oxygen is consistent with the finding that the brain meets its extremely high energy needs primarily through aerobic glucose metabolism in both normal and pathological states (Lassen, 1959). A virtually continuous supply of oxygen is thought to be critical for normal brain function. For example, the human brain contains at any one time a total of only about 7 ml of oxygen which at the normal resting rate of consumption of about 50 ml per minute would last less than 10 seconds; and symptoms of anoxia would occur sometime before complete exhaustion (Tower, 1958).

While the entire brain may show an increase in oxygen uptake during a condition such as severe anxiety (King, Sokoloff and Wechsler, 1952), more commonly any change in metabolic activity remains a local phenomenon because of the nature of the causal chain tying blood flow to neural activity level. Localized alterations in blood flow occur with activation of specific sites in the brain (Sokoloff, 1957). Increased activity in the brain means increased energy requirements, and these are met primarily through aerobic metabolism (Lassen, 1959). This increased aerobic metabolism means increased production of CO_2 and an increased pCO_2 of the venous blood, and cerebral vasodilation appears to be almost completely dependent upon the pCO_2 (Wyke, 1963).

However, under acute anoxia the brain will turn to anaerobic metabolism with large increases in glucose utilization and CO_2 production (Tower, 1958). Although the efficiency of energy production

without oxygen is much less than when oxygen is provided, this capacity for anaerobic metabolism should tend to give oxygen and glucose a considerable measure of functional equivalence. Further, since pCO_2 of the venous blood determines the degree of vasodilation and since vasodilation should govern flow rate, then all of these factors must necessarily be involved in the organism's reaction to anoxia.

Brain oxygen and glucose relations. - Different areas of the brain vary in their sensitivity to the effects of oxygen or glucose lack, but the changes in brain and behavior associated with deficiencies of either of these two factors tend to be the same regardless of which variable is manipulated. For example, Tschirgi (1960) has pointed out that during acute anoxia the electrical activity of the cerebral cortex survives for only 14 to 15 seconds, that of the caudate nucleus, 25 to 27 seconds, the ventromedial thalamus, 28 to 33 seconds and that of the R. F. of the medulla from 30 to 40 seconds. Tschirgi's listing of the course of brain anoxia follows closely the relationships defined by the patterns of regional sensitivity to insulin shock. First there is a depression of the cerebral hemispheres and of the cerebellum; then release of the subcortical diencephalon, the subcortical motor nuclei, the thalamus and the hypothalamus, release of the mid brain, next the upper, and the lower medulla (Himwich, 1952).

The intimacy of the relationship among CO_2, O_2 and glucose is also reflected in the fact that a similar phasic response of the nervous system occurs regardless of which variable is manipulated. There is a three-stage reaction to CO_2. With 3.5 to 7 per cent CO_2 in respired air there is some depression of cortical activity. From 5 to 20 per cent

produces a generalized reticular activation which reverses the original depression. Finally, at levels above 25 per cent there is a general CO_2 narcosis which is associated with the inactivation of the RAS (Wyke, 1963).

Some differences become evident, however, when other variables are considered. Small, Weitzner and Nahas (1960) ventilated dogs with 5, 10 and 15 per cent CO_2 and obtained large increases in cerebrospinal fluid pressure (CSFP) within a 10 min. interval which lasted for as long as 90 minutes without any consistent changes in arterial or venous pressure at any time, until termination of the hypercapnia at which time CSFP rapidly returned to normal. Ventilation with 8 per cent O_2 brought a more modest rise (84 per cent) in CSFP which approached its maximum within 5 minutes accompanied by dramatic increases in arterial and venous pressures. Yet during 90 seconds of asphyxia there was a 175 per cent rise in CSFP together with the same large increases in arterial and venous pressures that were observed with the hypoxia alone. Sludging, if it reduces flow rate and plugs small vessels, should produce an effect comparable at least to the combined effect of anoxia and hypercapnia as in asphyxia and not that of anoxia alone.

Other experimenters have been impressed by the similarity of functional effects upon the brain of hypoxia and of hypoglycemia. Sugar and Gerard (1938) pointed out that hypoglycemia may make an important contribution to the damage associated with sudden anemias, while Gellhorn, Ingraham and Moldavsky (1938) saw hypoxia and hypoglycemia acting synergistically to produce convulsive seizures.

Wyke (1963) has suggested that the observed sedative effect of hyperventilation associated with cerebral hypoxia is the result of

severe vasoconstriction of the brain. He also noted that these behavioral effects are correlated with slowing of the EEG and that both of these effects are maximal during coincident hypoglycemia and hypoxia and minimal when arterial concentrations of both O_2 and glucose are high. McFarland and Forbes (1940) have found that the ingestion of glucose counteracted the tendency for the stimulus threshold for light to rise in hypoxic anoxia. Perhaps the most dramatic demonstration of the functional equivalence of oxygen and glucose and of the importance of blood flow has been offered by Neely and Youmans (1963). They reported that dogs survived 30 minutes of continuous perfusion of a buffered glucose solution followed by replacement of the blood.

Brain blood flow and behavior. - Blood flow rate appears to be a critical variable in every consideration of brain oxygen-glucose relations, for the transport of both oxygen and glucose and the removal of CO_2 and of the acid residues of oxygen-free metabolism depend upon the integrity of vascular function.

The involvement of blood flow changes in the response to cerebral anoxia has been recognized and studied. For example, Geiger (1958) has insisted that the nervous system's ability to tolerate a deficiency of oxygen may depend upon the maintenance of a blood flow sufficient to remove the toxic waste products produced by the anaerobic metabolism. Gellhorn and Kessler (1942) have provided some experimental support for the importance of blood flow in brain function. They made rats comatose with injections of insulin following bilateral extirpation of the adrenal medulla. They applied electric shock to the brain and found that this resulted at once in a recovery from coma, with a return of normal

behavior and normal EEG in spite of the fact that the blood glucose had not risen above the coma level. It was hypothesized that stimulation of the sympathetic NS resulted in an increase in blood flow to the brain.

Swank and Nakamura (1960) have reported that hamsters given stomach loads of butter-fat become increasingly inactive during the hours following the ingestion but become active again and develop convulsions beginning approximately 3 hours after feeding. These behavioral changes were correlated with decreases in the pO_2 of the brain tissue and with increases in the viscosity of the blood; the pO_2 fell and blood viscosity increased. Swank and Escobar (1957) injected high molecular weight dextran into dogs and obtained major increases in the viscosity of the blood together with paralysis and electrocardiographic changes lasting between 1 and 6 days. The most severe effects were obtained in those animals which were given a long-acting anesthetic prior to the treatment. It should be noted that the high molecular weight dextran also produced sludging of the blood (Thorsen and Hint, 1950). Cullen and Swank (1954) injected high molecular weight dextran into six hamsters and observed extravascularization of trypan blue from both cortical and subcortical vessels 6 hours later.

The consequences of acute cerebral circulatory arrest have been reported by a number of investigators. Weinberger, Gibbon and Gibbon (1940) found permanent damage to the cerebral cortex in cats following 3 min. 10 sec. of complete circulatory arrest, while 3 min. 25 sec. produced a softening of the cortex. This sensitivity of the cerebral cortex to impairment of blood flow to the brain has been a common finding in acute experimental ischemia (Wright, 1965). Dogs exposed to 6 min. of apparently complete cerebral circulatory arrest have been found to

show no measurable behavioral decrement as determined by measures of learning, retention or psychomotor performance, whereas 8 min. of circulatory arrest resulted in impaired learning but had no effect upon retention or psychomotor performance (Nielson, Zimmerman and Cooliver, 1963).

Wright (1965) produced complete cessation of cerebral blood flow in rabbits and cats. Only one of four cats survived 7.5 min. and none out of four survived 10 min. of cerebral vascular occlusion. On the other hand none of three rabbits survived even 6 minutes. This difference might have been due to species differences in susceptibility to the treatment. Wright suggests that the difference in survival expectancy between the two groups of animals may be due to the difference in the two methods used. Only the arterial inflow was occluded in the cats. This treatment left the brain pale and bloodless. Both arterial and venous flow was occluded in the rabbits, leaving the vascular beds of the brain filled with blood for the entire period. Since more oxygen and glucose should have been available to the brain in the latter instance, the authors speculated that the lower survival time for the rabbits may have been due to thrombi forming in the small vessels of the brain during the period of vascular occlusion.

The possibility that species differences in response to cerebral vascular occlusion may have overshadowed any effects due to differences in the techniques used is suggested by the finding as noted above that dogs may tolerate 8 min. of cerebral circulatory arrest without severe neurological damage while cats and rabbits could not even be revived after 6 to 8 minutes. With regard to this question Wright (1965) has pointed out that arterial network interconnecting with the carotid

system is extremely complex and that major differences in these systems are common both among species and among individual animals within any given specie. Consequently the effect of the treatment may vary considerably among individual animals while different species may actually require different techniques for producing even a near total cerebral ischemia. Typical maximum periods of reversible ischemia reported for various species include 8 min. for the dog (Boyd and Connolly, 1961) and 5 to 7 1/2 min. for the cat (Wright and Ames, 1964), but no more than 5 min. for the rabbit (Hirsch, Bolte, Schandig and Tonnis, 1957).

Cerebral blood flow conditions approximating those to be expected during severe red cell aggregation may best be produced experimentally by means of an extended period of less than total flow impairment. For example, extensive infarction of the basal ganglia and cerebral hemispheres together with gross behavioral deficits has been produced in the dog by occluding the middle cerebral artery for a 2 hour period (Cyrus, Close, Foster, Brown and Ellison, 1962). In those dogs which received infusions of low molecular weight dextran before the occlusion the extent of tissue damage was significantly reduced and the behavioral effects much less extreme. Since the effect of the dextran appears to be that of reducing or preventing cellular aggregation and stasis of the blood (Gelin, 1956), the differences in extent of tissue damage between the controls and the dextran-treated animals should represent the effects of sludging and vascular stasis.

The above findings would seem to emphasize the role of thrombus formation in the process of cerebral tissue damage during ischemia as opposed to anoxia per se. Recent work by Neely and Youmans (1963) has provided additional support for such a position. These experimenters

cleared the dog brain of blood by raising the CSFP to 400 mm Hg which was at least twice that of the highest expected blood pressure level and found that all of five animals exposed to up to 25 min. of such treatment "were able to see, stand and hear the next day and survived at least 48 hours." Six other animals whose brains were kept bloodless for from 30 to 60 min. all had return of normal blood pressure and pO_2 of the arterial blood, but neurological abnormalities were evident and none of the animals survived over 24 hours. Completeness of the ischemia was verified by injecting radioactive sodium into the blood stream and testing the brain for radioactivity 15 min. later. No activity was present in the brain tissue as compared to a high activity for muscle tissue overlying the brain.

The authors suggested that tissue damage associated with the arrest of blood flow is related to the presence of anoxic blood in the vessels and that the method used here prevented such damage through clearing the blood from the brain before thrombi had time to form. Thus the absence of blood served to protect the brain from injury. With glucose available in the static blood the brain is presumed to produce lactic acid via anaerobic metabolism of the glucose. The pH of the blood is lowered and the clotting tendency is increased, an assumption consistent with the finding by Crowell and Houston (1961) that even the presence of heparin will not prevent the clotting of acedotic blood. When no blood is present, this process is restricted, since brain tissue lacks the stores of glycogen found in other tissues.

The results from the above study indicate that the brain is highly tolerant of all manipulations except stasis of the blood. Yet the permanent plugging of small vessels is precisely the function that

Knisely (1951) has ascribed to blood sludge. The brain then should be particularly sensitive to the effects of sludge.

The problem of adaptation to sludge. - There are few reports of work dealing directly with the problem of adaptation to sludge. A number of observations on adaptive changes following exposure to anoxia have been reported. The hypothesis of the functional equivalence of sludge and anoxia suggests that major adaptive changes do occur during sludge. The hypothesis further suggests that the literature dealing with adaptation to anoxia should be relevant to the problem of adaptation to a condition of blood sludge.

Resistance to hypoxia. - Increased resistance to hypoxia may be produced by a variety of conditions. Bartlett and Phillips (1960) showed that rats previously adapted to physical restraint survived acute hypoxia better than controls, a relationship which held whether the animals were restrained or not during the actual testing. Aclimatization produced by discontinuous hypoxia has been found to increase significantly the resistance to acute hypoxia in rats (Thorn, Clinton, Farber and Edmonds, 1946). Similar changes have been observed in humans (Luft, 1961; Van Liere and Stickney, 1963).

Prolonged exposure to mild hypoxia also results in a heightened tolerance for acute hypoxia. For example, in humans suffering from chronic respiratory impairment the increase in cerebral blood flow during arterial hypercapnia and anoxia was much less than would have been predicted for a normal subject with similar levels of blood gasses (Lassen, 1959).

No doubt a number of factors contribute to the adaptive changes

resulting from hypoxia. Van Liere and Stickney (1963) have suggested that quantitative changes in metabolic activity of the tissues may represent one means of promoting adaption. This view finds support in a recent report by Hamberger and Hyden (1963) to the effect that one of the enzymes of the electron transport system (cytochrome oxidase) showed an increase of several hundred per cent following exposure for an extended period of time to a moderately reduced oxygen concentration in the respired air.

Balke (1944) reported that in humans two weeks of training in a mildly hypoxic environment of about 10,000 ft. altitude resulted in a nearly one-third greater increase in maximum level of oxygen consumption and capacity for work than could be obtained by training at sea level. Failing to demonstrate changes in hemoglobin, myoglobin or pulmonary ventilation which might have accounted for this increase, Balke concluded that the capillary blood flow in the muscles must have improved. Since the testing situation was prolonged, the capacity of the subjects to accumulate an oxygen debt such as has been found to concur with exercise (Grollman, 1955) should have had little or no influence on the results.

Adaptive changes in the blood-vascular system. - A variety of evidence recommends that at least part of the mechanism of adaptation to anoxia involves changes in the size, shape and number of blood vessels, especially capillaries. Such changes appear to be common during the chronic and severe sludging which occurs in chronic diabetes mellitus (Ditzel, 1955). Blood vessel changes associated with this condition include capillary elongation (tortuousness, twisting) and shifts

in arteriovenous ratios. These changes were found to compare closely with those obtained in normals with the inhalation of 5 per cent CO_2 mixtures (Ditzel, 1964).

The dependence of vascular changes in chronic sludging upon tissue oxygen and CO_2 levels is also suggested by the nature of the changes occurring during adaptation to the relatively hypoxic conditions at high altitudes. Liebesny (1922) reported that persons who travel from low to high altitudes show changes in the condition of the blood as a result of the altitude change. The blood flow is impeded, and the vessels, because of the apparent clumping of the cells within, have a beaded appearance. Simulated altitude studies have shown that hypertrophy (increases in diameter and tortuosity) of the vasculature is the usual response to hypoxia. In acclimated animals the capillaries may double in diameter. The number of functional capillaries may also be increased such that the total increase in vascularization becomes a function of both hypertrophy and hyperplasia (Van Liere and Stickney, 1963; Korner, 1959). The net effect of these changes in vascularity should be to increase the diffusion gradients at the tissues and to permit continued integrity of function, particularly of the brain, at reduced levels of oxygen and other nutrients and at the same time remove more rapidly any toxic waste products.

Also, Swank (1956) found that dogs receiving repeated fat meals developed a "surprising" degree of adaptation which lasted for as long as a year. As a result, instead of showing the usual increased blood viscosity and red cell aggregation (Swank, 1951) following the ingestion of the high fat meal, the animals showed essentially normal flow conditions even after large test meals, and "remained active and vigorous". (Swank, 1956).

Endocrine relations in adaptation to sludge. - Endocrine response during adaptative changes occurring as part of the stress syndrome may have a considerable effect upon the entire vascular system. First, the proportions of the various molecular weights of plasma proteins are sensitive to the circulating levels of adrenocortical steriods (Ditzel, 1959), while the proportions of these protein fractions, the relatively low molecular weight albumin and the higher molecular weight globulins and fibrinogen, tend to determine the sludge level of the blood (Ditzel, 1959; Fahraeus, 1929; Thorsen and Hint, 1950).

The adrenocortical hormones apparently affect the functional capacity of the blood vessels as well as the blood itself. The significance of adrenocortical activity in producing and maintaining the functional integrity of the blood-vascular system in the face of environmental stressors is suggested by Zweifach's (1961) description of vascular function in the adrenalectomized animal.

> . . . a rise as small as 2° C. in the temperature of the fluid bathing a tissue exposed for microscopy causes a rapid stasis in the small venules and veins. Colloidal carbon in the circulation accumulates along the intercellular borders of the capillaries, emphasizing the abnormal status of this constituent. The capillaries undergo stasis following even minor mechanical manipulation with micro-needles. The endothelium cells swell and appear to have lost their normal tone. Numerous petechae develop along the venous capillaries. Intravascular thrombi fail to regress following even minor micro injury. Although topical application of cortisone or of adrenal cortical extract reverses within several hours the vasodilation and depressed vascular reactivity typical of such vascular beds, the fragility of the endothelium and the vessel wall as a whole is not significantly lessened . . . (p. 109).

The effect of these hormones on blood flow has been demonstrated in very dramatic fashion by Bergen, Hunt and Hoagland (1952) who showed that a 61 per cent decrease in cerebral blood flow together with a 46 per cent fall in brain oxygen consumption which developed in

adrenalectomized rats returned to normal in 2 to 3 hours following treatment with lipoadrenal extract.

Adaptation. Comments. - The restrictions upon the adaptive process should be repeated. Wyke (1963) has pointed out that with continued exposure to high levels of CO_2 in the respired air there is a gradual reduction of symptoms, indicating adaption to the highly abnormal conditions. In anoxia, however, the symptoms may change with time, but they do not go away. The effects of acute anoxia are suggestive of alcohol intoxication, whereas chronic anoxia produces a condition resembling physical and mental fatigue (Barcroft, 1920). The equating of the effects of acute anoxia with alcohol intoxication seems rather appropriate here, since alcohol is reported to be an effective agent for the production of intravascular aggregation (Bloch, 1956; Knisely, 1951). This latter observation suggests that any adaptive advantages gained from anoxia may also accrue to the periodic user of alcohol.

In summary, the evidence available with regard to adaptation seems to offer a strong argument for the dependence of sludge effects upon the prior stress history of the animal. Control for this factor seems to be recommended for any study concerned with the effects of sludge.

Present Status of the Sludge Problem

One observation which may be derived from the preceding pages is that there is a dearth of experimental data bearing directly on the question of the significance of sludge. This deficiency represents the basic criticism of the present status of the sludge problem.

The criticism is hardly original. Laufman (1951) called for more

data when he insisted that it should be first determined whether sludge has any detrimental effects on the organism before the question of how is taken seriously. While Laufman is to be commended for his concern with objectivity, his failure to see these two questions as representing two levels of analysis of the same problem instead of two separate problems may have added to the confusion. Ditzel (1959) also called for more data when he pointed out that "our knowledge concerning the pathophysiological significance of pronounced intravascular aggregation per se is inadequate, and concentrated effort should be exerted for the elucidation of this important problem," (p. 57).

There was only a very limited response to this latter appeal. The difficulty lay in the fact that the question was not "whether" to attack the sludge problem, but "how" to attack it. This difficulty has recently been summarized by David Long (1962).

> The pathophysiologic relevance of intravascular aggregation has been disputed for years. This dispute will probably continue for as long as our methods for evaluating function are so gross and imprecise for organs with a large functional reserve. Furthermore there are no methods for quantifying the magnitude of intravascular aggregation of blood corpuscles. In the meantime microcirculationists remain impressed when large aggregates of corpuscles occlude arterioles and venules preventing capillary circulation to focal areas. (p. 579).

Since behavioral studies on the problem of blood sludge are almost nonexistent, the present status of the sludge problem should represent even more of a challenge for the psychologist than for the "microcirculationist."

THE BEHAVIORAL EFFECTS OF SLUDGE: THE HYPOTHESIS AND APPROACH

An extensive literature search produced no reports of systematic observations made on behavior during experimentally controlled blood sludge. In the absence of such studies, an effort was made to formulate some predictions about the behavioral consequences of sludge through defining the mechanism of the action of sludge.

Evidence was offered for the following argument. Blood sludge promotes a condition of stagnant anoxia through impaired blood flow and the plugging of small vessels. The behavioral effects of all anoxias, including stagnant anoxia, are the same, but the effects vary as a function of intensity, rate of onset and duration. In particular, major changes in response to anoxia seem to occur with adaption during prolonged exposure. However, so long as anoxia is involved in severe blood sludge, that condition should be reflected in behavior.

Accordingly it was predicted that severe sludge in the rabbit is associated with behavioral changes which could range from a moderate depression of activity with increased response latencies and short term memory deficits to a more severe condition approaching stupor or even coma.

Attempts were made to test this hypothesis as well as to make observations on a variety of other behaviors which might be sensitive to the effects of sludge. These efforts are described below in three parts.

Part I. The problems associated with the development of a method

for the study of sludge-behavior relations are discussed together with the solutions to these problems.

Part II. Results from two separate experiments are offered as evidence for the validity and reliability of an in vitro technique for the evaluation of the in vivo condition of the blood.

Part III. The relationship between blood sludge and behavior is explored through a series of four experiments covering a variety of behaviors beginning with simple maintenance behaviors and extending to behaviors of increasing complexity.

Part I. Preliminary Problems

The four requisites for the study of the behavioral correlates of red cell aggregation are 1) an appropriate subject, 2) a means for inducing the aggregation, 3) a method for measuring the degree of aggregation and, finally, 4) a behavioral test.

These four factors, together with the attempts to specify and combine them, are discussed below.

Appropriate subject

The rat might have been the preferred subject for the present series of experiments because of the large number of standardized behavioral testing situations already available for use with this animal. However, the rat has what appears to be a species specific sensitivity to dextran (Adamkiewicz and Adamkiewicz, 1960), since only a small percentage of animals from a few colonies of Wistar rats have been found to be non-reactors (Harris and West, 1963). Further, use of highly viscous solutions with the rat requires anesthesia and surgical manipulation.

Because the anesthesia and the high molecular weight dextran may have complementary effects, the use of the rat in studying the effects of sludge on behavior might make increasingly difficult the task of isolating the effects of the sludge per se.

A non-rodent, the rabbit, (order Lagomorpha), was finally chosen as the best possible compromise. The ear veins of the rabbit are accessible without the need for anesthetics or surgical manipulation and they are large enough even in an animal of 750 gms body weight to tolerate continuous infusions of the highly viscous dextran solutions. The number of alternative sites for access to the vascular system available in the ears of the rabbit make the animal a particularly attractive choice. Successive infusions or blood samples can be scheduled with little risk of exhausting all alternative infusion sites before the experiment is completed. Further, infusion and blood sampling techniques have been developed to a point where the animal seems to be almost completely unmindful of the treatments and as a result there is minimal confounding from incidental aspects of experimental manipulations.

The choice of the rabbit was also recommended by the fact that there is already a considerable amount of information available on the use of this animal in the study of red cell aggregation and particularly in experiments where the aggregation is induced by infusions of high molecular weight dextran (Fajers and Gelin, 1959; Gelin, 1956; Gelin, 1959; Gelin, 1962; Lofstrom, 1959; Lofstrom and Zederfeldt, 1957; Thorsen and Hint, 1950; Zederfeldt, 1957). In none of these studies has any unusual sensitivity to the dextran been reported.

A method for inducing the sludging

As a means for inducing the aggregation high molecular weight dextran (HmDx) seems to have obvious advantages. First, except when used with the rat, there appears to be a minimum of confounding secondary effects. As previously noted, no hypersensitivity had been reported in any of the studies involving the use of dextran with the rabbit.

Infusion of HmDx (2,000k mean molecular weight) into rabbits has been shown to induce a number of physiological effects. The most dramatic effect of the infusion of HmDx into the blood stream appears to be the aggregation of the red blood cells, the severity of which is determined primarily by two easily controlled factors, dose level and molecular weight of the dextran (Gelin, 1956; Thorsen and Hint, 1950).

HmDx appears to have a prolonged effect upon the condition of the blood. Gelin (1959) has reported that a single injection of 1 gm/kg body weight in the rabbit induced severe sludge, increased blood viscosity and heightened ESR which lasted for 5 to 6 days. The duration of the effect of HmDx is presumed to be related to the fact that it is removed only slowly from the vascular system.

There appears to be little loss into the extravascular compartment of colloidal materials above a molecular weight of 412,000 (Mayerson, Wolfram, Shirley and Wasserman, 1960). Normally the dextran not lost through the kidney seems to be taken up gradually by various cells in the body and eventually broken down into glucose. Most of this dextran appears to be removed from the circulation by the reticuloendothelial system of the liver and spleen (Grotte, 1956; Osol and Farrar, 1955). However, when these phagocytic reticuloendothelial cells become saturated

following large doses of foreign colloidal particulate matter (such as dextran) the endothelial cells in the capillaries and venules in other areas of the body may become phagocytic. Because these endothelial cells are unable to metabolize the ingested particles, the results of the infusion of foreign colloidal material into the blood stream may be a generalized pathological condition of the entire vascular system (Zweifach, 1961). Support for this view is offered by the finding that a single injection of HmDx into the rabbit produces no obvious pathological lesions of the viscera even though the sludging remains severe for several days whereas successive injections of HmDx have consistently produced tissue damage to liver, kidneys, heart and lungs (Gelin, 1956; Gelin, 1959) or seriously impaired the healing to wounds (Zederfeldt, 1957).

There is some evidence, however, that the phagocytic response may interact with the sludging effect. Knisely, Bloch and Warner (1948) have reported that when particles of India ink are injected into the vascular system of the frog they become covered with a "sticky coating" and are thereupon ingested immediately upon contact with the phagocytes of the liver sinosoids. A similar fate apparently befalls the masses of parasitic red cells of the monkey during Stage III of Knowlesi malaria. These cell masses are also phagocytized in the liver. Knisely et al. (1948) estimated that in one case the rate of destruction was sufficient to have destroyed within a 3 hour period up to one third of all circulating red cells.

On the other hand, it seems that the mere presence of the foreign colloids in the blood stream does not result in pathologic changes in the vascular endothelium, or at least none severe enough to promote

damage to surrounding tissues. For example, Fajers and Gelin (1959) found that additional dextran infusions in the form of solutions of low molecular weight dextran (LmDx) prevented the tissue damage produced by the infusions of HmDx alone, and Zederfeldt (1957) found no interference with wound healing when LmDx infusions were given together with dose levels of HmDx which when given alone over four successive days caused significant retardation of healing.

Gelin (1956) has reported, also, that anemia occurs with severe red cell aggregation whether the aggregation is induced by tissue trauma or by infusions of HmDx, although he related the reduction in circulating cell volume only to vascular stasis and the packing of cells in capillaries and venules. There does seem to be some tendency toward specificity in this reaction. For example, the introduction of bacterial endotoxins into the vascular system is reported to increase phagocytic activity and tissue damage in the endothelia of the lungs, kidneys, adrenals and intestines (Zweifach, 1961), those organs which Fajers and Gelin (1959) found to suffer damage during intravascular aggregation induced by infusions of HmDx.

All dextrans have been observed to increase the apparent viscosity of the blood. The magnitude of this increase appears to vary directly with the molecular weight of the dextran used (Gregersen, Peric, Usami, Chien, Chang and Sinclair, 1963). Further, the dependence of blood viscosity upon shear rate also seems to vary directly with the molecular weight of the dextran (Rand and Lacombe, 1964).

Some of the effect of the HmDx appears to be related to the fact that it also acts as a plasma expander. For example, Gelin (1956) found that following a single injection of 1 gm of HmDx the urinary output fell

from a preinjection level of 240 ml per day to a low of 20 ml on the first posttreatment day and rose gradually thereafter to 75 ml on the second day and to 150 ml on the third day after the injection.

When HmDx is used for inducing sludging, there are available two methods for isolating those effects of the dextran which may be independent of the effect of the induced aggregation. First, a separate control group receiving infusions of a dextran with a molecular weight of between 75,000 and 80,000 may be used. Second, the blood sludge induced by the infusion of HmDx may be reversed by treatment with a low molecular weight dextran (LmDx) having a molecular weight of less than 40,000.

Dextran of a molecular weight between 70,000 and 80,000 (approximately that of blood albumin) does not induce red cell aggregation. However, it does act as a plasma expander, and so it may therefore be used to control for the plasma expander effect of HmDx. In normal subjects there is no significant effect of erythrocyte sedimentation rate (ESR) and perhaps only a slight rise in blood flow associated with a minor decrease in peripheral resistance while subjects with high sedimentation rates show little if any reaction to infusion of dextran of this weight other than perhaps an extremely slight increase in plasma viscosity (Gelin and Thoren, 1961).

LmDx also acts as a plasma expander, but its effect is shorter and more dramatic than for the higher molecular weight dextrans. The effect lasts only about 1 1/2 hours for a 10 per cent solution and not more than 3 hours with a 15 per cent solution when the dose volume is held constant. As a result, a significant concentration of LmDx remains in the blood stream for a much longer period, and so the flow-promoting, sludge-reducing effects of LmDx should last for a somewhat longer period

(Gelin, Sölvell and Zederfeldt, 1961).

Together with the plasma expansion there is a marked increase in diuresis, but no significant urinary losses of sodium or chloride nor changes in blood levels of these factors when salt-free solutions were used (Gelin, Persson and Zederfeldt, 1961). Excretion of the LmDx in the urine is rapid at first, but drops suddenly to extremely low levels even before the diuresis is reduced. Finally, no significant changes have been observed in the levels of the various plasma protein fractions during this period (Gelin, Sölvell and Zederfeldt, 1961).

The determination of the effect of the dextran per se should require also an evaluation of the influence of the large doses of saline solution which serve as the vehicle for the dextran. The saline appears to have no significant plasma expansion effect (Gelin, Sölvell and Zederfeldt, 1961). Neither have any measurable pathological effects been found from frequent and successive infusions of saline solutions (Zederfeldt, 1957). Nor does it seem to have any effect upon HmDx-induced red cell aggregation when the saline is used in place of the LmDx (Cullen and Swank, 1954), or upon the sludging occurring during eclampsia which has been reduced by intravenous albumin (Odell, Aragon and Pottinger, 1947). On the other hand, Brooks, Dragstedt, Warner and Knisely (1950) have reported some apparent improvement from saline infusions in sludging following severe thermal burns. Robbins (1963-1964) found a reduction in survival expectancy in saline injected rats made to swim to exhaustion. Further, preliminary observations with rabbits have indicated a depression in spontaneous activity following saline infusions in volumes required to match those of the dextran injections.

Since no conclusive evidence is available regarding the effect of

infusions of physiological saline into the rabbit, the present series of studies provided for control of this factor in every instance. In every experiment, the effects of dextran per se were also controlled by means of the first of the two methods described, that of matching the volumes of HmDx infusions with solutions of dextran with a molecular weight approximating that of blood albumin (77 k molecular weight).

Evaluation of level of sludge

Measurement of the degree of aggregation of the red cells has long been recognized as perhaps the most serious obstacle to the study of the effects of the aggregation. Fahraeus (1929) equated intravascular aggregation with erythrocyte sedimentation rate (ESR) but a number of more recent studies indicate that the two measures have only a limited correspondence (Bigelow, Heimbecker and Harrison, 1949; Ditzel, 1955; Gelin, 1959).

Knisely (1951) has shown a continuing dependence on direct in vivo observations of blood flow. He and his coworkers have emphasized that the blood flowing through any single artery is a statistically valid sample of all the blood in the body (Bloch, 1953; Bloch, 1956; Knisely, Bloch, Eliot and Warner, 1950; Knisely, 1951). Exceptions to this conclusion are to be expected only in cases of severe sludging where considerable settling of cellular elements may occur (Knisely, 1961). The observations on which this conclusion is based were made on several species and large numbers of animals, and the tissues studied included the intestinal mucosa, the mesenteries, liver and the surface of the brain; and it was concluded from these observations that "at all times the blood coming down the arterioles of uninjured bulbar conjunctiva is a

statistically valid sample of all the flowing arteriole blood in the body" (Knisely, Eliot and Bloch, 1945, p. 221).

It may be possible, however, that Knisely and his group never made simultaneous observations of both mesentery and conjunctiva during severe sludging. At least they do not state specifically that they had done so (Knisely, Eliot and Bloch, 1945). On the other had, Bloch (1956) reported finding no differences in the appearance of the blood flow between these two tissues in the rabbit, cat, dog or rhesus monkey while under nembutal anesthesia nor did he find any changes in blood flow in conjunctival vessels as a result of the anesthesia; but he made no mention of the condition of the blood in these animals at the time of the observation. It may be assumed that these were all "normal" animals since Heimbecker and Bigelow (1950) found from the simultaneous comparison of these two areas in the rabbit during severe induced sludging that in the rabbit "the degree of sludging in the conjunctiva and nicititating membrane was invariably more marked than that observed in the omentum and mesentery". A number of such comparisons made by the present writer on the rabbit and rat have tended to support Heimbecker and Bigelow's (1950) findings.

Yet if the objective were that of validating in vitro measures by in vivo observations then any differences in observed severity of sludging among the various tissues should be immaterial so long as the induced changes in level of aggregation vary in a consistent manner in any given tissue.

Actual determination of the condition of the blood via in vivo observation in the experimental animal presents a dual problem. The observation almost invariably represents a highly traumatic experience

for the animal, and it can be expected to affect the condition of the blood flow as a consequence, thereby at the same time setting up highly abnormal conditions for viewing the aggregation since changes in the flow rate appear to influence the observable level of aggregation (Ditzel, 1959; Hirschboek and Woo, 1950). Thus, any differences in handling the animals might be reflected in differences in the apparent level of in vivo aggregation. In addition, the actual observation is made difficult by the rapid breathing and the struggling to escape. The method is therefore seriously inadequate for use in the study of behavioral variables.

The use of an anesthetic might seem to offer a partial solution to the problem. However, there are indications that recovery from the anesthetic varies with the severity of the induced aggregation (Robbins, 1963-1964; Swank and Escobar, 1957). Further, successive observations would necessitate successive applications of anesthetic, with an increased probability of confounding.

The greatest problem, as David Long (1962) has recently pointed out, is met in trying to evaluate that which is finally observed. The usual approach has been that of using either four or five separate categories based on some criteria, presumably size of aggregates, which left a great deal to subjective judgment (Hirschboek and Woo, 1950; Odell, Aragon and Pottinger, 1947; Robertson, Wolf and Wolff, 1950). As Hirschboek and Woo (1950) have pointed out, the arbitrary standards used for in vivo quantification of sludge are "quite crude", and while the method may be of value when applied by a single observer it remains extremely difficult to make any kind of meaningful comparison among observers. Dissatisfaction with these highly subjective approaches promoted

development of a grading system for red cell aggregation which involved four categories of aggregation and which also took into account the location of the aggregation, that is, whether the clumping occurred in the venules or arterioles (Ditzel, 1955; Ditzel, 1959; Ditzel and Sagild, 1954).

It is felt, however, that in spite of the attention paid to the development of in vivo methods for evaluating sludge any such technique must involve a high degree of subjectivity.

One alternative to direct observation of the blood flow seems to be the use of blood smears. The presence of red cell aggregation has been determined by this method for many years (Fahraeus, 1929; Hunter, 1835; Jones, 1843; Lister, 1858; Norris, 1869). In fact, Jones (1843) even saw in the blood slide a means for estimating the sedimentation rate, since he pointed out that "in order to know if a patient's blood has a buffy tendency or not, it is sufficient to take a drop of blood from the finger tip, press it between two pieces of glass and observe whether or not it shows a dotted appearance. And just as there are differences in the degree to which the buffy coat is developed, corresponding degrees may be distinguished by the distinctiveness with which these dots make their appearance." (Jones, 1843, cited by: Fahraeus, 1929, p. 244). The "buffy layer" refers to the layer of fibrin which appears on top of the column of red cells when blood with a high ESR is allowed to stand so that the clumped cells settle to the bottom of the tube before clotting can occur (Fahraeus, 1929).

While there are no known reported instances of a complete dependence upon this method for the quantitative evaluation of red cell aggregation, a number of investigators have used the method to validate

observations made with other techniques (Fahraeus, 1929; Fahraeus, 1958; Gelin, 1956; Thorsen and Hint, 1950). Gelin (1956, 1962) compared blood flow in the conjunctiva of the rabbit with blood smears and found what appeared to be a close relationship. The comparisons were made before and after reversal of HmDx-induced sludging by means of infusion of LmDx. Fahraeus (1958), too, was impressed with the possibilities of this technique for the evaluation of the condition of the blood. Extensive preliminary work by the present author has indicated that the method may offer a higher degree of reliability and objectivity and simplicity than can be achieved by any other means. The validity of this assumption is demonstrated in the first of the present series of experiments.

Behavioral tests

The decision to use the rabbit brought with it the problem of defining suitable behavioral testing situations. While the rabbit has been highly recommended as a subject for behavioral studies (Zarrow, Sawin, Ross and Denenberg, 1962), most of the work dealing with rabbits has involved classical conditioning experiments (Fromer, 1963; Schneiderman and Gormezano, 1964) and observations of maternal behavior (Deutsch, 1957; Sawin, Denenberg, Ross, Hafter and Zarrow, 1960).

The neglect of the rabbit in behavioral research may be related largely to its apparent timidity. Fink (1954), who tested a single rabbit on his "Arrow" maze during a comparative study of maze performance which ranged from turtle to man, found the rabbit to show an extreme degree of initial timidity in the testing situation. However, in spite of the animal's initial reaction to the testing situation, its total performance in the maze placed it at a level between the rat and the cat.

More recently Livesey (1964) tested three rabbits (sophisticated) on a double alternation problem in the WGTA and found their overall performance to be comparable to that of cats tested under similar conditions (Stewart and Warren, 1957). Livesey (1965) also compared directly the performance of rabbits, rats and cats on the double alternation problem and again found the performance of the rabbit to compare favorably with that of the cat, while the rat required approximately twice as many trials to reach criterion of 80 per cent correct responses over 50 consecutive series of responses as did the rabbit or the cat. DeBaron (1962) successfully used rabbits in an instrumental conditioning situation in which the animals were required to discriminate between a continuous and an intermittent buzzer. Voronin and Napalkov (1959) have reported using the rabbit in conditioning response chains containing up to seven or eight links, with only the final link reinforced by the US (food). Jackson (1965) has conditioned chained responses in a number of rabbits intended for exhibition purposes.

No other studies involving conditioned behavior in the rabbit have been found. Consequently it was necessary to develop suitable testing situations for this animal which is not only exceptionally timid but extremely curious as well.

Part II. Measuring the Blood Sludge

An extensive series of preliminary observations had indicated that the appearance of the blood smear obtained and viewed under standard conditions varies in a consistent manner with the condition of the blood in vivo. Accordingly it was decided to verify this relationship by means of systematic comparisons among in vivo and in vitro observations

made under standard conditions. The relationship is demonstrated through a series of two experiments, each emphasizing different aspects of the problem.

Experiment 1 was designed to test the validity of the blood smear technique as a means for estimating the intravascular aggregation tendency of the blood. In vivo and in vitro photographic observations were made before and after treatment with saline, 77k dextran or HmDx. Comparisons among matching sets of these photographs showed that the condition of the smear varies in a consistent fashion with changes in the in vivo appearance of the blood. This experiment also provided an opportunity to make direct comparisons on the in vivo condition of the blood following each of the several treatments.

Experiment 2 was designed (a) to test the reproducibility of the results obtained with the blood smear technique for the evaluation of sludge, and (b) to provide standards for a blood smear rating scale. Sets of two blood smears each were obtained following successive fractional doses of sludge-inducing HmDx. Photographs obtained from two locations on each slide from each set of slides were compared for a demonstration of the reliability of the technique. Four photographs were chosen as standards to represent blood sludge levels ranging from no sludge (+1) to severe sludge (+4).

The two experiments are presented below.

Experiment 1. Validation of the blood smear technique by comparisons of in vivo and in vitro observations

Method

Subjects. - Four albino rabbits, approximately 20 weeks old and

weighing between 2500 and 2800 gms, were tested by means of blood smears for the absence of significant levels of blood sludge and assigned at random to four treatment conditions, the same conditions used throughout the entire series of experiments: normal control, saline-injected control, 77k dextran control and the HmDx-treated (sludged) condition.

Apparatus. - The motion pictures of blood flow were taken on Kodachrome II film at 64 frames per second using a 16 mm reflex camera and a Leitz ophthalmic binocular bi-objective microscope with a 12X objective and an 18X eyepiece. The tissue being photographed was transilluminated with a Knisely (1936) type quartz rod illuminator equipped with a 1000 watt light source and a water heat filter. The end of the 5/8 in. diameter quartz rod was placed 2 in. from the exposed tissue. Attached to the microscope stand was a paraffin lined cork board with a 2 cm. hole over which a section of the mesentery was pinned for observation. A small adjustable stand was used to hold the animal in position under the cork board. All observations were made with the microscope in the horizontal position, an observational technique highly recommended by Knisely and his coworkers (Knisely, Warner and Harding, 1960).

Blood smears were photographed on Ansco Hypan film with a Leica 35 mm camera on a tri-ocular microscope using a 40 X dry objective, a 10X eyepiece and a Mikas micro attachment with a 1/3 intermediate adapter. Areas of standard density were located on the blood smears with the use of a photometer and densitometer equipped with a 1.96 mm aperture.

Animals were anesthetized with hexabarbital sodium ("Evipal

sodium") dissolved in tap water to a concentration of 50 mg per cc and applied intravenously. The HmDx used for inducing the sludge was a 2,000,000 molecular weight dextran obtained from Pharmacia, Uppsala, Sweden. The 77,000 molecular weight dextran was obtained from Pharmachem Corp., Bethlehem, Pennsylvania. Both dextrans were prepared in 50 cc lots as 10 per cent solutions in normal saline and all solutions were autoclaved for 45 min. at 118° C. before use. Each lot was used for a single treatment only, and it was discarded and replaced if not used within 5 days. Both the treatments and the anesthetic were administered through a 23 gauge needle joined to a two-way valve by a 50 cm length of 0.023 polyethylene ("Intramedic") tubing. A constant temperature water bath was used to maintain all solutions at approximately the mean body temperature of the animal (40° C.)

Procedure. - Each animal was first anesthetized. A 10 cm area was shaved along the ventral midline where a 6 cm incision was made in a bloodless operation using surgical scissors. The animal was placed on the adjustable stand under the cork board attached to the microscope stand and a section of the mesentery suitable for observation and photography was pinned to the cork board with the desired field of view extending across the hole in the board. The exposed tissue was bathed continually with isothermic saline throughout the entire period of observation.

The schedule of treatments and observations was as follows. Ten feet of film was exposed as soon as camera and microscope were properly focused. The appropriate treatment was then administered during a 10 to 12 min. interval. An additional 10 ft. of film was exposed one hour

after the beginning of the treatment. During this one hour period the position of the microscope and camera remained unchanged, thereby permitting a comparison of treatment effects against a constant background. Blood smears were taken immediately after each film series and were coded for later processing.

Areas of standard density were located and marked for each of the smears. The blood slides were stained for 45 sec. using Wright's stain followed by application of buffer. After the slides were dry, 35 mm photographs were taken from the marked areas. A matching set of photographs representing in vivo observations was made by enlarging selected single frames from each of the 10 ft. sections of exposed 16 mm film.

Results

Acceptable motion pictures showing the condition of the blood before and after treatment were obtained for the three animals receiving infusions of the different solutions, but not from the normal control animal. Although normal blood flow was maintained in the untreated animal throughout the entire period as determined by direct microscopic observation of the mesenteric vessels, camera malfunction rendered the exposed film useless.

All blood smears taken from the three treated animals were successfully processed and photographed. These photographs, matched with enlargements obtained from the 16 mm film, are presented in Fig. 12 (Appendix) to permit (a) comparison of the in vivo condition of the blood and the appearance of the blood smear, and (b) comparisons of the condition of the blood following each of the several treatments.

Comparisons among these photographs show the following. All

photographs taken during the pretreatment period show a complete absence of blood clumping either in the vessels or on the smears. After treatment with saline neither observation indicated any change from pretreatment conditions. The posttreatment photograph for the 77k dextran treatment shows a slight aggregation tendency for the blood smear. The matching photograph (in vivo observation) for this condition shows increased streaking of the particles present in the blood stream, indicating an increase in rate of flow. The distribution and the size of these particles suggest no major change in intravascular blood sludge level following treatment. By contrast, both photographs for the posttreatment HmDx condition show a severe aggregation of the blood. The smear shows large formless masses in which the outlines of the individual cells are lost, while the in vivo observation reveals large cell masses separated by spaces containing only plasma. Also, the cell masses show a tendency to settle at the bottom of the vessel leaving only plasma at the top.

Discussion

Comparison of the in vivo and the in vitro observations on the condition of the blood before and after each of the three treatments revealed a close correspondence between the two sets of observations under all conditions studied. This close correspondence is regarded as a demonstration of the validity of the blood smear technique as a means for evaluating the in vivo aggregation tendency of the blood.

Both sets of photographs revealed major changes in the condition of the blood following treatment with HmDx but not after either of the other treatments. These changes consist of the presence of large cellular aggregates in the blood stream, dramatic slowing of blood flow and

settling of the cellular aggregates to the bottom side of the vessel.

This difference in the appearance of the blood which occurred between the HmDx condition and both controls is regarded as a demonstration of the adequacy of the present technique for the control of the independent variable, the level of blood sludge.

It should be pointed out that while only one animal was used for each of the three conditions in the present study, the results from these observations were found to be entirely consistent with those from a large number of preliminary observations made under comparable conditions.

Experiment 2. Demonstration of the reliability of the blood smear technique and development of a rating scale for evaluation of blood smears

Method

A single normal sludge-free unanesthetized male albino rabbit weighing approximately 2300 gms was given the standard 1 gm/kg body weight dose of HmDx in the form of ten equal injections at 12 min. intervals, a period of time considered sufficient to assure an essentially complete mixing of the blood following each injection (Pritchard, Moir and MacIntyre, 1955). The infusions were made into the marginal vein of one ear. Two blood smears were taken from the marginal vein of the other ear immediately prior to each injection to permit comparison of the condition of the blood smear with the magnitude of the cumulative HmDx dose. Each slide was coded for later identification. The two sets of slides were processed in the manner described in Experiment 1. Two points of standard density were identified on each smear. A single photograph was taken of each of the two locations on each smear from each set of slides. From the eleven sets of photographs representing all dose levels, four

sets were chosen to serve as standards for use in rating the blood smears taken from those animals serving as subjects in the several studies on the behavioral consequences of sludge.

Results

Inspection of the eleven sets of photographs taken from blood smears obtained during the course of the successive infusions of HmDx revealed a high degree of similarity among the four photographs of each set but major differences among the several sets.

The differences among the sets were found to vary consistently with HmDx dose. Beginning with (1) a random distribution of individual cells before treatment there is evident an increasing (2) clustering of cells into rouleaux, followed by the development of (3) networks of rouleaux masses interconnected by chains of rouleaux with the individual cells becoming more difficult to identify in the cell masses, until finally with maximum dose levels the interconnecting rouleaux formations become ragged and beaded or are missing altogether and the appearance of the smear is that of (4) formless isolated masses within which the outlines of the individual cells and of the rouleaux are lost.

Photographs most nearly representative of the conditions described above were found to be those obtained from smears taken (1) before treatment, (2) after 20 per cent of standard dose, (3) after 50 per cent of standard dose and (4) after 100 per cent of the standard dose of 1 gm of HmDx per kg body weight. These four sets of photographs, listed by order of increasing severity of blood sludge, are presented in Fig. 13. The other seven sets of photographs showed the same internal consistency found in the above four and they were excluded only to avoid overburdening

this report with an excess of detail.

Discussion

Each set of four photographs, representing two observations per smear from each pair of smears, were found to show an almost complete absence of variability while major differences occurred among successive sets of photographs. These findings indicate that (1) the appearance of the individual blood smear is highly uniform when thickness of smear is held constant, that (2) random variability among successive blood smears is very low when the aggregation tendency of the blood is held constant, and that (3) the reproducibility of the results using blood smears is independent of the blood sludge level at the time of the observation.

The four sets of photographs which were presented in Fig. 13 represent the four standard sludge levels for the blood smear rating scale. These levels might be described as normal, mild, moderate and severe and they are designated as sludge levels +1 through +4, respectively.

The use of only four categories of blood sludge maximizes interclass differences and should make the scale both simple to use and effective as a tool for the task which has been regarded as perhaps the greatest problem in research on blood sludge - the quantification of the aggregation tendency of the blood.

Part III. Behavioral Studies

With standardized techniques available for the production and measurement of blood sludge in the rabbit and with meaningful behavioral testing situations defined and modified for use with the rabbit, the exploration of the relationship between sludge and behavior was carried

out through a series of four separate experiments.

Experiment 1. Observations on the effects of HmDx-induced blood sludge on food intake, water intake, spontaneous home-cage activity and exploratory behavior.

Experiment 2. A study of the effects of blood sludge on response latency in a shock-escape learning situation.

Experiment 3. A study of the effect of sludging on learning behavior defined in terms of response latency and running time in a maze-runway situation.

Experiment 4. A study of the effect of dextran-induced sludge on performance on a bar pressing task under a DRL schedule using a 20 sec. minimum delay interval.

The results from these several experiments provided no evidence for any significant behavioral effects attributable to the presence of severe HmDx-induced sludge in the rabbit.

Experiment 1. The effects of dextran-induced intravascular aggregation of red blood cells on food intake, water intake, body weight, home-cage activity and exploratory behavior in the albino rabbit

Experiment 1 was intended primarily to test the hypothesis that severe blood sludge is associated with a significant decrease in levels of spontaneous activity. However, because of the scarcity of data relevant to the question of the relationship between sludge and behavior, the present experiment includes observations on several behaviors not directly related to the hypothesis but which may nevertheless vary with the level of sludge.

The possibility of major changes in behavior as a function of duration of the sludge pointed to the need for observing behavior over

an extended period of time following treatment with the HmDx. In addition, while the severity of the experimentally induced sludge was known to vary with time since treatment, no systematic observations had been reported on the time course of the change. These two considerations suggested that the study of the behavioral effects of sludge should begin with a series of observations on both the dependent and the independent variables covering a period of several days before and after the sludge treatment.

Experiment 1 was designed to provide for the continuous monitoring of a number of variables which were expected to vary as a function of sludge. All observations were made over a 6 day period beginning 2 days before treatment. The observations included (1) food intake, (2) water intake, (3) body weight, (4) home-cage activity and (5) exploratory behavior. Coincident observations were made on the condition of the blood over the course of the 6 day period using the blood smear technique for measurement of the severity of the sludge.

Method

Subjects. - Twenty young albino rabbits (obtained from Holsenbeck's Rabbitry, Jacksonville, Florida), weighing between 1500 and 2500 gms, were tested for absence of red cell aggregation and assigned to five replications of four animals each on the basis of body weight. Within each replication the rabbits were assigned at random to four groups: Group I, normal controls; Group II, saline-injected controls; Group III, 77,000 molecular weight dextran-injected controls and Group IV, the group receiving infusions of the sludge-inducing high molecular dextran (HmDx).

Apparatus. - The observations on several aspects of behavior required the development of the following apparatus.

Activity cages. - The four individual cages housing the animals during testing were 10 in. wide by 12 in. high by 40 in. long, with sides, ends and the hinged tops made of 1/4 in. hardware cloth and the floors of 1/2 in. hardware cloth. Two standard glazed pottery food and water containers (5 in. diameter by 3 in. deep) were placed side by side at one end of each cage. The end of the cage was extended over the dishes to allow free access to the dishes by the animals from the inside and easy removal by the experimenter from the outside. The animals were given Purina Rabbit Chow and tap water.

Home-cage activity measurements were obtained with a transistorized (Silicon controlled switch, G. E. 2N58) photoelectric relay device activating an Esterline Angus event marker recorder operating continuously at a chart speed of 3 in. per hour. The photobeam controlling the photosensitive resistor (Clairex, 1 megohm) crossed the cage 3 in. above the level of the cage floor and 9 in. from the end of the cage opposite to the food and water dishes. Interruption of the light beam triggered the transistor and activated a recorder pen. Once the transistor had been triggered, the pen circuit became independent of the photo-resistor and could be inactivated only by interruption of the pen circuit itself. A narrow (5 in. wide) section of the cage floor located between 10 and 15 in. from the end of the cage containing the food and water dishes was hinged at its inner edge and made movable across a 0.5 mm arc at its outer edge to permit operation of a nearly silent microswitch which interrupted the recorder pen circuit when the section of

cage floor was depressed. The apparatus thus provided a virtually foolproof system for the measurement of home-cage activity: when the animal moved to the rear of the cage it activated the pen circuit which then remained on until the animal moved to the front of the cage and interrupted the pen circuit by depressing the floor section and opening the microswitch.

Each cage was suspended between 2 in. high "runners" over a litter pan spread with Pel-E-Cel. The cages were placed side by side in individual compartments made from 1/4 in. plywood. These compartments were 48 in. high by 60 in. long by 16 in. wide. A single door of 1/4 in. plywood permitted access to all of the cages at once.

Two levels of illumination were provided by a single 7 watt frosted bulb and one 40 watt bulb at the top center of each compartment, with the two levels controlled for all four compartments by a single s.p. d.t. switch accessible from the outside.

"Square maze". - The observations on exploratory behavior were made in a four compartment "square maze", a unit functionally similar to the circular maze used by Gwinn (1949) on rats. This unit was 33 in. square by 14 in. high and stood on 8 in. long legs. Its panels were of 1/4 in. plywood and painted gray. Each of the four compartments was 16 in. square by 14 in. high measured from the top of the removable hardware cloth floor section. Square doorways, equipped with sliding panel doors of 1/4 in. plywood, were located between each two adjacent compartments. These doorways were 8 in. square, and reached to within 2 1/2 in. of the tops of the floor sections. The sliding doors were so placed that they were always behind the panel as the S moved in a clockwise direction from one compartment to the next. The wire cloth floor

sections were 3/4 in. narrower than the compartment at each side and were suspended only by their corners. Each compartment had a separate hinged cover of 1/16 in. screening framed with 3/4 in. by 3/4 in. wood, with a layer of cheesecloth above the screen to minimize the possibility of the animal seeing beyond the limits of the apparatus. A single 7 1/2 watt bulb was placed at the center of each screened cover to provide individual lighting for each compartment. Separate pull strings were attached to the sliding doors between the compartments, but all doors were connected to a single switch which controlled the operation of an electric timer such that raising any or all doors activated the timer while all doors had to be lowered before the timer circuit switch opened.

Rabbit holder. - In this experiment as in all other experiments described here, a V-shaped stand was used to hold the animal while treatments were being administered or blood smears taken. This stand was 18 in. long with sides 6 in. wide and made of 1/4 in. plywood. A barrier was used at one end only, and the animal was placed with its head directed toward the unobstructed end of the unit. When this stand was placed on a table with the open end slightly beyond the end of the table a restless animal placed in the device would take one look at the floor and retreat until it backed up to the barrier at the other end, while the more timid one would back up to the barrier at once and stay there. The unit was found to be extremely effective, and no major difficulties were experienced in treating or testing any of the large number of rabbits used in the several experiments reported here or in the many exploratory studies which preceded the work discussed in this report.

Dextran treatments. - The high molecular weight dextran (2,000,000 molecular weight) was obtained from Pharmacia, Uppsala, Sweden, and the 77,000 molecular weight dextran from Pharmachem Corp., Bethlehem, Pennsylvania. All solutions were made fresh weekly as 10 per cent solutions in normal saline and autoclaved for 45 min. at 118° C. before use. All infusions were made at the rate of 10 cc per kg body weight, thereby providing for a dextran dose of approximately 1 gm per kg body weight. These solutions were administered into the marginal ear vein through a 2 cm length of a 23 gauge needle inserted into the end of a 40 cm length of .023 in. polyethylene tubing whose other end was attached via a 23 gauge needle to a B & D two-way valve. This arrangement permitted the use of a large (20 cc) syringe as a reservoir with a smaller (1 cc to 5 cc) syringe providing the necessary infusion pressure for the high viscosity dextran solutions. A constant temperature water bath maintained the solutions and syringes at approximately the mean body temperature of the rabbit (39.7° C.).

Procedure. - At least four animals (one replication) were brought into the laboratory at one time. Immediately upon arrival each animal was weighed and a blood smear taken to determine the aggregation tendency of the blood. Any animal showing an abnormal condition of the blood as defined by the condition of the blood smear was replaced at once. The four animals chosen for each replication were then placed into individual cages in the laboratory and provided with free access to food and water during an adaptation period of at least three days.

At the end of the adaptation period, and beginning at 7 p. m., blood smears and body weight measurements were obtained, and the animals were assigned at random to the four experimental cages within the

plywood compartments. These weighings and blood smears were repeated at 12 hour intervals for the duration of the 6 day observation period which was standard for all replications. Daytime lighting (7 a.m. - 7 p.m.) for the compartments was provided by the 40 watt lamps and the 7 p.m. to 7 a.m. lighting by the 7 watt lamps.

After a 48 hour standardization period the animals were assigned at random to the four experimental conditions and the various infusions were given immediately following the taking of the blood smears. Only one infusion was given to any one animal within each replication. Observations were continued at 12 hour intervals until the end of the sixth 24 hour period for a total of 4 days of posttreatment observations. At the end of this period the four animals were removed from their experimental cages, a final series of blood smears and body weights taken and the cages thoroughly cleaned and scrubbed preparatory to beginning the next replication.

For observations of food and water intake during the 6 day period covered by each replication, the food and water containers and their contents were weighed at the beginning and at the end of each 12 hour period. The differences between these two measures were taken as the amounts consumed. Control was provided for evaporation loss. Fresh food and water was supplied for each 12 hour interval.

Because of the number of different measures obtained during these 12 hour intervals, the handling procedures will be described in detail. Measures of exploratory behavior were obtained as follows. All lights except those in experimental cages and in the square maze were shut off. The single large door of the experimental compartments was raised. The animals were taken from their cages one at a time, carried to the square

maze and placed carefully into compartment 1. After a 10-sec. delay all doors of the maze were opened at the same time and kept open for 60 sec. During this time the animal was free to move from compartment to compartment and his movements were traced out by E on diagrams of the maze. These diagrams served as the basic data for the observations on exploratory behavior.

As each animal was tested in the square maze it was placed in a temporary cage to await completion of the entire test series. Next, body weights and blood smears were obtained, with the animals being handled in the same order as previously. The animals were again returned to their temporary cages while food and water containers were weighed and refilled and the litter pans under the experimental cages cleaned and washed (a.m. only). Following these operations, and at the end of 1/2 hour after the opening of the door to the experimental cage compartments, the animals were returned to their respective cages to remain undisturbed for another 11 1/2 hours. The 1/2 hour period which was allowed for the observations and handling was seldom exceeded except when the various injections were given in addition to the regular handling.

Results

The group mean results for the six different measures (including sludge ratings) obtained in Experiment 1 are presented separately in Tables 1 through 6 and are shown graphically in Figs. 1 to 6. Results for individual animals for all observations are presented in Tables 14 through 19 in the Appendix.

Food intake. - Group mean values for food intake (in gms) for

each of the four groups and for each 24 hour interval of the 6 day observation period are shown in Table 1 and Fig. 1. It is evident from Fig. 1 that the largest drop in food intake on the first posttreatment day occurs following treatment with the sludge-inducing HmDx. However, by the second day following treatment the mean food intake for this group rose to a level almost identical to that for the other two treated groups. Further, not all animals in the HmDx-treated group contributed to the initial posttreatment fall in food intake levels. Food intake for one of the animals in this group actually increased following sludging (see Table 14), while only two animals showed a considerable decrease at this time.

Table 1

Mean Daily Food Intake (gms) During 2 Days Before and for 4 Days After Treatments with Five Animals per Group

Treatment group	Before treatment		After treatment			
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	92	108	102	109	111	111
Saline	95	96	89	91	104	106
Dextran	97	100	100	91	94	102
HmDx	98	106	85	92	104	109

In summary, mean daily food intake for the HmDx-treated group fell to below control group levels for the first posttreatment day only.

Water intake. - The results from the measurement of water intake by the four treatment groups during the 6 day observation period are shown as mean values in Table 2 and are presented graphically in Fig. 2.

Table 2

Mean Daily Water Intake (gms) During 2 Days Before and for 4 Days After Treatments with Five Animals per Group

Treatment group	Before treatment		After treatment			
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	165	192	191	174	179	172
Saline	180	169	169	172	194	166
Dextran	228	243	243	274	232	231
HmDx	215	201	201	165	200	188

Individual results are found in Table 15. Fig. 2 shows that the patterns of water intake for the dextran-treated groups are similar in that both show a fall in water intake immediately following the injections and a rise by the second posttreatment day. However, evaluation of these results is complicated by the fact that levels of water intake exceeding 500 per cent of "normal" were reported for one of the animals from the dextran control group. Therefore, the results for the HmDx-treated animals should be compared only with the results for the normal and saline groups. This latter comparison shows that the mean water intake for the HmDx-treated animals returned to approximately normal values by the second posttreatment day and continued to rise in linear fashion to a maximum on the third posttreatment day.

Body weight. - The results from the twice daily measurements of body weight are presented in Table 3 as group mean values for each of the 6 days of observations. The table also includes the average beginning body weights for the four groups.

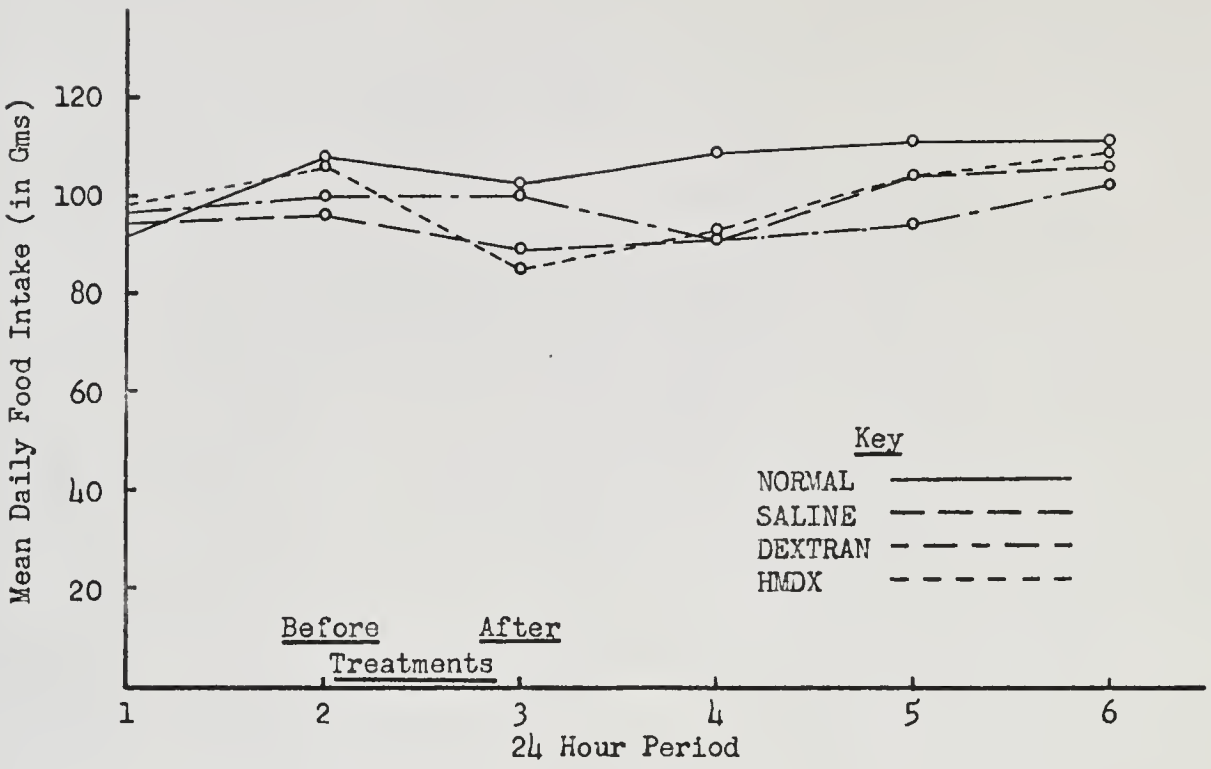


Figure 1. Mean Food Intake per 24 Hours for Each of Four Groups of Albino Rabbits with Five Animals per Group.

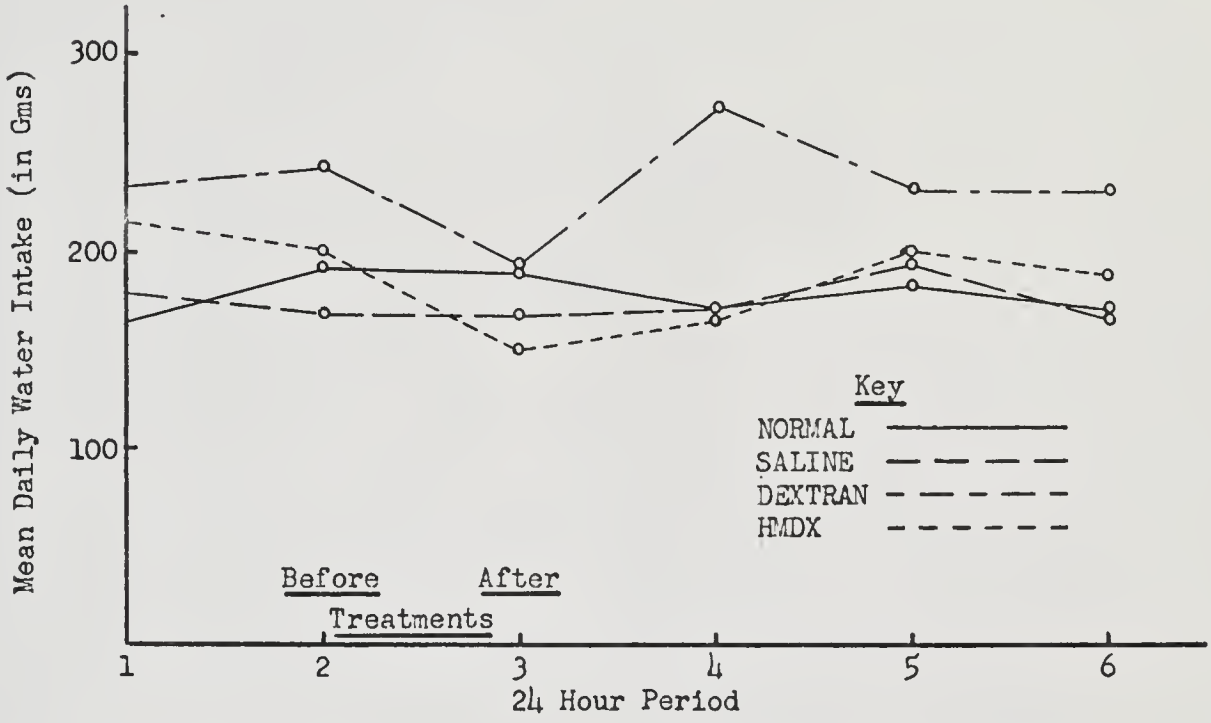


Figure 2. Mean Water Intake per 24 Hours for Each of Four Groups of Rabbits with Five Animals per Group.

Table 3

Mean Body Weight (gms) During 2 Days Before and for 4 Days After Treatments with Five Animals per Group

Treatment group	Before treatment			After treatment			
	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	2058	2074	2085	2106	2122	2146	2172
Saline	2121	2105	2128	2148	2169	2199	2228
Dextran	2151	2145	2159	2161	2182	2217	2258
HmDx	2153	2169	2189	2204	2222	2245	2284

These group results are shown graphically in Fig. 3 to facilitate inter-group comparisons. Fig. 3 shows that following treatment the mean body weight for the dextran-treated animals showed a fall in the rate of weight increase from which the animals did not recover until the fourth day after treatment. On the other hand, those animals treated with the high molecular weight dextran showed no change in rate of weight increase during the posttreatment period.

Home-cage activity. - Home-cage activity was defined in terms of the number of round trips made by the animal in its long narrow cage during a given period of time. Measures of this behavior were obtained directly from the chart of the Esterline Angus event marker recorder. Results for individual animals for 12 hour and 24 hour intervals are reported in Table 17 (Appendix). Group means values for successive 24 hour periods of the 6 day observation period are presented in Table 4 and Fig. 4. As shown in Fig. 4, group mean differences are minimal for the 24 hour period immediately prior to the treatments and for each of the 4 days of the posttreatment period.

Table 4

Mean Home-cage Activity (pacing) During 2 Days Before and for 4 Days After Treatments with Five Animals per Group

Treatment group	Before treatment		After treatment			
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	93	53	69	57	52	51
Saline	109	57	57	48	51	43
Dextran	132	62	61	53	48	58
HmDx	114	65	51	58	52	47

Table 17 indicates that while two of the five HmDx-treated animals showed substantial decreases in level of activity for day 3 as compared to day 2, two of the animals from the dextran control group showed similar changes in activity levels. In summary, these results offer no evidence for any changes in activity level following treatment with HmDx.

Exploratory behavior. - Results of observations made on exploratory behavior during the course of successive 1 min. exposures to the square maze situation are presented in Table 5 as group mean values shown separately for each of the 6 days of observation. These results are presented graphically in Fig. 5. Individual scores for each testing session are reported separately in Table 18. Fig. 5 reveals that the saline group and the HmDx group showed almost identical performance for each of the 6 days of observation, while both of these groups showed a considerably higher level of activity in terms of mean number of compartments entered than did normal or dextran control animals.

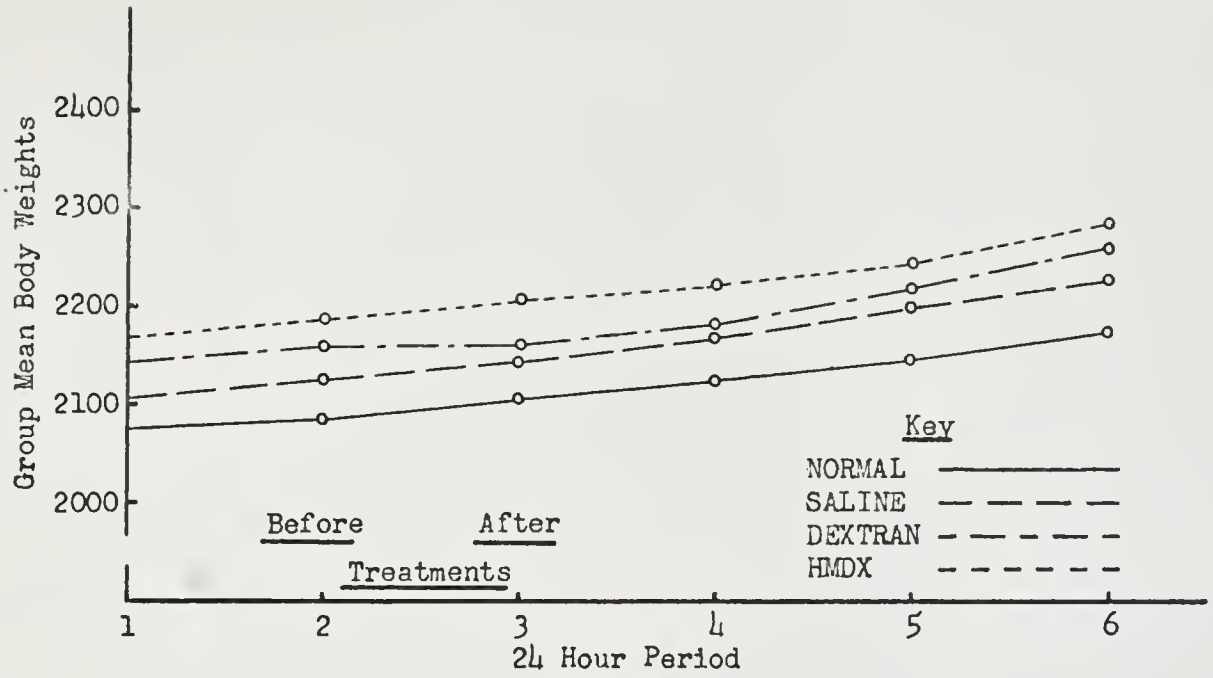


Figure 3. Mean 24 Hour Body Weights for Each of Four Groups of Five Albino Rabbits During 6 Days of Observation with Treatment Given at the End of 2 Days.

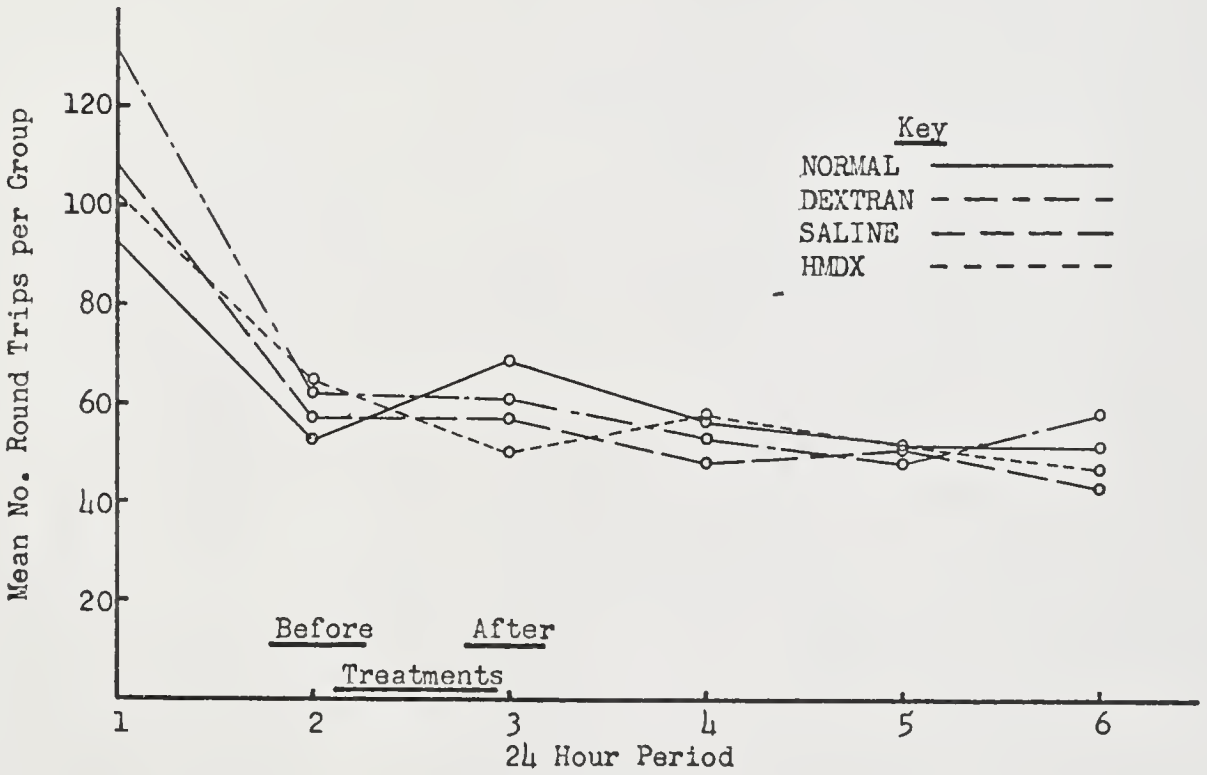


Figure 4. Mean Home-cage Activity (No. Round Trips) for Four Groups of Five Albino Rabbits Before and After Treatments During the 6 Day Observation Period.

Table 5

Mean Exploratory Behavior During 2 Days Before and for 4 Days After the Treatments with Five Animals per Group

	Mean no. compartments entered per two 1 min. test periods					
	Before treatment			After treatment		
Treatment group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	7.4	5.8	5.2	4.4	3.8	2.4
Saline	7.2	9.2	7.2	5.6	3.4	3.0
Dextran	7.2	6.8	4.4	2.4	2.6	3.2
HmDx	6.8	9.2	6.8	5.6	4.8	3.4

In spite of the size of the differences among the treatment means, no formal analysis of the results was carried out because of the obviously large within group variability and the skewness of the distributions of individual scores (see Table 18).

Blood sludge levels. - Mean daily sludge ratings based on observations (blood smears) taken at 12 hour intervals during the experiment are presented in Table 6 for each of the four treatment conditions.

Table 6

Mean Blood Sludge Levels During 2 Days Before and for 4 Days After Treatments with Five Animals per Group

	Blood smear ratings, lowest (+1) to highest (+4) sludge levels					
	Before treatment			After treatment		
Treatment group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	1.0	1.0	1.0	1.0	1.0	1.0
Saline	1.0	1.0	1.0	1.0	1.0	1.0
Dextran	1.0	1.0	1.0	1.0	1.0	1.0
HmDx	1.0	1.0	4.0	3.8	3.0	2.2

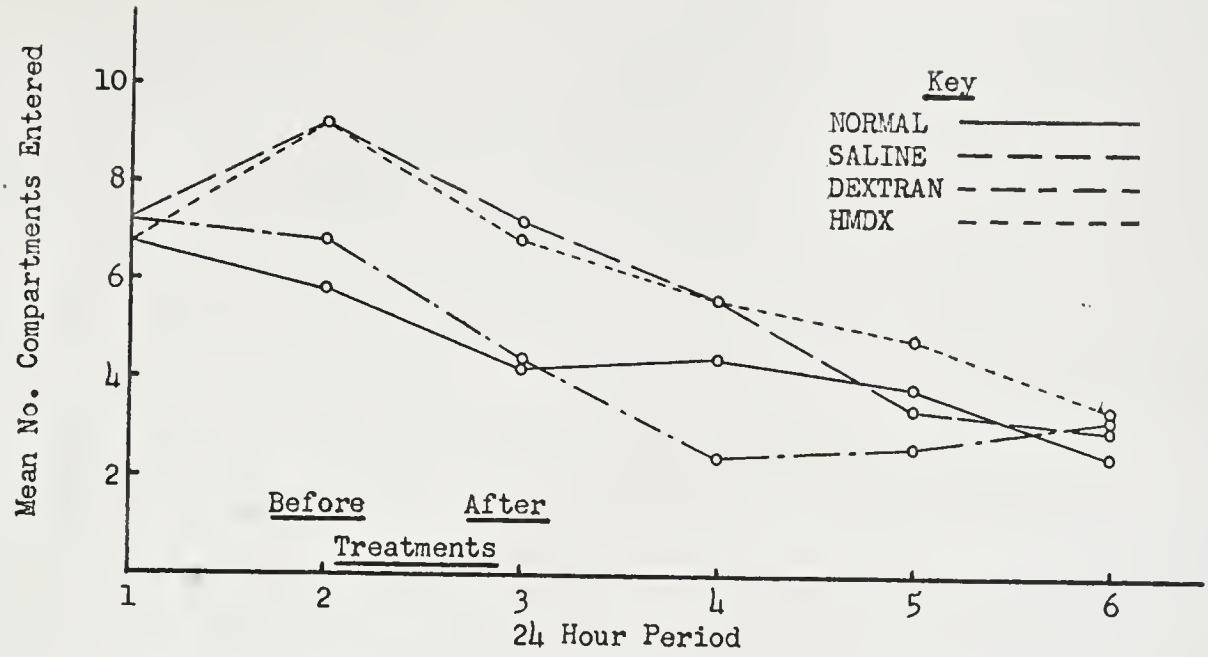


Figure 5. Mean Number of "Square Maze" Compartments Entered by Four Groups of Five Albino Rabbits Tested Daily at 12 Hour Intervals Over the 6 Day Period Beginning 2 Days Before Treatments.

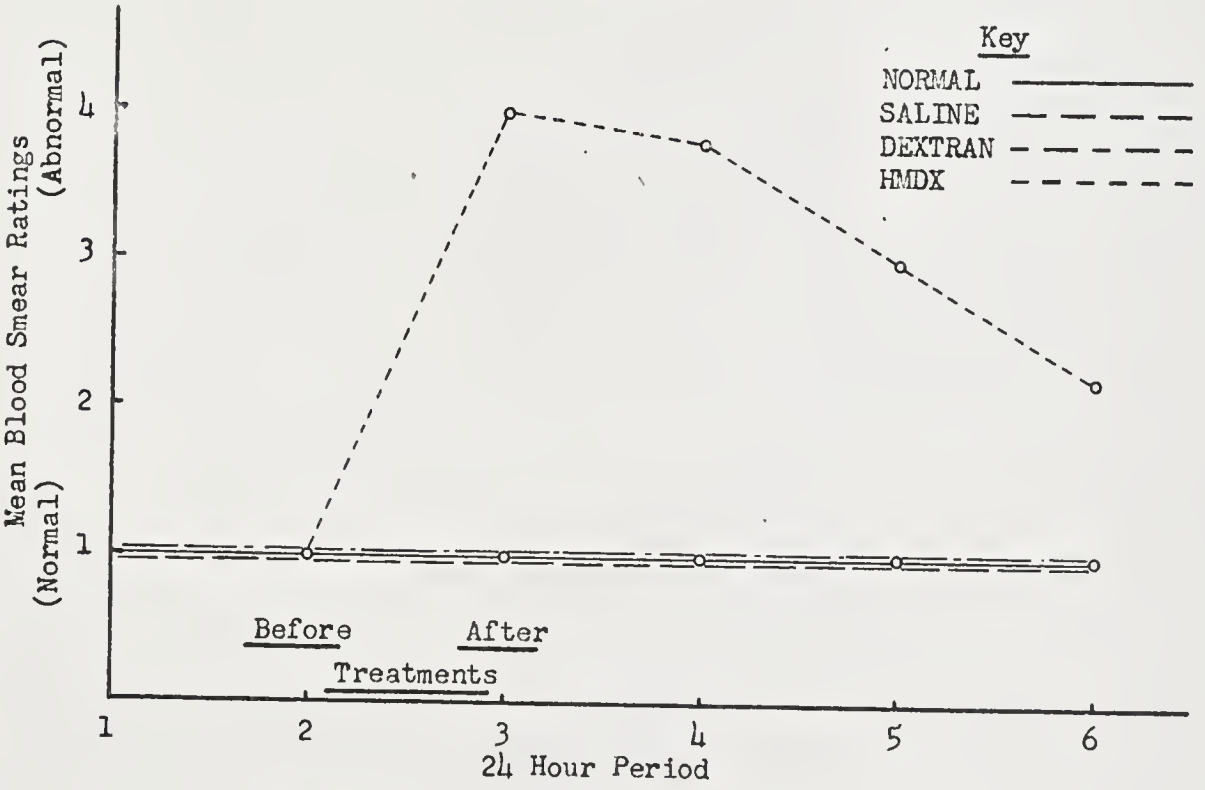


Figure 6. Mean Blood Sludge Levels During 2 Days Before and for 4 Days After Treatments for Four Groups of Five Rabbits.

Individual observations are reported in Table 19. These sludge ratings indicate a high degree of reliability for the technique since normal blood is reported for all animals and all conditions except for the HmDx-treated animals following treatment.

Further, the course of recovery from the sludging effect of the HmDx seems to be both gradual and uniform among all animals used in the present study. The almost total absence of random variability in the results obtained with the use of the blood smear technique for evaluating the aggregation tendency of the blood might appear to indicate the presence of bias in measurement. Accordingly it seems appropriate to point out that the technique used here offered protection against bias, first, through the use of a coded marking system which effectively concealed the identities of the individual slides, and, second, through randomizing the order in which the blood smears were rated. The results of these observations indicate that the blood sludge induced through the rapid infusion of 1 gm of HmDx remains severe for at least 2 days following the infusion after which it begins to improve until by the end of the fourth day after treatment most animals show only mild clumping and rouleaux formation.

Discussion

The results from the various observations made on the four groups of rabbits before and after the several treatments suggest that, while a modest and short-lived reduction in food and water intake may follow the onset of severe red cell aggregation in the animal treated with high molecular weight dextran, such reduction is not associated with a fall in the rate of weight change. Further, the reduction could not be a

universal effect since not all animals showed it. The transient fall in water intake was succeeded by an above normal increase in water consumption. This phasic response in water intake may be related to the plasma expanding characteristic of the dextran, and so the difference in rate of recovery from the mild depression in rate of water intake may be a function of the rate of loss of the dextran from the body. Thus the effect upon water intake may be largely independent of the condition of the red cell aggregation while the food intake should be presumed to follow water intake and may also be independent of the sludge. Impressive evidence for the independence of the changes in levels of water intake and severe red cell aggregation is to be found in the observation that the pattern of water intake for the HmDx-treated group in the present experiment appears to coincide with the changes in urinary excretion levels which Gelin (1956) reported for the rabbit following the 1 gm/kg dose of HmDx. As noted earlier in this report, Gelin found that the urinary output on the first posttreatment day almost stopped altogether. Then it began to rise gradually, until by the third day it had nearly reached pretreatment levels.

The results from the observations on activity levels also indicate the absence of any treatment effects associated with the blood sludge per se. Spontaneous home-cage activity levels as determined by the number of times an animal traveled the length of its cage and returned was found to be nearly equal for all treatment conditions for each day of observations.

With respect to the measures of exploratory activity the results were also negative in spite of the fact that consistent differences were found to occur among the several treatment means over the course

of the observation period. The HmDx and the saline groups showed comparable changes in levels of exploratory activity as a function of time, and these levels were consistently higher than those for the other two groups. However, these relationships among the group means developed before the treatments were given and they showed little change during the immediate posttreatment period. Also, the group results showed large within group variability and abnormal distributions of individual scores. While these two sets of observations, that of group mean changes independent of treatments and that of the large random inter-subject variability, may have discouraged a formal analysis of the results, they should permit the conclusion that if HmDx has any effect on exploratory behavior that effect is not toward the suppression of such behavior.

Summary and conclusions

Observations were made on patterns of food intake, water intake, weight changes, home-cage activity levels, exploratory behavior and blood sludge levels in young male albino rabbits before and after treatment with high molecular weight dextran to induce severe red blood cell aggregation (sludge). Controls were provided for the effects of the saline and of the dextran per se. The blood sludge resulting from the 1 gm/kg body weight dose of HmDx was found to remain severe for at least 2 days following treatment and to improve gradually thereafter, but no behavioral consequences could be related to the presence of the severe sludge.

Experiment 2. The effects of sludge on shock-escape learning in the albino rabbit

The generality of the negative findings from the previous experiment was seriously restricted through the concentration on the study of simple maintenance behaviors. This observation pointed to the need for sampling a broader range of behaviors. The present experiment represents a first step in the study of the effects of blood sludge on behaviors of increasing complexity. This first step involved a simple shock-escape conditioning situation in a beginning effort to define the effects of severe blood sludge on learning.

Two problems were met in developing a testing situation for use with the rabbit. First, the hair pads on the animals' feet offer a high electrical resistance. This problem was resolved by using a high voltage shocker. Second, the rabbit, or at least the strain used in the present series of studies, shows a strong tendency to crouch and to lick its feet in the presence of shock rather than to seek escape from the situation. The results obtained from six replications of this experiment clearly reflect the failure to fully resolve this problem.

Method

Subjects. - Twenty-four albino rabbits (M. E. Holsenbeck's Rabbitry, Jacksonville, Florida) were obtained and handled in the same manner as described for Experiment 1. These rabbits provided six replications with animals matched for weight and aggregation tendency of the blood within each replication but with treatments (normal, saline, 77k dextran and HmDx) assigned at random within each replication. Body weights of the animals ranged from about 1500 to 2500 grams.

Apparatus. - The testing situation used here was the square maze described in Experiment 1, Part II, but with several additional features which were required because of the use of the shock. The "shocker" unit, adapted from the one described by Licklider (1951), was a standard 12 volt automobile ignition system operated by a thyratron-controlled 110 volt electric motor to provide a pulse rate of approximately 15 per second. The high voltage output of the system was estimated to be over 20,000 volts. A phototimer was used to provide a 2 sec. delay interval between the opening of the compartment door and the activation of the "shocker". The phototimer controlled a relay in the primary circuit of the shocker unit. The system operated as follows: raising any door of the square maze activated an electric stop-clock and the phototimer. As the door remained open beyond the 2 sec. delay period, the phototimer relay circuit was energized, thereby causing the contacts of the relay in the primary circuit of the shocker to be closed, thus activating the primary circuit of the shocker and producing the high voltage pulses at the output of the ignition coil. This high voltage output was fed through a high voltage 4-contact rotary switch and separately to the hardware cloth floor sections of each of the four compartments of the square maze. The wiring used here was standard high tension automobile ignition wire. Capacitance of the high voltage system was increased by the addition of a 4 in. wide strip of 0.5 mm thick aluminum along the two outer walls of each of the individual compartments of the maze. The high voltage system was grounded to these strips.

The preparation and the composition of the various solutions used in treating the animals and the techniques and materials used for the infusions and blood smears were the same as for Experiment 1.

Procedure. - Following the prehandling and an extended period of adaptation to the laboratory as described under Experiment 1, the four animals of each replication were assigned at random to the four treatment conditions. In addition, the treatment and testing order for each replication was randomized and all handling during the experimental period was performed in the same order.

On the day of testing each animal was removed from its cage, weighed, tested for aggregation tendency of the blood (Smear A), given the assigned treatment and returned to its cage. Then, 1 hour after the beginning of the injection the animal was placed into compartment 1 of the test apparatus. (Only the lights in the apparatus were on at this time and daylight was maximally excluded from the room.) Ten seconds later one of the two doors of the compartment opened. The opening of the door caused the switch controlling the stop clock and the phototimer to be closed, thus initiating the 2 sec. delay period for the shock being applied to the floor of the compartment containing the animal. Successful escape from the shock required the animal's jumping through the open door into the adjoining compartment. The door was released as the rabbit's hind legs touched the floor of the next compartment. Since lowering the door also opened the switch controlling the timers, this act also provided the end point for the measurement of response time.

Testing consisted of three series of three trials each, with 10 min. between series and 10 sec. between trials. By directing the shock always to the compartment containing the animal and by raising the compartment doors successively in a counterclockwise direction the three trials of each session moved the animal from compartment 1 to compartment 4. Finally, 10 sec. after the end of trial 3 the cover of

compartment 4 was raised and the animal gently lifted out and returned to its cage. A total of three blood smears was obtained from each animal; one before treatment (Smear A), another before testing (Smear B) and a third after testing was completed (Smear C). This approach permitted an effective monitoring of the conditions of the blood throughout each phase of the experiment. All blood smears were rated in a random order using the method and the 4-point rating scale described under Experiment 2, Part II.

Results

Combined results for the six replications of four animals each are presented as group mean values in Table 7 and 8. Table 7 shows the group means for first trials for each of the three successive 3-trial series. Group means for combined trials for each series are presented in Table 8. Standard deviations are included. Results for individual animals are found in Table 20 (Appendix).

Table 7

Mean Response Latencies (in sec.) for First Trials from Three Successive Series of Three Trials Each for Four Groups of Albino Rabbits in a Shock-escape Situation with Six Animals per Group

	First trials					
	Series 1		Series 2		Series 3	
Treatment group	Mean	SD	Mean	SD	Mean	SD
Normal	62.8	61.0	10.9	6.9	10.4	6.0
Saline	99.6	84.1	39.9	73.2	34.9	62.9
Dextran	62.7	70.6	21.7	25.6	12.3	13.8
HmDx	44.2	64.4	12.2	15.8	8.8	7.3

Table 8

Mean Shock-escape Latencies (in sec.) for Combined Scores for Three Successive Series of Learning Trials with Six Animals per Group

Treatment group	Series 1		Series 2		Series 3	
	Mean	SD	Mean	SD	Mean	SD
Normal	101.8	72.5	31.4	13.5	29.8	10.0
Saline	130.6	86.4	91.6	146.1	109.9	188.2
Dextran	137.6	145.7	51.7	54.5	41.7	50.0
HmDx	107.8	115.3	33.8	11.3	26.2	11.0

The mean values found in Tables 7 and 8 are presented graphically in Figs. 7 and 8, respectively. Both reveal typical learning curves for all treatment conditions with the exception of the saline group from Fig. 8. The U-shape of the learning curve in this one instance is the result of extremely long latencies shown by a single animal during the third series of trials (see Table 20).

Comparisons among group performance levels as shown in Fig. 7 and Fig. 8 show a close correspondence between changes in response latencies as a function of trials for the normal and the HmDx (sludged) group. Even the ranges of the individual scores for both the first trials of each series and for series totals are comparable. Intergroup comparisons involving the saline and the dextran control groups are complicated by the presence of one extremely high scoring animal in each of these two groups. However, the results for the remainder of the animals from each of these two control groups seem to compare favorably with the group values shown for the normal and the HmDx-treated group.

Blood sludge ratings for each of the three blood smears (Smears A, B and C) are shown separately for individual animals in Table 21

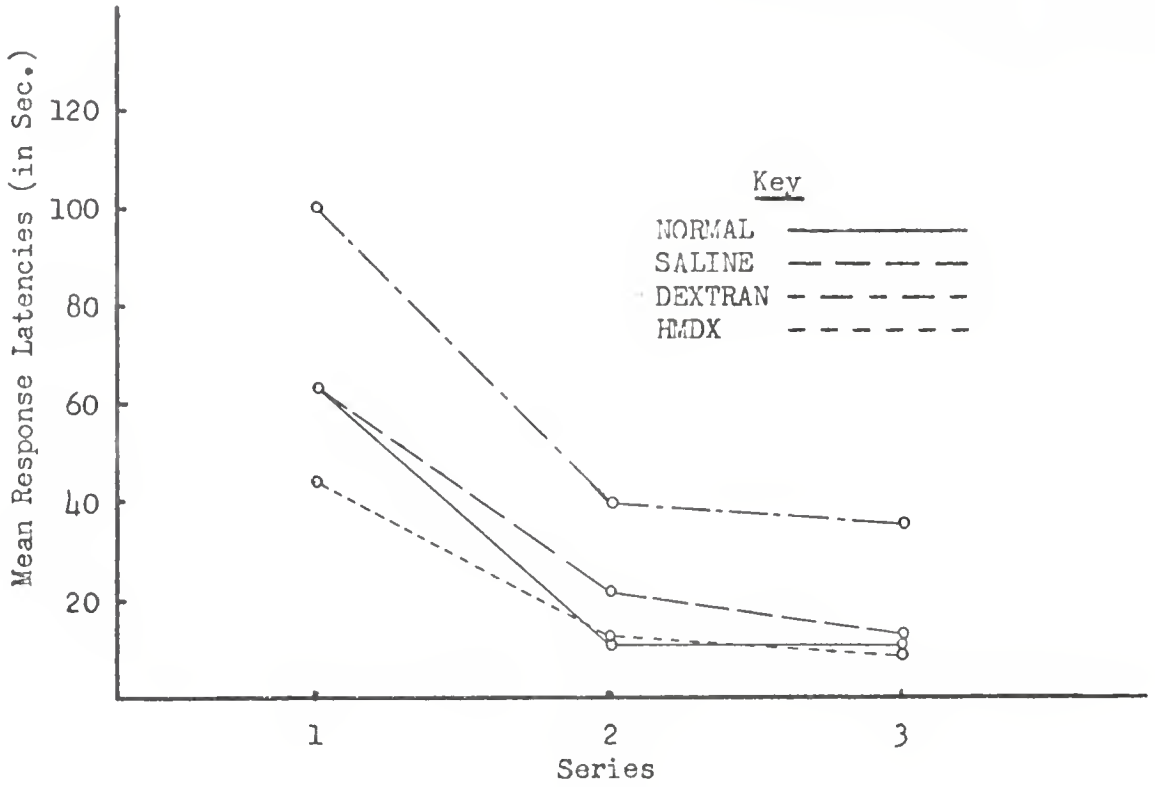


Figure 7. Mean Response Latencies for First Trials for Successive Series of Shock-escape Learning Trials

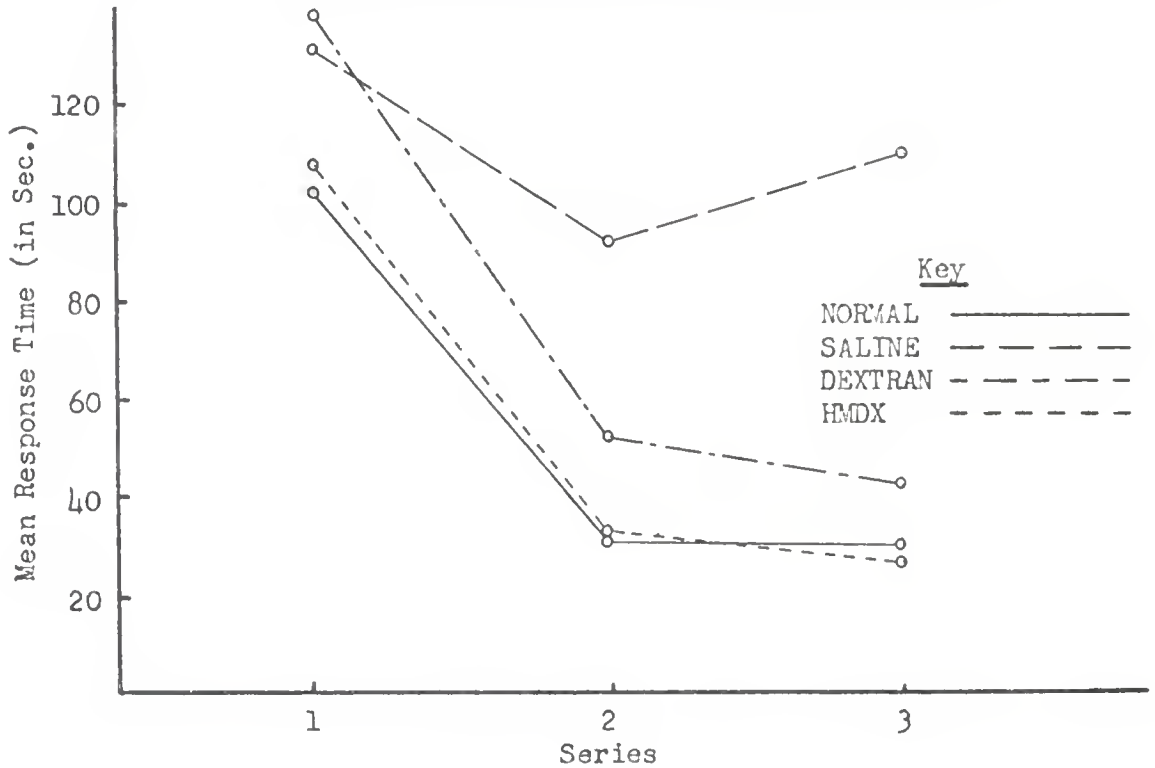


Figure 8. Mean Total Response Latencies (in Sec.) for Four Groups of Six Albino Rabbits for Each of Three Successive Series of Three Trials Each in a Shock-escape Learning Situation.

and are presented in summary form as group mean values in Table 9.

Table 9

Group Mean Blood Smear Ratings Before (Smear A) and After (Smear B) Treatments and After Shock-escape Conditioning Trials (Smear C) with Six Animals per Group

	Sludge ratings: lowest (+1) to highest (+4)			
Smear	Normal	Saline	Dextran	HmDx
A (pretreatment)	1.0	1.0	1.2	1.0
B (posttreatment)	1.0	1.0	1.3	4.0
C (posttesting)	1.0	1.0	1.2	4.0

Table 9 shows that the blood was essentially free of any aggregation tendency in all groups prior to treatment and remained so following treatment in all control groups, whereas severe aggregation resulted in the HmDx-treated group following treatment. Of the 24 rabbits used in the present experiment only the dextran-treated rabbit from replication 2 showed any pretreatment sludge tendency of the blood (sludge level + 2) as measured by the blood smear technique, and this same level of sludge tendency remained constant throughout the period of behavioral observations. The dextran control animal from replication 5 also showed some sludging tendency of the blood (sludge level + 2), but only during the immediate posttreatment period. These two animals also showed lower response latencies for trial series 2 and 3 than did the other four animals of the same treatment group.

Discussion

The high degree of correspondence between the means and variances from each of the three series of test trials for the normal control group

and the HmDx-treated group showing severe sludge as determined by the blood smear technique offers strong evidence that learning as measured under the conditions of the present experiment is not seriously affected by the sludge. Neither is there evidence of any major changes in reaction times, particularly in the direction of increased latencies as would be predicted by an hypothesis relating blood sludge to cerebral anoxia, a condition which should be expected to promote a stuporous state (Levy, 1920). In fact, not only did the sludged animals show on their first exposure to the shock-escape situation a mean response latency comparable to that of the normals, but the shortest response latency on first exposure to the test situation was obtained by an animal from the HmDx group. Interestingly, of the six animals from the dextran control group, the two best performers in terms of response latencies were the two which showed mild blood sludge (sludge level +2) when tested by means of blood smears immediately before exposure to the learning situation.

The importance of the large individual differences in performance levels should be considered. First, as suggested by the preceding discussion, this extreme variability of the scores discouraged a formal analysis of the data. Further, as indicated earlier, the common response of the rabbit exposed to shock was that of crouching and retreating to a corner of the compartment. Consequently, the latency measure used in the present study may have been more of a measure of readiness to make a particular response than a measure of readiness per se.

Summary and Conclusions

Twenty-four young male albino rabbits representing four separate treatment conditions, a sludged group and three control groups, were

tested in a shock-escape learning situation beginning 1 hour after treatments. No evidence was found that HmDx-induced sludge had any effect on shock-escape learning.

Experiment 3. The effects of dextran-induced sludge on food reinforced maze learning in the albino rabbit

While Experiment 2 was intended as a study of learning, the results were inconclusive for several reasons. The large variability of the individual scores could have concealed modest treatment effects. At the same time the tendency of the animals to crouch instead of to run raised the question of what the response latency measures signified. Finally, the use of the shock-escape situation may have produced behavioral changes which reflected the confounding of adaptation to the UCS with instrumental conditioning.

The present study represents an attempt to obtain minimally confounded measures of learning under conditions of severe sludge. Food instead of shock was used as the UCS. This change in procedure should have minimized the probability of the animal making any response to the CS (raising of the compartment doors) other than the one being measured. Further, the use of food as the UCS should have assured the full dependence of the measured response on the CS.

Method

Subjects. - A total of 24 male albino rabbits weighing between 1700 and 2300 gms was used for six separate replications of a random replications experimental design with four treatment conditions: normal control, saline control, dextran control and HmDx (severe sludge).

Apparatus. - The present behavioral testing situation involved a modification of the basic square maze unit used in Experiment 1. The modifications included the removal of the shocker, the installation of a small food cup at the outer corner of each compartment and the addition of a second electric timer.

Each food cup was 1 in. deep with tapered sides and a flat bottom 2 in. in diameter resting 1 in. above the compartment floor. Food pellets (250 mg Purina Rabbit Chow pellets) were introduced into these cups through polyethylene tubes which extended from a common point at one side of the unit to each of four short metal tubes which penetrated compartment walls directly over the food cups. The second electric timer was connected in parallel with the electric stop clock already in the circuit operated by the compartment doors. A switch operated by the experimenter permitted operation of either of the two clocks but only while the compartment doors were open.

Procedure. - All animals for a single replication were obtained at the same time, and all were tested for absence of significant blood sludge before being accepted into the laboratory. Following a 5 day minimum pretreatment adaptation period the animals were placed on a food deprivation schedule consisting of one 46 hour period followed by two periods of 70 hours each, with treatments and behavioral testing beginning at the end of the second 70 hour period. Drinking water was freely available at all times except during treatment or testing.

The procedure followed on the test day was as follows. All animals were removed from their home cages, weighed, tested for absence of blood sludge by means of blood smears and returned to their cages. Four animals, matched by weight and condition of the blood, were randomly

assigned to the four treatment conditions and to separately randomized testing orders. In accordance with the treatment and testing schedule each of the four animals was removed from its cage, a fresh blood smear taken (smear A), the appropriate treatment administered, and the animal again returned to its cage. Fifty-eight minutes after the beginning of the treatment the animal was again removed from its cage and another blood smear (smear B) taken, but this time the animal was placed immediately into the square maze.

A standardized testing procedure was used for every series of trials. All the lights in the laboratory were shut off 1 min. before testing was begun. The animal was always placed into compartment 1 of the maze. Following a 10 sec. delay interval, three of the four compartment doors were raised simultaneously to open a path from compartment 1 to compartment 4 in a counterclockwise direction and at the same time to start the electric timer #1. As soon as the animal hopped through the first doorway and its hind feet touched the floor (compartment 2) the experimenter switched over to timer #2. This procedure provided separate measures of start latency and running time.

The food reinforcement was introduced into the appropriate food cup before each trial. For trial 1 the 250 mg Purina Rabbit Chow pellet was present in the food cup of compartment 4 before the animal was introduced to the apparatus. For trial 2 the food reinforcement was released through the plastic tubing into the appropriate food cup (compartment 3) at the moment that the doors were lowered at the end of trial 1. This method prevented the animal from orienting to the correct food cup on the basis of the sound of the pellet striking the food cup. Timing of the 30 sec. interval was begun only after the animal found the pellet in the food cup.

The learning trials consisted of three series of five food reinforced trials followed by one set of five extinction (non-reinforced) trials. The first of these series began 1 hour after treatment and each successive test series began at 1/2 hour intervals. No intertrial handling was necessary during the five trials of any one series. The animals simply kept circling in a counterclockwise direction, with the goal box for the first trial of any series becoming the starting box of the next trial such that the starting box moved clockwise until, with the final trial (trial 5) of a series, compartment #1 was again the starting box. A final blood smear (smear C) was taken upon completion of the four successive test series.

Results

Group mean values obtained from the measurements of starting latency and running time are presented in Tables 10 and 11. The means shown in Table 10 are for first trials only and are shown separately for each of the three series of reinforced trials and for the single extinction series. These results are presented in Fig. 9. Data for individual animals are found in Table 22 (Appendix). Similarly, group means for totals for the five trials from each of the several series of trials are presented in Table 11 and in Fig. 10. However, the values shown for individual animals in Table 23 represent sums for the five trials from each series rather than individual trial scores. Standard deviations are reported in all tables.

Some of the values shown in Tables 10 and 11 are based on the results from less than 6 animals. Such discrepancies occurred because testing was discontinued within any given series for any animal which

exceeded 500 sec. on either of the two measures of performance while no series total scores were included in the results for individual animals unless all five trials of the series were completed. Because some animals completed some but not all of a series of trials, fewer omissions are found in the results for first trials as shown in Table 10 than in Table 11 which reports series totals. Further, it should be noted that the results for first trials as shown in Table 10 and Fig. 9 permit meaningful comparisons among all four series of observations since both response latency and running time for this first trial of the extinction series should have been independent of the conditions of the reward.

Table 10

Mean Response Latencies and Running Times (in sec.) for First Trials From Each of Three 5-Trial Food Reinforced Test Series and One Extinction Series (4) for Four Groups of Rabbits with Six Animals per Group

Treatment group		Mean response latency				Mean running time			
		1	2	3	4	1	2	3	4
Normal	M	66.1	10.3	10.5*	10.8*	91.9	73.1	28.0*	9.1*
	SD	23.0	7.1	8.7	6.9	73.6	128.7	30.8	5.1
Saline	M	59.2	8.7	8.2	10.4	65.3	32.2	42.7	23.2
	SD	28.6	5.5	4.8	9.5	36.1	51.1	74.5	35.1
Dextran	M	59.5	19.2	13.6	5.4*	59.5	16.3	35.7	16.6*
	SD	35.2	14.1	11.4	2.8	33.2	11.9	38.0	10.8
HmDx	M	44.0	17.6	8.4	17.1	79.6	32.8	25.1	9.7
	SD	18.0	5.6	5.3	21.0	42.9	35.1	37.7	3.3

* N = 5

Table 11

Mean Total Response Latencies and Running Times (in sec.) for Each of Three Successive 5-Trial Series of Food Reinforced Learning Trials and One Extinction Series (4) for Four Groups of Six Albino Rabbits Tested in a 4-Compartment "Square Maze"

Treatment group		Mean response latency				Mean running time			
		1	2	3	4	1	2	3	4
Normal	M	114.1	34.5*	42.1*	47.4*	241.9	76.5*	134.1*	68.2*
	SD	48.8	16.0	19.4	18.8	131.8	35.3	79.5	76.7
Saline	M	99.0	44.6	35.2	46.1	176.5	135.9	133.8	96.8
	SD	35.8	23.3	13.9	33.2	74.8	152.0	126.5	59.3
Dextran	M	91.6	53.7	51.3	33.6*	209.3	120.8	103.0	145.2*
	SD	41.2	22.1	28.3	14.5	137.5	61.7	93.5	129.1
HmDx	M	231.4	66.8	47.4	40.8*	146.0	121.2	119.2	87.6**
	SD	311.7	38.4	25.2	26.9	39.6	77.9	97.2	53.2

* N = 5

** N = 4

The within groups variability appears to be relatively high for both sets of measures as indicated by the standard deviations shown in Tables 10 and 11. Some of this variability should be related to the separate replications because of the use of the random replications design. However, most of the variability seems to be uncontrolled. For example, the two obviously deviant mean scores occurring for the sludged group as shown in Fig. 10 are readily accounted for on the basis of single extreme scores. If the single high latency score (921.1) for the HmDx-treated animal from replication 1 was ignored, then the mean for the sludged group for the sum of all trials of series 1 would be 92.4

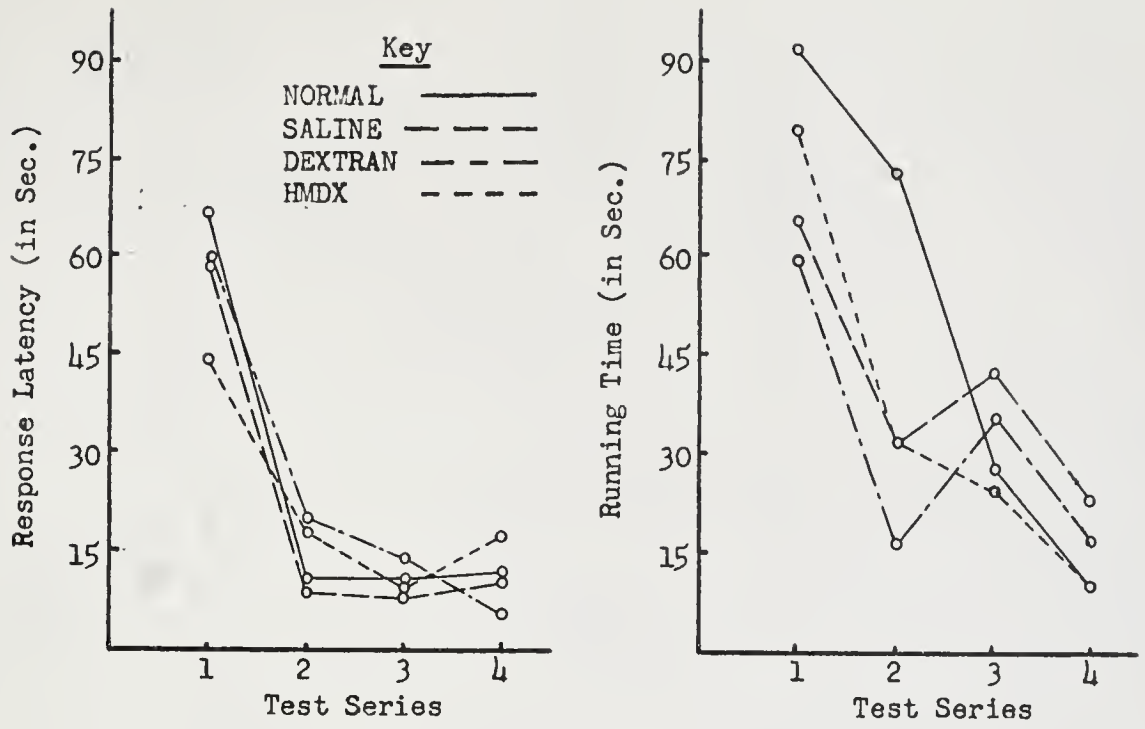


Figure 9. Mean Group Results Using First Trials for Measures of Response Latency and Running Time from Four Groups of Six Albino Rabbits Receiving Three Series of Food Reinforced Training Trials and One Extinction Series.

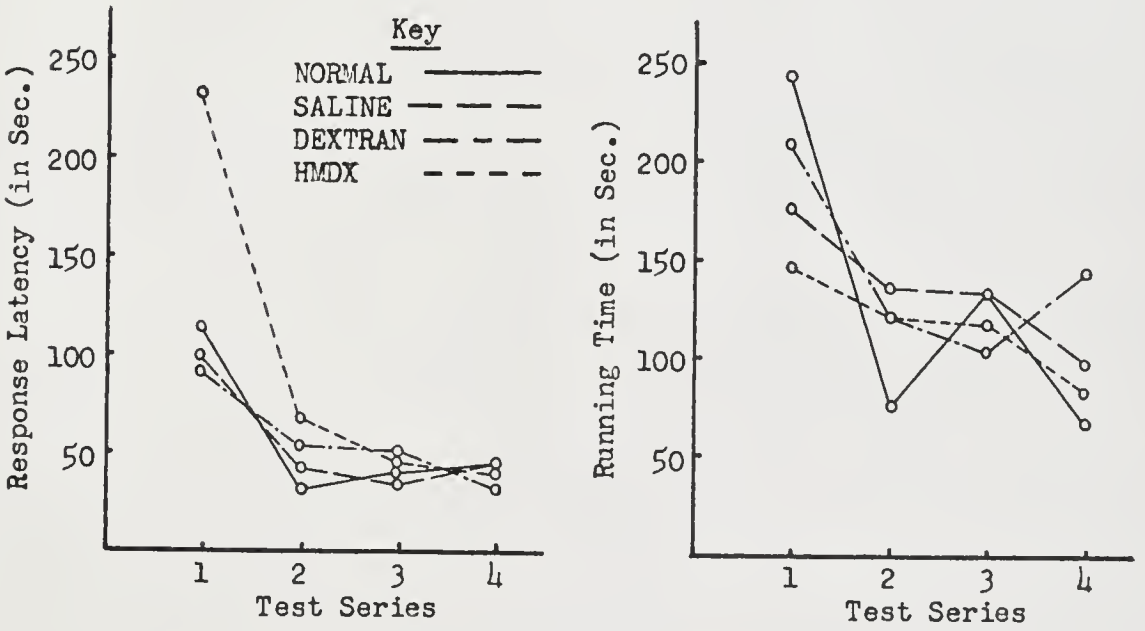


Figure 10. Mean Group Results for Measures of Response Latency and Running Time Obtained from Four Groups of Six Albino Rabbits Tested in a "Square Maze" with Three Series of Positively Reinforced Learning Trials and One Extinction Series.

instead of 231.4 (see Table 23). This adjusted value would be almost exactly equal to the mean for the dextran control group (91.6) which is the lowest of the four for this series. In similar fashion the adjustment of the mean running time value for the dextran control group for series 1 by the omission of a single extremely high score would serve to equate the results for the sludge group and the dextran control groups. No other single deviant mean scores for the sludged group are seen in Figs. 9 and 10. Neither is there evidence of trend differences. Because of the several extreme scores together with the absence of any major differences among the means for the HmDx group and the various control groups, no formal statistical analysis was performed on the data.

Observations on the condition of the blood in all animals used in the experiment are reported as group mean values in Table 12 while Table 25 also shows the ratings for individual animals.

Table 12

Mean Blood Sludge Levels Before (Smear A) and After Treatments (Smear B) and After Four Series of Training Trials (C) in the "Square Maze". with Six Animals per Group

Smear	Time	Normal	Saline	Dextran	HmDx
A	Pretreatment	1.0	1.2	1.0	1.2
B	Posttreatment	1.2	1.2	1.7	4.0
C	Posttesting	1.2	1.2	1.0	4.0

These observations show that even the normal animals had mildly sludged blood and that many of the animals (67 per cent) treated with the 77,000 molecular weight dextran had a temporary increase in blood sludge levels in the immediate posttreatment period which in every instance returned to normal by the time behavioral testing was completed.

The results from the measurement of food intake during the 2 hour feeding period following behavioral testing are shown individually in Table 24. The mean food intake for the several groups during this 2 hour period was found to be as follows: normal control group, 21.3 gms (± 4.0), saline control group, 21.5 gms (± 5.2), dextran control group, 22.2 gms (± 4.0) and HmDx group, 26.3 gms (± 6.7). Analysis of variance on these results yielded a nonsignificant F ($p > 0.05$).

Discussion

The testing situation used in the present experiment was intended to provide a standardized learning task as a background against which to measure the effects of sludge on learning. The results for the several treatment groups presented in Figs. 9 and 10 reveal consistent changes in behavior as a function of trials whether that behavior is measured in terms of response latency or running time. The situation therefore should have been appropriate for the study of the behavioral effects of sludge. While the blood sludge level in the severely sludged animals appears to have been effectively controlled by means of the HmDx infusions, blood smears obtained from the dextran control group show that these animals are more sensitive to the effects of the 77k molecular weight dextran than the animals from Experiment 1. This increased sensitivity of the blood to the 77k dextran may have been related to the prolonged period of food restriction which preceded the treatment. However, this increased responsiveness to the control dextran infusions should not have influenced significantly the results from the experiment since (1) no animal from the dextran control group showed sludge levels greater than that found to occur following 20 per cent of the standard dose

of HmDx (sludge level #2) and since not even the standard dose of HmDx seems to have any effect upon behavior under the conditions of the present experiment.

Food intake levels during the immediate posttesting period were not affected by the condition of severe blood sludge induced by the infusions of HmDx. This latter finding might appear to be inconsistent with the results obtained in Experiment 1 where mean food intake appeared to be mildly depressed following the HmDx treatment. However, the differences in the conditions under which the respective observations were made may account for the differences in results. The observations in Experiment 1 were made on animals with continuous and free access to food, and measurements of food intake were made for 12 hour intervals rather than the 2 hour period used in the present study. It may be suggested, therefore, that in the present experiment the HmDx associated anuria did not have time to affect the measures of food intake during the short time period intervening between treatment and the measurement of food intake. Actually, the present finding of a higher mean level of food intake for the HmDx-treated group might be regarded as strong supporting evidence for the conclusion reached in Experiment 1 that HmDx-induced blood sludge per se has no significant effect on food intake.

Summary and conclusions

The effect of severe HmDx-induced blood sludge on behavior was studied in rabbits using a food reinforced maze-runway learning problem. Separate measures were obtained for starting latency and running time. Neither measure provided any evidence for an effect due to the presence of the sludge.

Additional groups provided control for the effects of the saline and of dextran per se. Blood smears permitted effective monitoring of the condition of the blood. Equivalence of motivational levels among groups was verified by the finding of no significant differences in posttest food intake levels.

It was concluded that severe HmDx-induced blood sludge has no effect on learning behavior under the conditions of the test.

Experiment 4. The effects of dextran-induced sludge on a conditioned bar press response with differential reinforcement for low response rates (DRL)

A careful review of the preceding experiments suggested three conditions as guidelines for the development of a maximally sensitive testing situation within the framework of the limitations imposed by the use of the dextran and the rabbit. First, the emphasis on behaviors of increasing complexity should be intensified. Second, maximum control should be achieved over all sources of variance. This was suggested by the observation that, if sludge had any effect at all on behavior, the effect was a rather modest one and therefore easily concealed by the random variability in individual performance levels.

The third condition required a shift in emphasis in terms of the mechanisms underlying the behaviors being studied. The testing situations used in the previous studies consistently emphasized excitatory processes and favored reduced response thresholds and perseverative response tendencies. Thus this latter condition called for the use of a behavioral testing situation which was differentially sensitive to changes in the animal's ability to inhibit responses.

The three conditions could be satisfied by a testing situation in

which treatment effects are evaluated against a stable performance base line using a conditioned bar press response maintained under a DRL shcedule (differential reinforcement of low response rates) which made reward contingent on a minimum 20 second interresponse interval (Ferster and Skinner, 1957).

The rabbit, the dextrans, the conditioned bar press response and the DRL schedule were combined to test under controlled conditions the effect of HmDx-induced blood sludge on response inhibition. This experiment is reported below.

Method

Subjects. - Eight rabbits weighing between 2500 and 3000 gms were used for two replications of a latin square design in which each animal of each set received all treatments. Both sets of animals had been used previously in the positive reinforcement learning study reported under Experiment 3. Those animals used in the first replication of the present experiment had received infusions of HmDx in the previous study while three of the four animals from the second replication had received injections of 77k dextran previously and the fourth had received only saline. The one saline animal was used as a replacement for one 77k dextran treated animal whose performance following training failed to reach the minimum acceptable level of 25 per cent "correct" responses.

Apparatus. - The bar press situation was essentially a "Skinner box" apparatus with its proportions tailored to requirements of the rabbit. The experimental box was a standard rabbit cage (manufactured by Wahmann, Baltimore, Md.) with the door removed and replaced by a panel

containing the food cup, bar and lighted panel. This box was placed into a larger plywood compartment containing a 1/8 hp. blower to provide ventilation. The unit was located in the animal colony room. The box (cage) measured 21 x 18 x 13 in. high and the door, 9 x 14 inches. The "bar" was of 1/4 in. stainless steel tubing, bent in the form of a U which extended 2 in. into the box with a length of 4 inches. The 3 in. wide stainless steel food cup was set into the door panel at a height of 3 1/2 in. above the floor and 3 1/2 in. below the bar. Immediately above the bar was the 2 3/4 in. square of frosted glass behind which was a 7 watt light bulb.

The programmer was located in an adjoining room and far enough away so that the sounds of the relays could not be heard in the compartment. A pressure of approximately 50 gms was required to depress the bar over the 1 cm arc at which point a set of micro-switch contacts closed to activate the circuit of the programmer. Electronic counters accumulated total trials (bar presses) and correct trials (those preceded by a delay interval of at least 20 sec.) separately, and two pens of the Esterline Angus recorder (see Experiment 1) recorded these same events on a chart moving at 1 cm/minute. While the program allowed the release of a single 250 mg pellet of Purina Rabbit Chow into the food cup following a 20 sec. delay, activation of the circuit before the 20 sec. caused the phototimer to trip and begin the timing of the interval all over again. For training purposes the light behind the frosted glass was set to go on at the end of each 20 sec. of delay and to remain on until a response occurred. Pellets were dispensed automatically from a magazine located 16 in. above the food cup. The saline and the two dextran solutions were prepared and administered using the same equipment

and techniques as in the previous experiments. The same blood smear technique for evaluation of sludge was also used here.

Procedure. - Training on the DRL schedule began with the use of the light on the panel. The first replication was started with a 5 sec. interval which was gradually extended to 20 sec. at which time the light on the panel was made progressively dimmer with each trial until it ceased to be visible. Training of the animals for the second latin square was started with the light and the 20 sec. interval, and as the animal began to respond consistently to the light, i.e., to press only when the light came on, the light was removed suddenly and reinstated at intervals if the animal failed to maintain the delay interval.

A standard procedure was followed for handling the animals at each training trial. The rabbit was placed gently into the box. At the end of 30 sec. the bar was depressed manually to initiate a new delay interval and the door closed to make the bar accessible to the animal. As the four animals from each replication reached a stable performance level above 25 per cent reinforced responses per 1 hour test session, they were given 3 days of standardized trials each of 1 hour duration. On the fourth day the treatments were administered and the animals tested beginning 1 hour after start of injections. Blood smears were obtained from each animal just before treatment and immediately after testing. Treatment order was randomized independently for each latin square, and testing order was randomized separately. Each successive series of treatments was preceded by three daily 1 hour retraining trials. The extinction trials were not part of the original procedure. They were initiated by equipment failure but were continued to maintain standard conditions.

Retraining was begun from 8 to 15 days after the previous test series in order to allow time for the effects of the HmDx to be dissipated. A 22 hour food deprivation schedule was maintained continuously throughout each training and testing series and for 2 days preceding each series. Measurements of food intake during the 2 hour feeding period were obtained following each series of treatments.

Results

Results for the two latin squares are combined in Table 13. Total number of responses, number of reinforced responses, per cent reinforced responses and number of responses per reinforcement are reported separately as mean values for all animals for all treatment conditions for (A) the three daily 1 hour pretreatment testing sessions, for (B) the 1 hour posttreatment testing session and for (C) the extinction series 24 hours after treatment. These same values except for the extinction series are presented graphically in Fig. 11.

From Fig. 11 it is apparent that total responses remained essentially unchanged for the normal and the saline-injected conditions following treatments whereas both dextran treatments were associated with a decline in total responses, with the HmDx treatment showing the greater drop. The three infusion treatment conditions showed moderate and almost identical decreases in mean number of correct responses in the posttreatment period, but there was only a very slight fall in performance level associated with the normal control conditions. The mean per cent reinforced responses, representing the ratio (X 100) of reinforced responses to total responses, was necessarily affected by changes noted above. The net effect of these changes was that of leaving the ratio

Table 13

Mean Performance Measures for All Rabbits from Experiment 4 Shown Separately for Each Treatment Condition

	Pretreatment (3 day ave.)	1 Hour Post-treatment	Extinction
		Mean Total Responses	
Normal	175.6	170.4	80.4
Saline	156.5	151.1	90.0
Dextran	181.0	161.8	96.0
HmDx	149.9	114.6	89.6
		Mean Reinforced Responses	
Normal	84.4	80.4	44.0
Saline	84.6	68.9	44.9
Dextran	82.5	70.4	45.6
HmDx	78.8	66.5	45.5
		Mean Per Cent Reinforced Responses	
Normal	51.6	49.7	61.9
Saline	57.0	47.8	60.6
Dextran	48.3	45.8	51.5
HmDx	54.8	57.4	57.5
		Mean Number of Responses per Reinforcement	
Normal	2.1	2.2	1.8
Saline	1.9	2.3	1.9
Dextran	2.2	2.4	2.0
HmDx	2.0	1.8	2.0

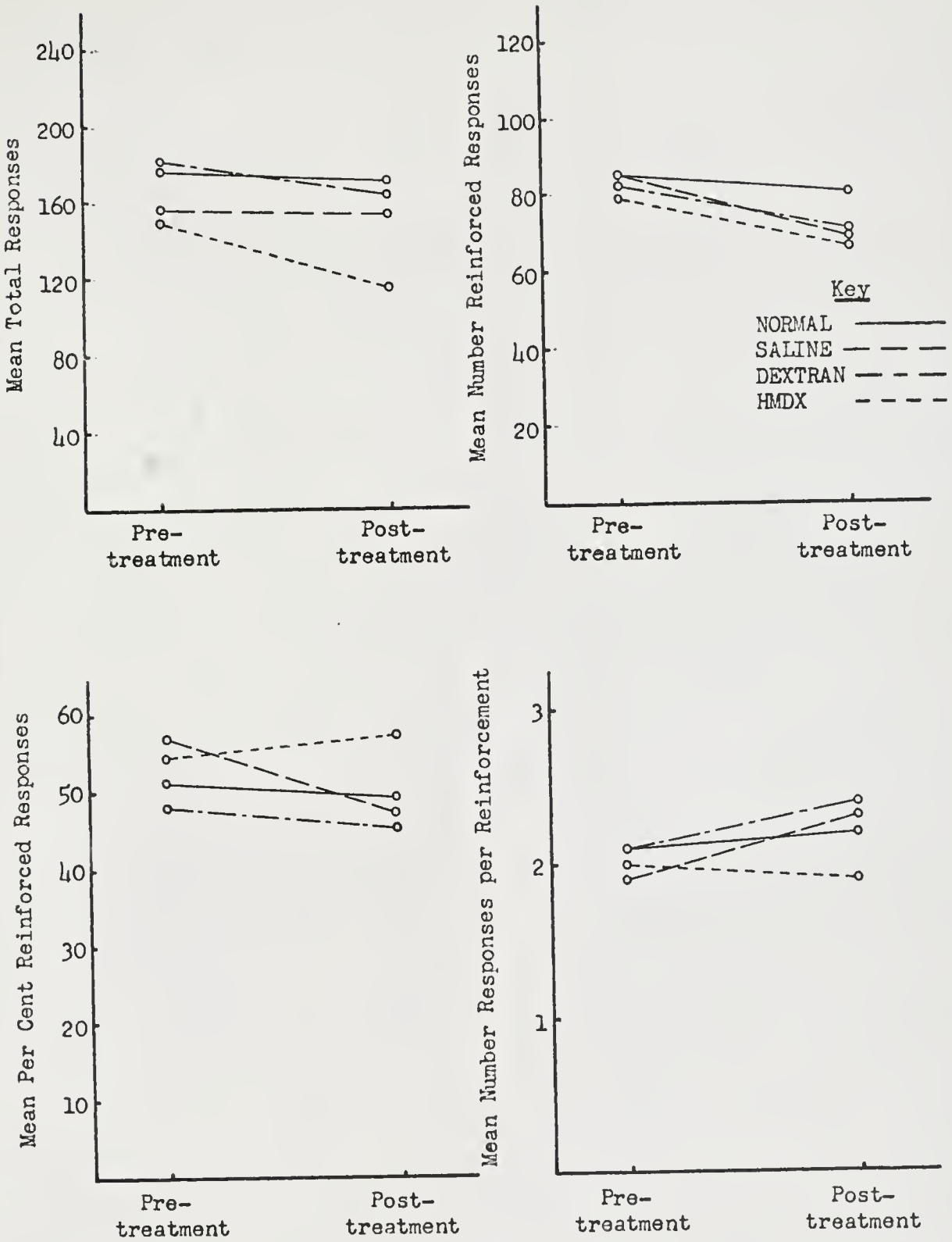


Figure 11. Mean Performance for All Animals Before and After Each of Four Separate Treatment Conditions. Results Shown Include Mean Total Responses, Mean Reinforced Responses, Mean Per Cent Reinforced Responses and Mean Number of Responses per Reinforcement.

for the normal control groups almost unchanged but only slightly less affected than that for the dextran control condition. The posttreatment means for the other two groups showed moderate changes, but in opposite directions. The percentage of successful (reinforced) responses increased for the sludge condition and fell following the saline treatment.

The significance of the mean differences shown in Fig. 11 was tested by analysis of variance using difference scores calculated from pre- and posttreatment observations on individual animals. Total responses, number of reinforced responses and per cent reinforced responses and their reciprocals were analyzed separately for each of the two latin squares and for the two squares combined. The results from these analyses are reported in detail in Table 27.

Analysis using all scores for each of the several measures showed that only the variance due to the squares x treatments interaction for reinforced responses produced a significant F ($p < 0.05$). Separate analyses of the individual latin squares for each of the several measures including the ratio of total responses to reinforced responses yielded a number of significant ($p < .05$) F ratios. For latin square 1 the variance associated with successive treatments series produced a significant F for measures of total responses and for number of reinforced responses but not for per cent reinforced responses. Subject differences for latin square 1 were found to be significant only for measures of total responses.

The only measure which produced a significant treatment effect was the number of responses per reinforcement (the reciprocal of per cent reinforced responses) and this was found only for latin square 1. The treatment means for this measure were as follows: HmDx-treated

condition, 0.77; dextran control, 1.07; normal control, 1.32 and saline control, 1.72. Multiple comparisons among these means using "Duncan's New Multiple Range Test" (Edwards, 1960) showed that the means for the two dextran groups differed significantly ($p < .05$) from the mean of the saline group. No other comparisons produced significant differences.

The results from the observations made during the successive extinction trials as reported in Table 13 offer no indication of any mean differences large enough to warrant formal analysis.

The observations made on the condition of the blood throughout each treatment and testing series are shown separately for each animal for each treatment condition in Table 28. Mean values are included in the table. The results shown in the table may be summarized as follows. No animal showed a deviation from normal unless injected with dextran. All animals injected with HmDx showed + 4.0 level sludge within 1 hour after beginning of treatment. This acute condition was found to remain unabated when measured after testing and after the extinction test series 24 hours later.

Treatment with 77k dextran produced mild sludging. For the animals from latin square 1 the blood sludge level changed from normal before treatment to a mean level of + 2 one hour after treatment, with one of the four animals showing no change while another showed moderately severe sludge (rated + 3) at this time. By the end of the 1 hour testing session the animal with the initial moderately severe sludge showed only a + 2 sludge, while all others remained unchanged. However, by the following day (Smear D) the blood for all four animals had returned to normal.

Similarly, three of the four animals from latin square 2 showed

increased blood sludge following treatment with 77k dextran. One of these three animals showed return of normal blood conditions by the end of the 1 hour testing period, but the other two animals showed no change even 24 hours later. It should be noted, however, that these changes represent only very moderate changes in the condition of the blood.

Measures of food intake during the 2 hour posttreatment feeding period were obtained following three of the four treatment series for latin square 1 and following each of the four treatment series for latin square 2. These observations, together with mean values for individual squares and for combined results, are presented in Table 29. The means based on the three observations for each treatment condition for latin square 1 are as follows: normal, 84 gms; saline, 76 gms; dextran, 82 gms and HmDx, 75 grams. A similarly ordered listing of treatment mean values, this time based on four observations, for latin square 2 as shown in Table 29 is the following: normal, 84 gms; saline, 81 gms; dextran, 79 gms and HmDx, 66 grams. The only apparently deviant mean value is that for the HmDx condition from latin square 2. Table 29 indicates that the relatively low value for this mean is largely the result of a relatively low score for animal #28. This animal received an infusion of HmDx during the first treatment series for this replication. Informal observations during the process of feeding and caring for the animals both before and after treatments suggested that food intake and fecal excretion rate for animal #28 increased considerably after recovery from the HmDx. It is suggested that animal #28 actually showed a "normal" level of food intake immediately following the HmDx treatment but that the rate of food intake became abnormally high later and that

these abnormal values tended to inflate the means for each of the control conditions thereby exaggerating the differences between the sludge condition and each of the controls. Accordingly the results shown in Table 29 may be interpreted as indicating no differential effect on post-treatment food intake levels from any of the four treatment conditions.

As previously noted, the original plan for the present experiment had called for successive sets of observations carried out over a number of days following treatments. Although equipment failure frustrated the original attempt, a single set of such observations was made over a period of 5 days following treatments in four experimentally naive rabbits trained, treated and tested under exactly the conditions described above. These observations are reported in Table 30. These include total responses for each 1 hour test session, number of reinforced responses per period and the per cent (X 100) reinforced responses. Successive observations on the condition of the blood showed the following: sludge level rose from + 1 to + 4 with HmDx treatment, but fell to +3 after the second day and remained at this level for the remainder of the 5 days of observations. All other animals showed normal blood at all times both before and after treatments. The results from the several measures of performance on the bar press task as reported in Table 30 give no indication of any treatment-related effects.

Discussion

The major result of this experiment is the absence of consistent treatment effects associated with the severe blood sludge. Out of twelve separate analyses, only the analysis of the ratios of total responses to reinforced responses for latin square 1 produced a significant

F ($p < .05$) for treatments; and even here the HmDx group did not differ from its dextran control condition.

When the above ratio scores for the two latin squares were analyzed jointly no significant treatment effects were found. Further, an analysis of the combined results in terms of the per cent reinforced responses (the reciprocal of the above ratios) produced an F ratio of .78 with 3 and 12 degrees of freedom for the treatment effect. Since an F ratio of 4.78 would be required to reject the null hypothesis at the 0.05 level of probability, the present findings lead to the conclusion that, under the conditions of the test, treatment with HmDx has no effect on bar press performance under a DRL schedule and that sludge has no effect on the animal's ability to inhibit its responses.

There is, however, some basis for suggesting that the results are not quite so clear-cut as the above conclusion indicates. Some explanation should be offered for the posttreatment increase in efficiency of performance as measured by number of responses per reinforcement (ratio of total responses to reinforced responses) which was found for latin square 1 but not for latin square 2.

Also, a significant squares x treatment interaction effect was found for number of reinforced responses, a measure which also might be considered a measure of efficiency of performance. This significant interaction effect should be the result of differences in the pattern of treatment mean differences between the two latin squares. The two dextran treatment conditions show the smallest posttreatment changes in number of reinforced responses in latin square 1 and the largest posttreatment decrease for this same measure in latin square 2. In both latin squares the more extreme value for the two dextran conditions was found

for the HmDx condition. The two latin squares may have differed in the way in which the animals reacted to the treatments.

Thus there may be some justification for suggesting that an HmDx or a sludge-related treatment effect may have been operative in the present testing situation. The animals in each square were matched on the basis of their prior experience with the treatments. The four animals from latin square 1 had been treated with HmDx while, of the four animals from latin square 2, three had received 77k dextran and one had been treated with saline only. Since the group from latin square 1 showed the superior performance following HmDx treatment, it becomes possible to speculate that prior treatment with sludge inducing HmDx may affect the response of the animal to a second treatment with HmDx.

These speculations find some support from observations previously made on rats (Robbins, 1963-1964). When rats were given successive dextran treatments followed by physical endurance swimming tests it was found that rats injected with HmDx for the first time showed a significant decrease in survival time whereas following a second treatment at a later time these same rats showed a slightly higher mean performance level on the second performance test than did the untreated animals.

Alternative explanations might be offered for the observed performance differences between the two squares in the present experiment. The differences in posttreatment performance levels might conceivably have been related to the differences in training procedures used on the two sets of animals; and, of course, they could have been due to chance. In fact, the significance of all differences is made suspect by the number of separate analyses performed on the data.

The experimental results provided some information on the extended

term effects of sludge. The record of performance on the extinction trials shows an apparent absence of treatment related differences. However, the blood smears showed maximal sludging for the HmDx-treated animals at this time. Further, the performance levels for the single replication of naive animals which were studied during the 5 days before and the 5 days after treatments showed no evidence of sensitivity to the treatments even though blood sludge was severe for 2 days after treatment and only gradually improved.

The present series of observations again showed a close relationship between HmDx treatment and blood sludge as evaluated by means of the blood smear technique. The technique permitted the verification of the condition of severe blood sludge in all HmDx-treated animals both before and after testing. As previously, the smears were rated in a randomized order with identities effectively concealed. Accordingly any statements concerning treatment effects may be related directly to the condition of the blood at the time of testing.

The frequent observations made on the condition of the blood before and after treatments revealed some sludging following treatment with 77k dextran. However, this effect, as indicated by the condition of the blood smear, was always much less severe than that produced by the HmDx.

Mean posttreatment food intake was found to be lower after infusions of HmDx than after any of the other treatments. However, most of this mean difference could be related to a single animal out of the seven for which data on food intake following HmDx infusion was available. Thus no major effect upon levels of food intake from the HmDx treatments is indicated by the data. Neither does a comparison of the posttreatment

food intake levels (Table 29) with the number of reinforcements obtained by the individual animals (Table 26) suggest a close relationship between these two measures. The observations on food intake should discourage any thought that a treatment related behavioral effect might have been masked by or confounded with a shift in motivational level.

Summary and conclusions

A conditioned bar press response and a DRL schedule were used to study the effects of severe blood sludge on response inhibition. The experiment was carried out with eight albino rabbits in two replications of a 4 x 4 (treatments x subjects) latin square. Blood sludge was induced by HmDx and verified with the use of blood smears. Severe blood sludge occurred following treatment with HmDx, but none of the several measures of performance, total response, number of reinforced responses, ratio of reinforced responses to total responses or its reciprocal, showed any evidence of sensitivity to the sludge.

GENERAL DISCUSSION

Significant behavioral effects were anticipated for the condition of severe blood sludge in the present series of experiments. The blood sludge was expected to promote a condition of stagnant anoxia. Behavioral effects generally associated with anoxia, such as depressed levels of spontaneous activity, increased response latencies and impairment of short term memory were therefore expected to occur with the HmDx-induced blood sludge.

The series of four experiments failed to show any behavioral changes attributable to the presence of the severe blood sludge. In Experiment 1 homecage activity and activity in a novel environment were found to be unaffected by treatment. The effect of sludge on learning was studied in Experiments 2 and 3. Response latency measures were used in both studies, while the spacing of blocks of trials permitted observations on treatment effects on short term memory. No differences due to treatments were found. Finally, in Experiment 4 the effect of sludge on response inhibition was studied using a conditioned bar press response maintained on a DRL schedule. No treatment effects were indicated.

The hypothesis concerning the behavioral effects of sludge was not supported by the experimental findings.

The basis for the failure of the results to support the hypothesis is not immediately apparent. Either the HmDx treatment used in the present series of experiments does not induce severe blood sludge, or the severe blood sludge does not induce stagnant anoxia, or the stagnant

anoxia does not have any behavioral consequences.

This failure should not have been due to an inability to control effectively for the level of blood sludge. The blood sludge induced by the infusion of 1 gm per kg body weight of HmDx was uniformly severe and persistent. By contrast, the dextran and the saline control conditions were associated with occasional transient mild sludge but more commonly with a complete absence of sludge. The blood smear technique for measuring the severity of the in vivo condition of the blood showed highly stable patterns of change in severity of blood sludge as a function of type of treatment and time since treatment. These observations, together with the evidence offered for the validity and reproducibility of the blood smear results as presented under Part II of this paper, permit the conclusion that effective control was maintained over the independent variable at all times. Yet no behavioral effects were found.

It should be noted that the results from the present study are not only inconsistent with the hypothesis but also at variance with incidental observations on behavioral changes following HmDx treatment reported by other authors. For example, Swank and Escobar (1957) reported that dogs treated with sludge inducing dextran developed generalized paralysis lasting from 1 to 6 days. The contrast between present findings and those reported by Swank and Escobar is obvious. The reconciliation of the results from these two sets of observations may represent a first step in explaining the results from the present study.

The two studies may be contrasted in terms of the experimental animal used. However, if sludge were expected to promote a condition of stagnant anoxia, then, with other factors held constant, the dog should have been more resistant to the sludging than the rabbit since

the dog has been reported to survive longer periods of cerebral ischemia than the rabbit (Boyd and Connolly, 1961; Hirsch, Bolte, Schandig and Tonnis, 1957).

Another difference is found in the dose levels and molecular weights of the dextrans used. The dogs received 1 1/2 gm per kg of a dextran with a mean molecular weight of approximately 85,000, whereas the rabbits were given 1 gm per kg of a dextran with a mean molecular weight of about 2,000,000. Systematic observations on the differential effects of dextran dose and molecular weight suggest that the smaller volume of the higher molecular weight dextran should have induced the more severe sludge condition (Thorsen and Hint, 1950).

There is another difference related to the dextran dose. Swank and Escobar (1957) anesthetized their dogs before treating them with HmDx whereas no anesthetic was used for the rabbits. Swank and Escobar acknowledged that the effects of their treatment were more severe with anesthetic than without, but the magnitude of the difference was not indicated.

The answers to the problem may be found with the difference in the response of the animal depending on whether or not an anesthetic is used. Thus the critical factor is probably not blood sludge per se, since Swank and Escobar (1957) reported the most dramatic behavioral effects following the use of Nembutal, an anesthetic which is free of any direct influence on the aggregation tendency of the blood. (Knisely, Bloch, Eliot and Warner, 1950; Knisely, Eliot and Bloch, 1945; Bloch, 1956). Thus if sludge does have an effect, that effect may be an interaction phenomenon, possibly involving changes in level of oxygen requirements for the brain and blood flow rates.

Additional information may be relevant to the question of why the severe blood sludge treatment in the present study was not associated with any behavioral changes.

First, HmDx-induced blood sludge may be a very special kind of sludge. For example, the following effects have been reported for dextran infusions as summarized by Gelin, Korsan-Bengtsen, Ygge and Zederfeldt (1961): increased bleeding time, reduction in platelet number and activity, retarded thrombin formation and a reduced concentration of fibrinogen. Further, the magnitude of these changes appears to vary directly with the molecular weight of the dextran. These findings indicate a significant increase in clotting time following treatment with HmDx.

Also, the fact that the interference with clotting mechanisms is a function of the molecular weight of the dextran suggests at least a partial explanation for the difference from the present study and those obtained by Swank and Escobar (1957) with dogs. The dogs received a much lower molecular weight dextran (85,000) than did the rabbits (2,000,000). This should have meant a greater tendency for intravascular clotting in the dogs than in the rabbits.

Another possibly relevant observation is that of the increased settling tendency of the blood during severe sludge (Fahraeus, 1929; Knisely, 1951; Knisely, 1961). A major increase in settling rate (ESR) after large doses of HmDx has been reported by Gelin (1956). This effect is evident in the observation from the posttreatment condition for the animal receiving the HmDx in Experiment 1, Part II, as shown in Fig. 12.

Here might be at least part of the explanation for the finding

that anesthetics exaggerate the effects of the HmDx. It may be that during anesthesia the animal's head is held low in relation to the rest of the body and that large numbers of red cells settled out and packed in the blood vessels of the brain. Associated with the settling tendency is an exaggeration of the "plasma skimming" effect (Gelin, 1963) which together with the settling effect should tend to reduce the cell count in the blood reaching the brain when the animal remains alert and keeps its head up. The cells settle and pack in the vessels at the lower side of the body while the blood to the upper regions of the body is mostly plasma.

Also, not only is there an absolute decrease in the number of red cells in the circulation, but the plasma expander effect of the HmDx (1 gm per kg dose) produces an increase in total blood volume of over 25 per cent on the first day after treatment. This level of increased volume is maintained with only slight reduction even on the third post-treatment day (Gelin, 1956).

The decrease in circulating blood cells should tend to minimize shifts in the viscosity of the blood due to the presence of the large HmDx molecules, since normally the viscosity of whole blood is largely a function of its cell content (hematocrit) (Thorsen and Hint, 1950).

HmDx has been found to increase the viscosity of the blood whether added in vivo or in vitro (Gelin, 1956; Rand and Lacombe, 1964). Thus, independent of its effect on hematocrit, the dextran should tend to reduce blood flow rate. Rand and Lacombe (1964) have emphasized that the addition of dextran to the blood makes the viscosity of the blood increasingly dependent upon flow rate in that the higher the flow rate the less resistance. Further, Hoyt and Soli (1965) have reported that the

addition of long chain polysaccharides to a liquid medium will reduce drag on turbulent fluid flow by as much as 65 per cent and will result in increased flow rate with constant perfusion pressure. Also, it was noted that the reduction in drag was greater at higher flow rates. It would seem then that the amount by which dextran increases viscosity of the blood depends increasingly upon the flow rate at which the measurement is made.

However, the 1 gm per kg dose of HmDx has fairly consistently been associated with severe impairment of blood flow (Gelin, 1962; Gelin and Shoemaker, 1961). For example, Gelin and Shoemaker made direct observations on the blood flow in the liver of the rabbit following HmDx treatment. They found changes in the conditions of flow which included periods of temporary stasis in the sinosoids and a marked decrease in cell content of blood flowing in central venules. However, circulatory breakdown came only with increasing doses of HmDx.

The effect of the HmDx on circulation of the brain was thought to approximate that found for other tissues. A number of reports have stressed that the blood flow in the conjunctiva of the eye is representative of the flow throughout the entire body (Bloch, 1956; Knisely, Bloch, Eliot and Warner, 1947; Knisely, Eliot and Bloch, 1945), and the effect of settling on regional flow should be comparable for both eye and brain. Observations on blood flow in the conjunctiva and the nictitating membrane of the rabbit following treatment with HmDx consistently show severely impaired blood flow and the presence of large aggregates in the vessels. Perhaps the most striking feature of the vascular beds of the eye at this time is the absence of cells. Most of the smaller vessels seem to have disappeared completely, while larger vessels show a halting

flow of widely spaced cellular aggregates (Gelin, 1956). This description is also consistent with the findings from a large number of observations made under similar conditions during the course of the extensive research activity which preceded the present series of experiments. This same condition was expected to occur within the brain.

The assumption of the equivalence of blood flow in the eye and in the brain during severe HmDx-induced blood sludge may not be valid. For example, Swank and Hissen (1964) found major differences developing between peripheral blood flow and blood flow to the brain in response to injections of serotonin into the arterial circulation of the dog's hind limb. A marked reduction in blood flow resulted. Yet when the same dosage was injected into the carotid artery an increase in blood flow was found. However, the increased flow did not prevent the extravascularization of trypan blue indicating increased cerebrovascular permeability which occurred when much larger dose levels of serotonin were used.

While these authors recognized that the effect of the serotonin might have been directly on the blood vessels of the brain, they felt that the effect was related to the condition of blood sludge induced by the serotonin. The aggregates, it seemed, slowed down blood flow in the muscles but raised it in the brain. The authors were impressed that the brain seemed to be protected in some fashion from ischemia, but they observed also that there was a definite limit on the severity of the aggregation which the brain could tolerate. Swank and Hissen (1964) seemed to feel that the improvement in blood flow to the brain during the serotonin treatment was related to an improvement in the flow characteristics of the blood as a result of the aggregation of the cells which served to increase the width of the peripheral plasma layer by

restricting the distribution of the red cells in the flow stream. This is the same argument for the advantages of blood sludge suggested earlier by Fahraeus (1929).

However, there is also support for the argument that the mechanism for the increase in blood flow through the brain following the serotonin treatment and the aggregation was primarily the result of a vasodilation induced by an increase in the pCO_2 of the venous blood. As noted earlier in this paper, there are few if any arteriovenous shunts in the brain. The blood which flows through the brain must pass through the capillaries. Vasodilation in the brain appears to be controlled by the pCO_2 of the venous blood. It should be expected, therefore, that the sudden appearance of the cellular aggregates in the brain temporarily plugged cerebral vessels, impairing flow and raising pCO_2 of the venous blood. Vasodilation of the cerebral vessels followed, and the resulting increases in the size of the capillaries brought essentially free flow through all vessels. It is to be expected that the vasodilation was associated with an increase in the total number of active vessels, since except under the most extreme conditions only a fraction of the total vascular bed is functional at any one time (Krogh, 1919). The authors themselves offer evidence for these speculations through their observation that the increase in blood flow to the brain after the serotonin injection was usually preceded by a transient decrease in flow. If changes had occurred solely in the characteristics of flow without changes in the condition of the vessels themselves, the flow change with treatment should have been immediate and consistent.

There is considerable support for the idea that capillary changes represent the critical factor in maintainance of blood flow during severe

blood sludge. Gelin (1956) has reported that during severe HmDx-induced sludge capillary flow improves with vasodilation. Hegedus and Shackelford (1963) have found that sympathectomized dogs with chronically impaired blood flow to the brain show a significant decrease in cerebrovascular resistance and an increase in blood flow rate to the brain when breathing a 5 per cent CO_2 mixture.

Also, Swank and Hain (1952) found that a dog with fever of unknown cause had cerebral capillaries which were two to three times normal diameter. Swank and Hain injected paraffin emboli (4-12 micra diameter) into the animal 1/2 hour before sacrifice and discovered that none of the emboli had been retained in the cerebral vessels. In contrast, normal animals retained large numbers of these emboli for extended periods of time. The dog with the fever should also have had severe sludge.

The above findings indicate that major changes occur in the vasculature of the brain during sludge. Further, since any plugging of the vessels by cellular aggregates should also have meant the retention of numbers of the inert paraffin emboli, the conclusion may be justified that the enlargement of the capillaries was sufficient to permit unobstructed passage of all emboli whether paraffin or cellular aggregates. These observations are consistent with the findings reported earlier in the discussion on adaptation in the vascular system.

Also relevant to this question of cerebrovascular adaptation to emboli is the observation that severely sludged rabbits are much more resistant to air emboli than normal animals. The author has found that the introduction of air emboli into the ear vein of the animal is a simple and humane method for sacrificing animals at the termination of

an experiment. A single 10 cc dose is uniformly lethal for the normal animal. However, the severely sludged animal often shows no response to injections of that volume and some times tolerates as much as 100 cc of air. In one instance an animal injected with approximately 150 cc over a 5 min. period showed no change in behavior during a subsequent 10 min. period, at the end of which time additional volumes were infused until breathing ceased. It seemed that the longer the condition of severe blood sludge continued, the more tolerant the animals are of the air emboli. This observation should indicate that the sludge may have served to generally reduce the animal's dependence on oxygen. Possibly major adaptive changes occurred at a biochemical level to increase the animal's capacity for anaerobic metabolism. The extreme sensitivity of respiratory enzymes to anoxia were noted earlier.

In summary, two possible explanations for the failure of the results from the series of experiments to support the hypothesis of significant behavioral effects from severe dextran-induced blood sludge were suggested: either (1) the effect of the experimentally induced sludge is counteracted by some secondary characteristics of the dextran or (2) sludge of the severity produced by the HmDx does not have any significant behavioral consequences.

With respect to the first alternative, it was noted that the condition of the blood following treatment with HmDx differs in several respects from that of sludge occurring as a concomitant to illness or injury. The HmDx results in increased plasma volume and hemodilution. It tends to cause a very high erythrocyte sedimentation rate in comparison to the severity of the aggregation. The HmDx also interferes with the clotting mechanism and reduces the amount of fibrinogen in the plasma.

Consequently the blood perfusing the brain of the normal alert animal during severe HmDx-induced blood sludge may have an unusually low hematocrit (cell count) and may be highly resistant to clotting even if temporary stasis should occur.

However, the secondary effects of the dextran do not provide a full explanation for the failure of the experimental results to support the hypothesis. In spite of a possible major decrease in the volume of cellular aggregates entering the blood vessels of the brain, a large number of such aggregates should have penetrated the brain during the extended period of severe aggregation which followed the HmDx treatment. Yet no behavioral consequences were observed. The response of the cerebrovascular system seems to be a vasodilation sufficient to pass the cellular aggregates of sludged blood, at least those of the size produced by the HmDx treatment used in the present experiment.

Finally, sludge has been defined in terms of the cellular aggregates present in the blood. The evidence from the present study indicates that a large number of aggregates were present in the blood and that many of them did enter the brain. These conditions should be sufficient for testing the hypothesis that blood sludge has a significant effect upon behavior. No behavioral effects were found.

If sludge does have an effect, that effect occurs as an interaction phenomenon involving a number of other variables which influence blood flow.

It is concluded that blood sludge per se has no significant behavioral effects.

SUMMARY

A series of experiments was carried out to investigate the effects of severe intravascular aggregation of red blood cells (sludge) on behavior in the rabbit. Cellular aggregates were expected to impede blood flow, to plug capillaries in the brain and to produce a condition of stagnant anoxia in the brain. Behavioral effects common to anoxia were expected from the severe sludge condition.

Observations were made on spontaneous activity, exploratory behavior, food and water intake, on instrumental conditioning using both negative and positive reinforcement and on conditioned operant behavior maintained under a reinforcement schedule providing for differential reinforcement of low response rates (DRL).

Standardized experimental conditions were used for all studies. The blood sludge was induced by the infusion of 1 gm per kg body weight of a 10 per cent solution of high molecular weight (2,000,000) dextran (HmDx) in normal saline. Three control groups were used: a normal untreated group, a saline-injected group and a group receiving a standard dose level of 77,000 molecular weight dextran which does not induce sludging although it has the plasma expanding capability of the HmDx. The condition of the blood was monitored with the use of blood smears on glass slides. A rating scale was developed for evaluating the blood smears.

Experiment 1 involved observations on spontaneous home cage activity, activity in a novel environment, food and water intake and

body weight over a 6 day period beginning 2 days before treatments. Five replications using the four standard treatment conditions were carried out. Food and water intake were mildly depressed for the sludged animals during the posttreatment period, but these effects were found to coincide with the course of the anuria which occurs with HmDx treatment while body weight was unaffected. It was concluded that sludge had no effect on any of the measures used.

Experiment 2 consisted of six replications of a shock-escape learning test using the same four conditions as in Experiment 1. Testing was begun 1 hour after the beginning of treatments and consisted of three series of five trials from each series and for total scores from each series were evaluated separately. No differences due to treatments were indicated.

Experiment 3 tested for the effects of blood sludge on maze learning using food reinforcement with rabbits on a 72 hour food deprivation schedule. The maze unit consisted of a 32 in. square box divided into four equal sized compartments and interconnected by small doorways equipped with sliding panel doors. Testing consisted of three series of five trials given at 30 min. intervals beginning 1 hour after treatment. Response latency and running time measures were obtained separately and were evaluated separately for first trials from each series and for total trials for each series. No treatment related effects were indicated for any of the above measures. Neither were any differences found for food intake during the posttreatment feeding period.

In Experiment 4 a conditioned bar press response with reinforcement contingent on a minimum interresponse interval of 20 sec. was used

to study the effect of blood sludge on response inhibition. Eight rabbits were used in a 4 x 4 (treatments x subjects) latin square design in which all animals received all four standard treatment conditions. A minimum performance level of 25 per cent reinforced trials maintained over three successive 1 hour daily test sessions was achieved by each animal before treatments were given. Blood sludge levels were evaluated before treatment and before and after each testing session by means of blood smears. Eight to 15 days were allowed between treatments. Analysis of variance was carried out on total trials, reinforced trials, per cent reinforced trials and on number of trials per reinforcement. No significant effect due to treatments were found for any of these measures.

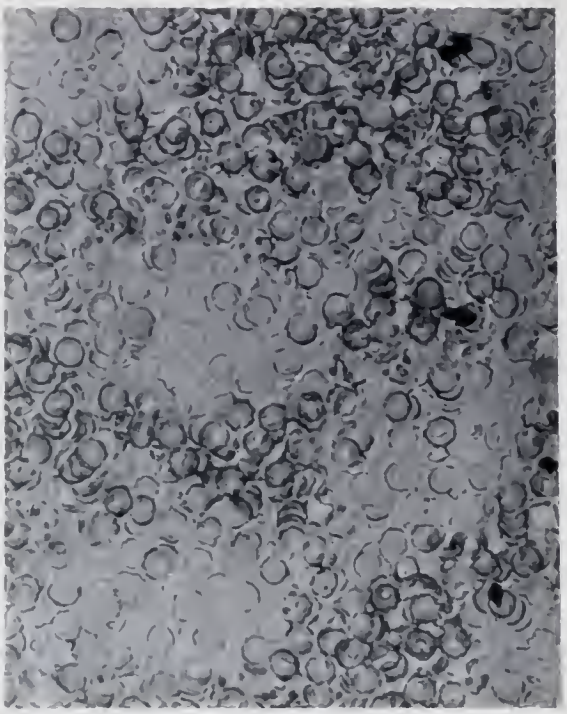
The negative findings from these several experiments were contrasted with relevant findings in the literature. It was suggested that the capillaries in the brain can dilate sufficiently to permit passage of the large aggregates produced by the HmDx treatment. Further, the plasma expander effect of the HmDx, its dramatic effect on erythrocyte sedimentation rate, and its interference with clotting mechanisms may prevent intravascular clotting of acedotic blood during temporary plugging of cerebral vessels and permit sufficiently rapid perfusion of the brains with anemic blood to sustain normal levels of brain energy metabolism.

APPENDICES

Appendix A

Data From Experiment 1, Part II

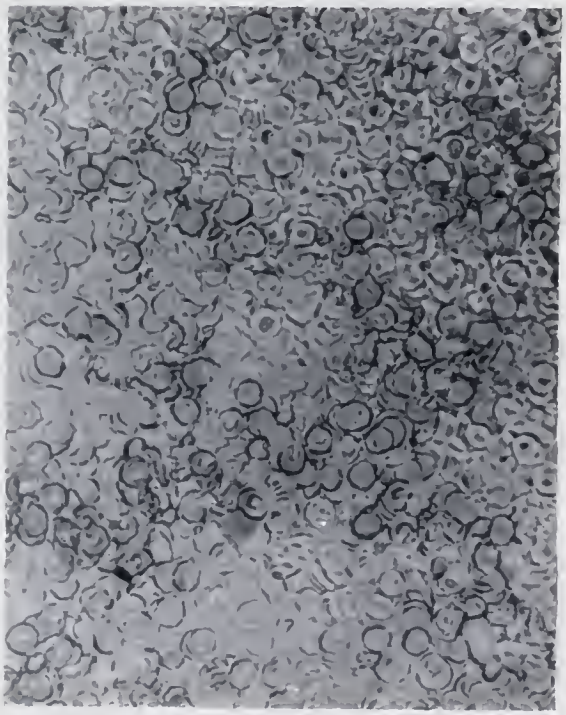
Figure 12. Comparison of Blood Smears and In Vivo Observations of the Condition of the Blood in the Mesentery of the Rabbit Before and After Treatment with Standard Dose Levels of Saline, 77k Dextran or HmDx.



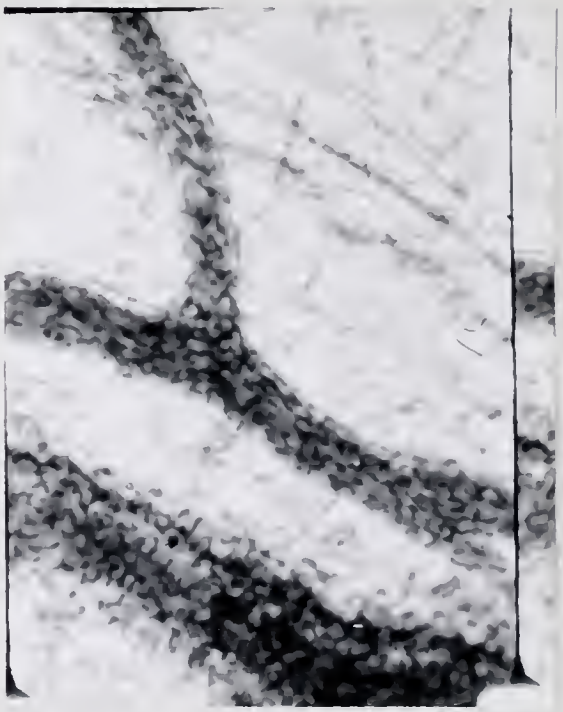
Blood Smear

In Vivo

Before Treatment With Saline

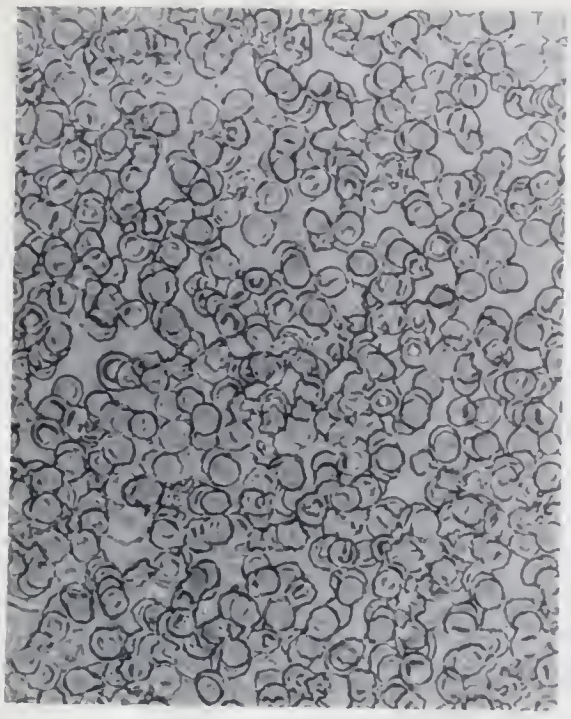


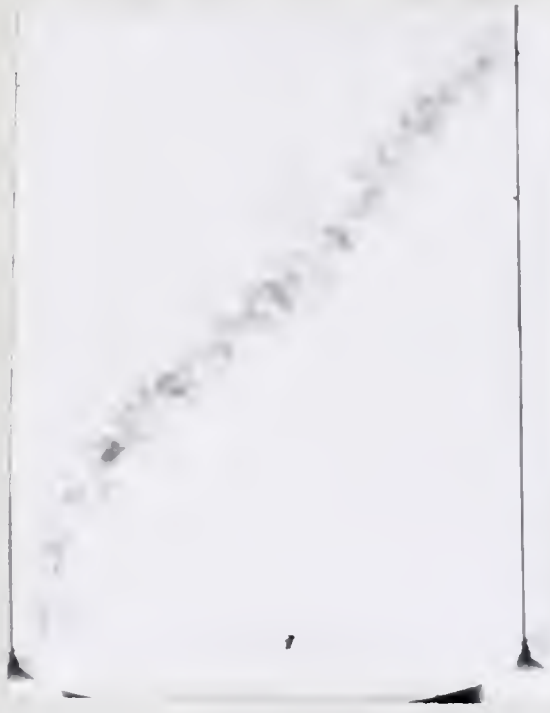
Blood smear



In Vivo

One Hour After Treatment With Saline

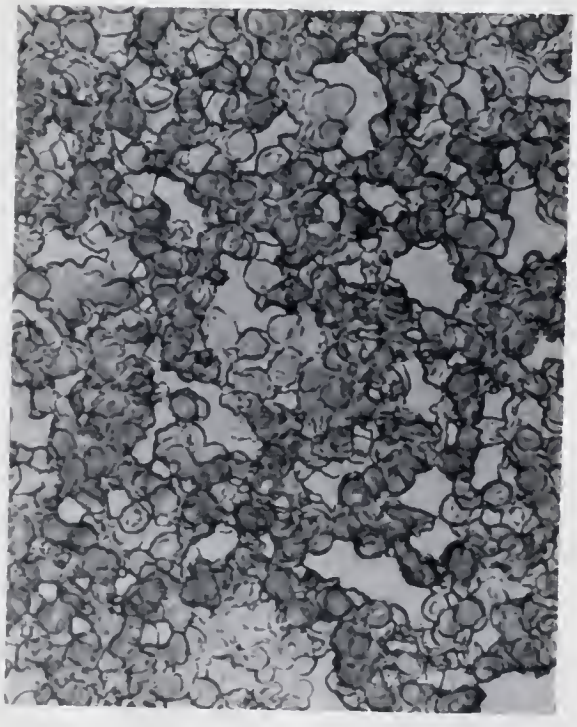


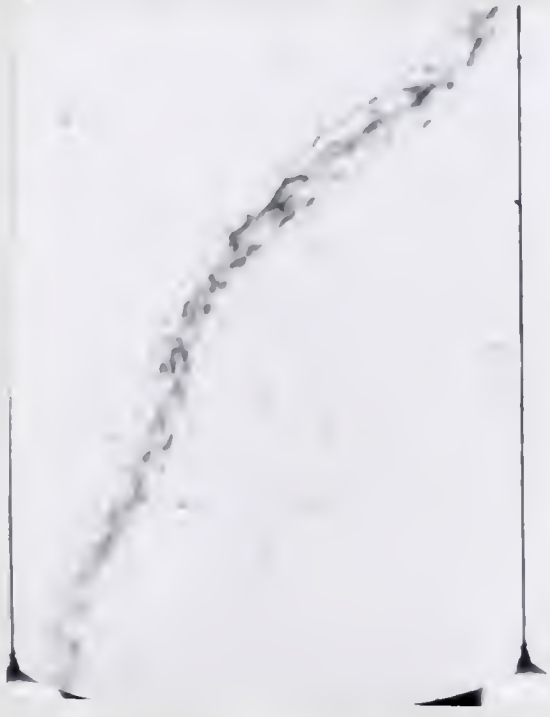


Blood Smear

In Vivo

Before Treatment With 77k Dextran



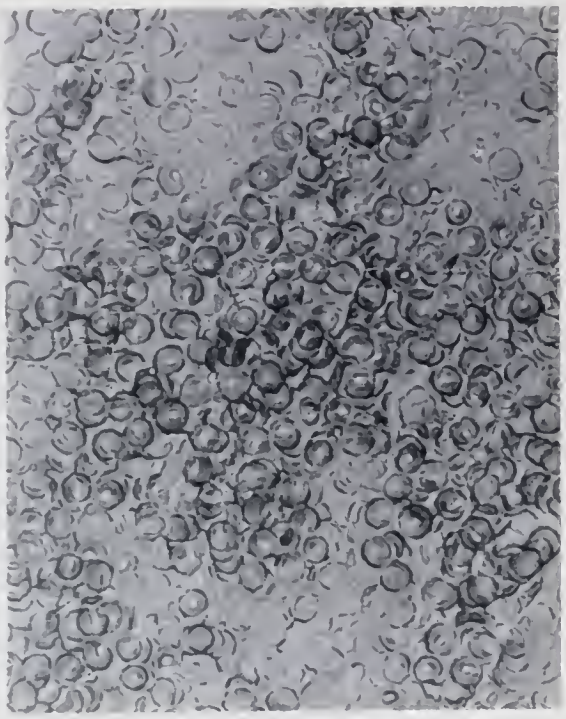


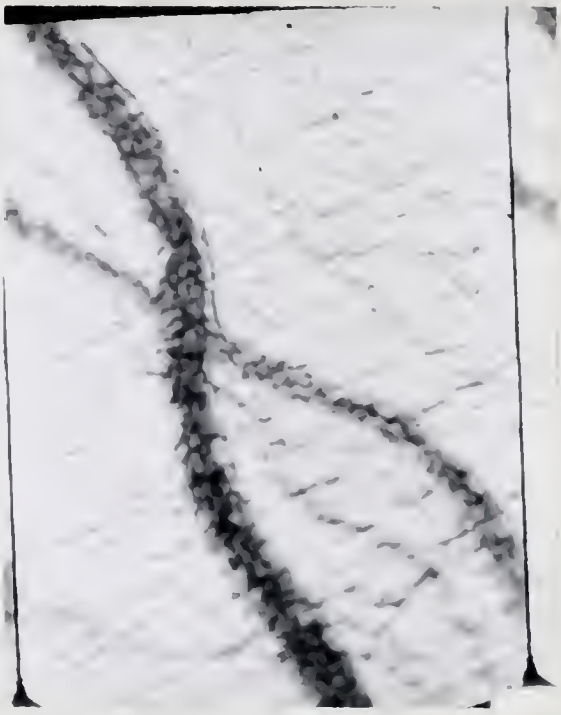
Blood Smear

In Vivo

One Hour After Treatment With 77k Dextran

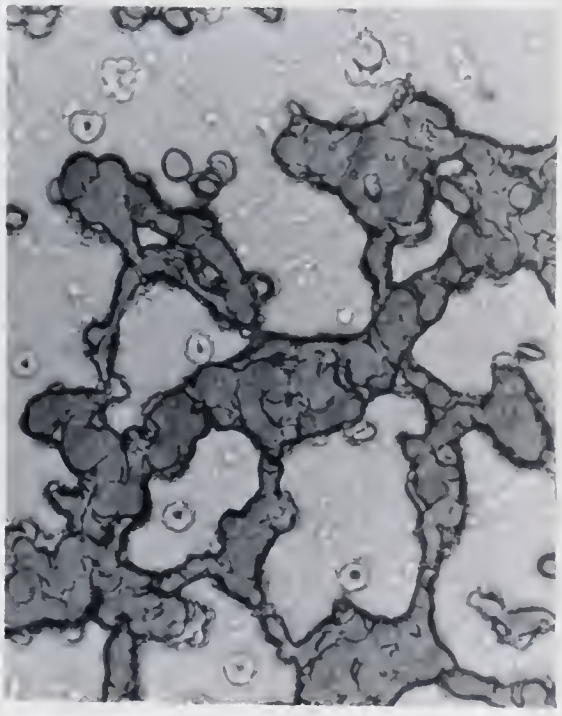
Figure 12 - Continued

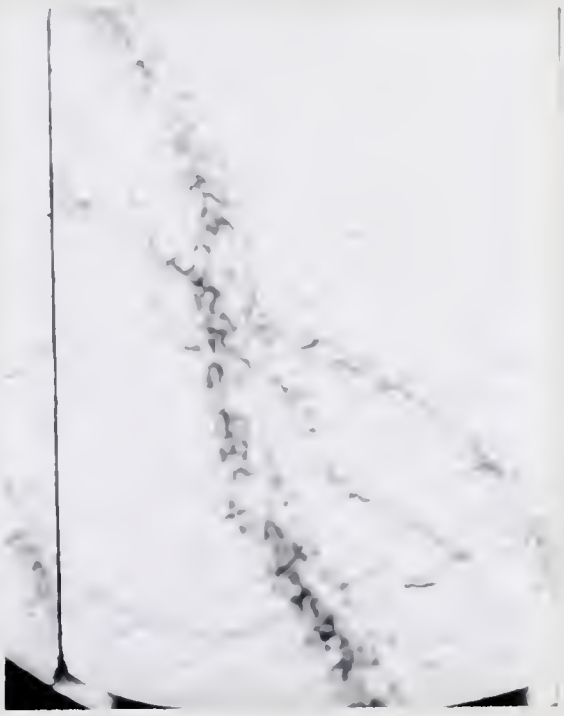




Blood Smear In Vivo

Before Treatment With HmDx





Blood Smear In Vivo

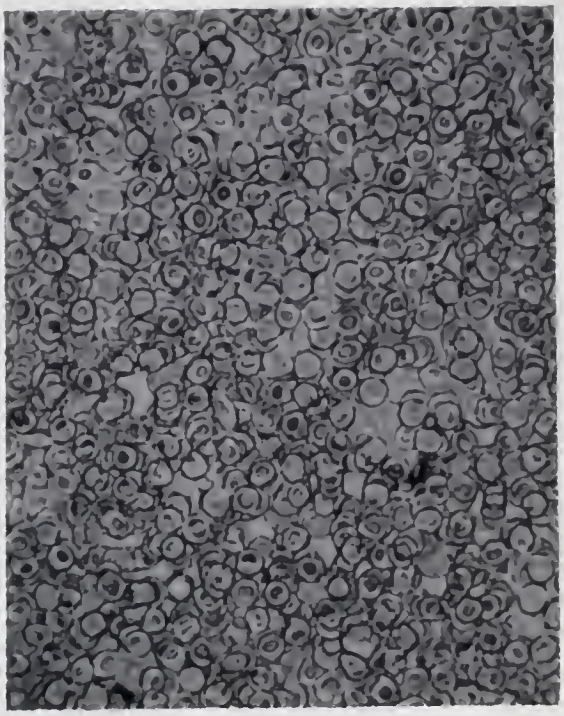
One Hour After Treatment With HmDx

Figure 12 - Continued

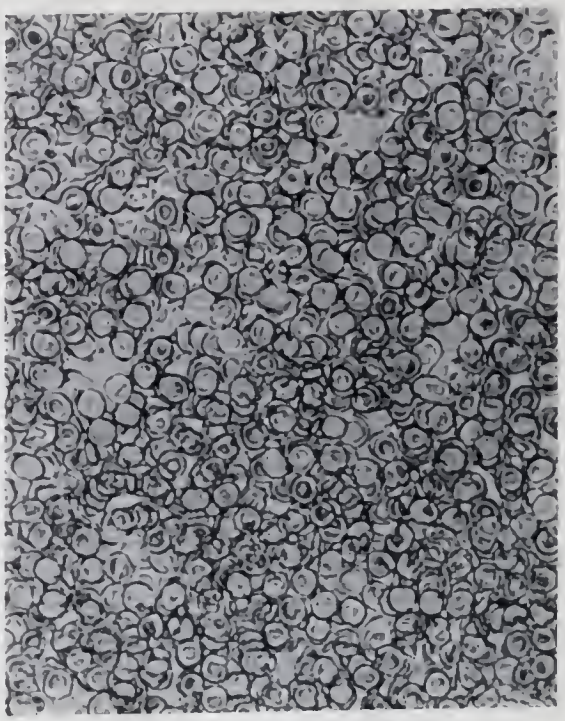
Appendix A - Continued

Data From Experiment 2, Part II

Figure 13. Condition of the Blood Smear Before Treatment and After Treatment with 20, 50 and 100 Per Cent of Normal Dose of HmDx. Each Set of Four Photographs Includes Two Observations From Separate (A and B) Locations From Each of Two Blood Smears (1 and 2) Taken at the Same Time. The Four Sets of Photographs Were Used as Standards to Represent Blood Sludge Levels +1 Through +4, Respectively, for Rating the Blood Smears Obtained During the Course of the Behavioral Studies.

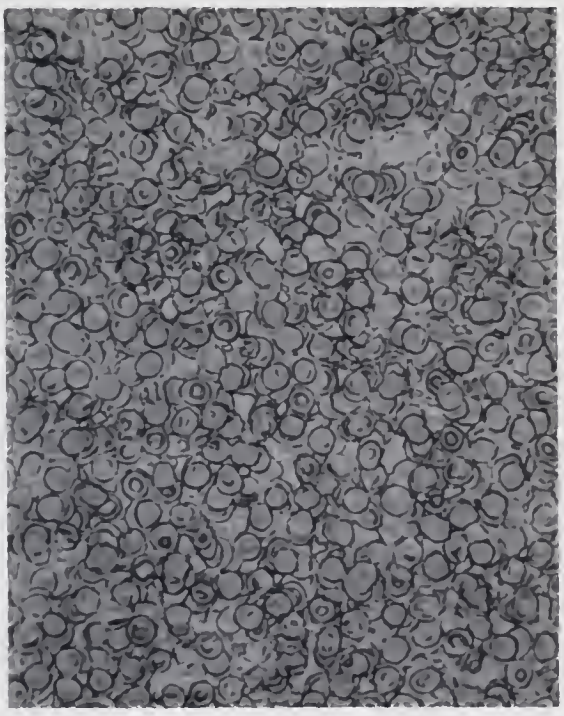


Location A

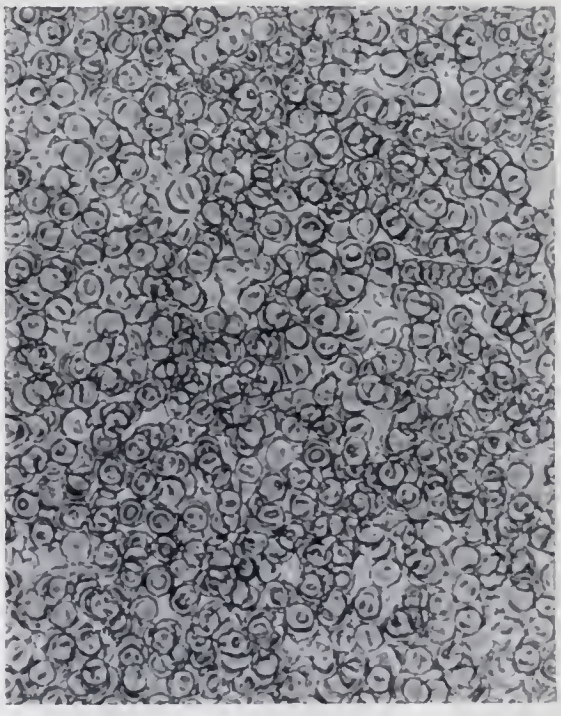


Location B

Blood Smear 1



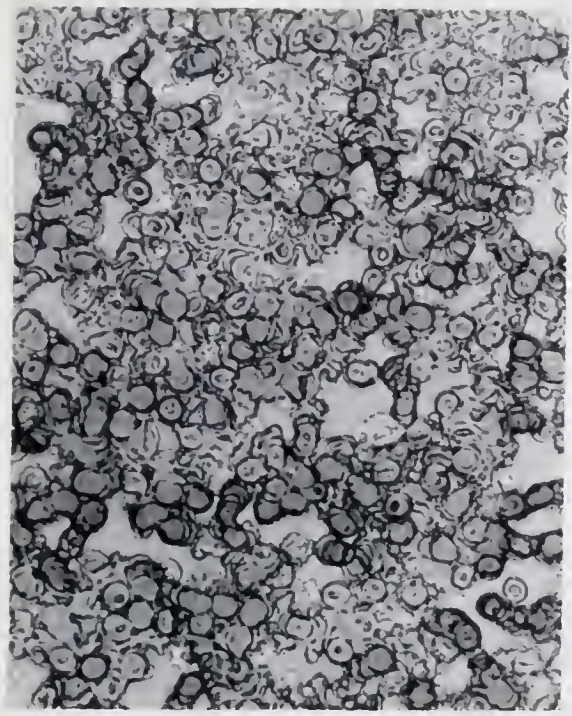
Location A



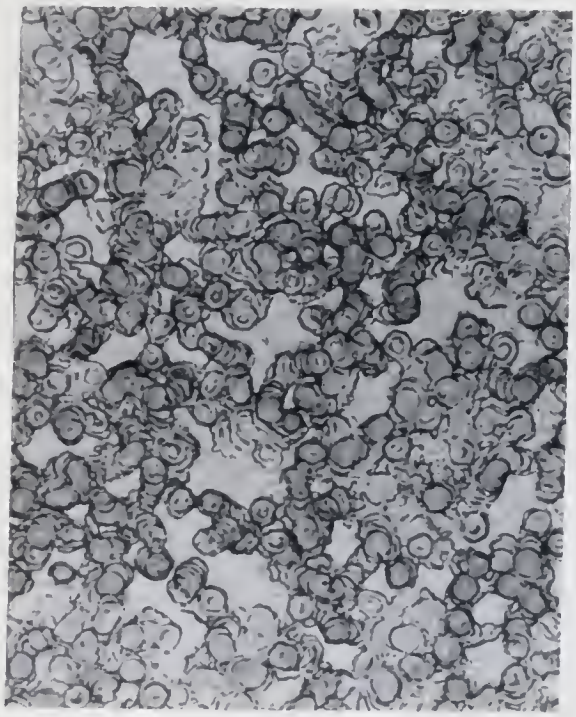
Location B

Blood Smear 2

Before Treatment: Blood Sludge Level, +1

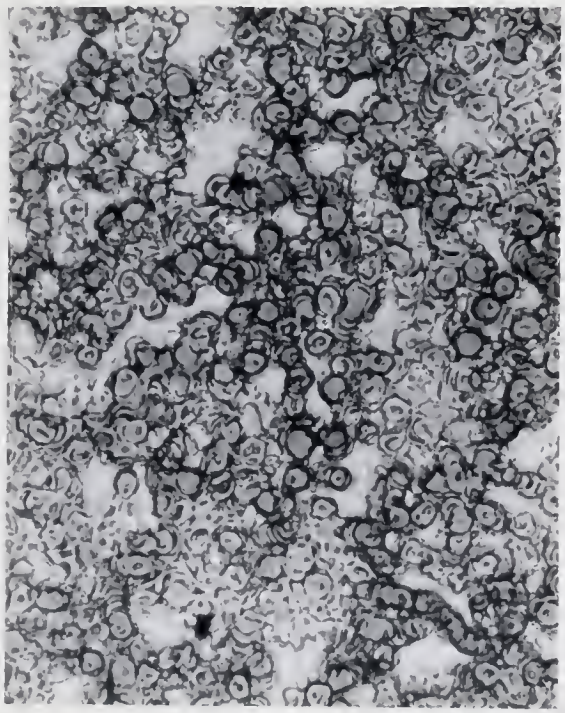


Location A

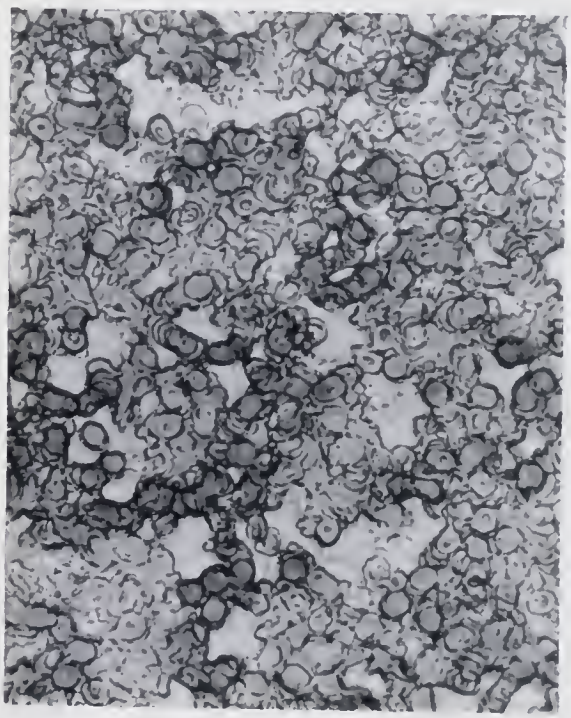


Location B

Blood Smear 1



Location A

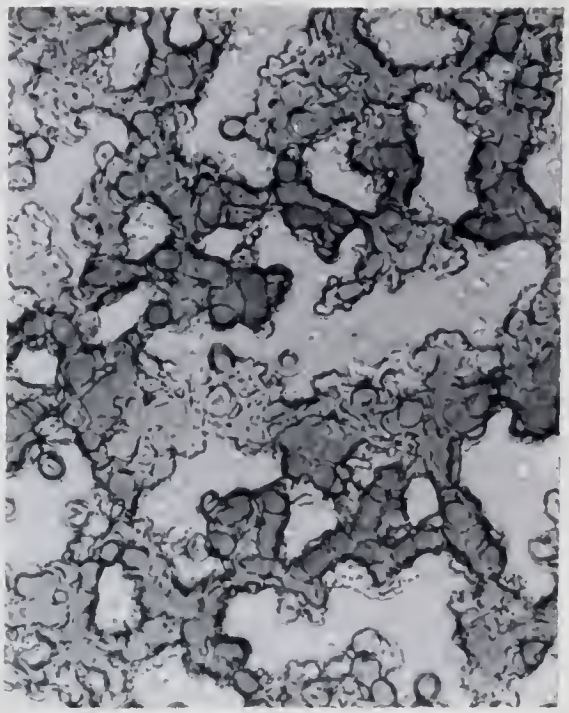


Location B

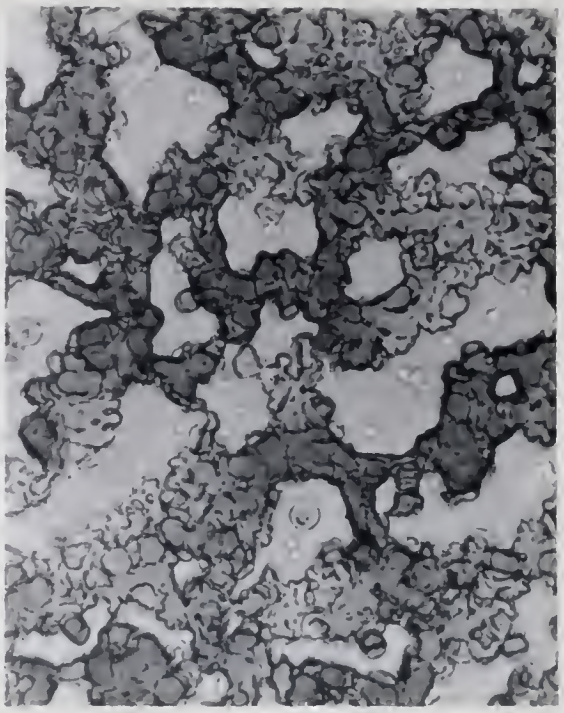
Blood Smear 2

After 20 Per Cent Normal Dose of HmDx: Blood Sludge Level, +2

Figure 13 - Continued

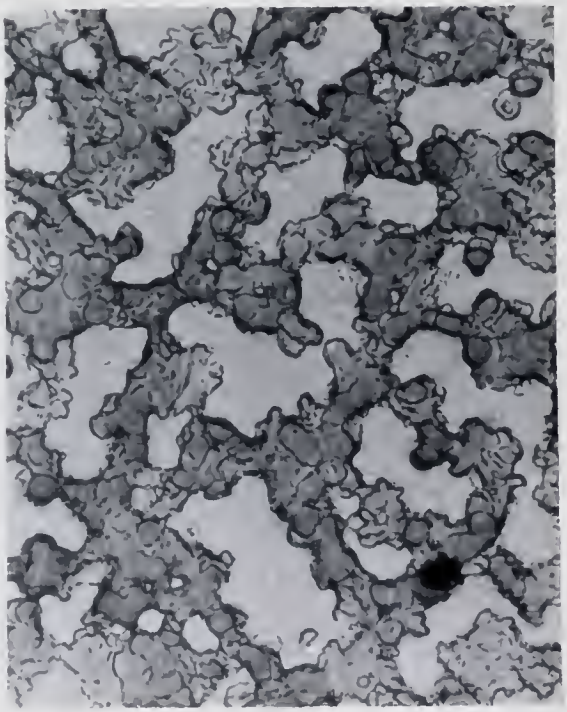


Location A

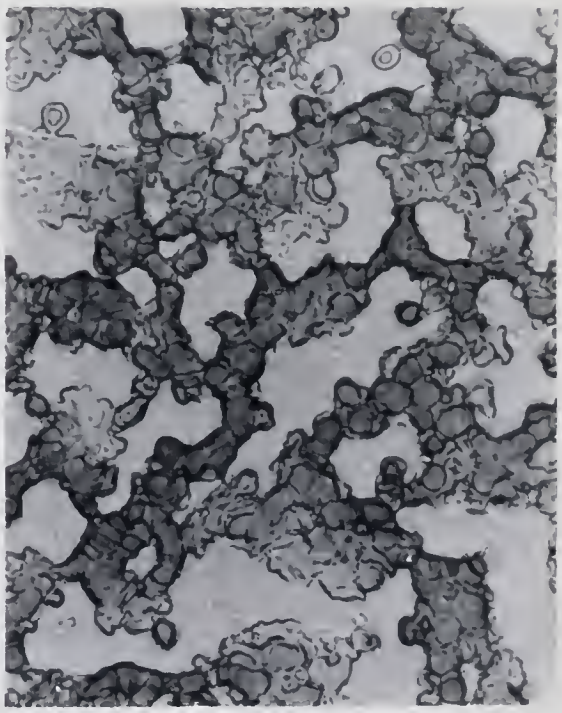


Location B

Blood Smear 1



Location A

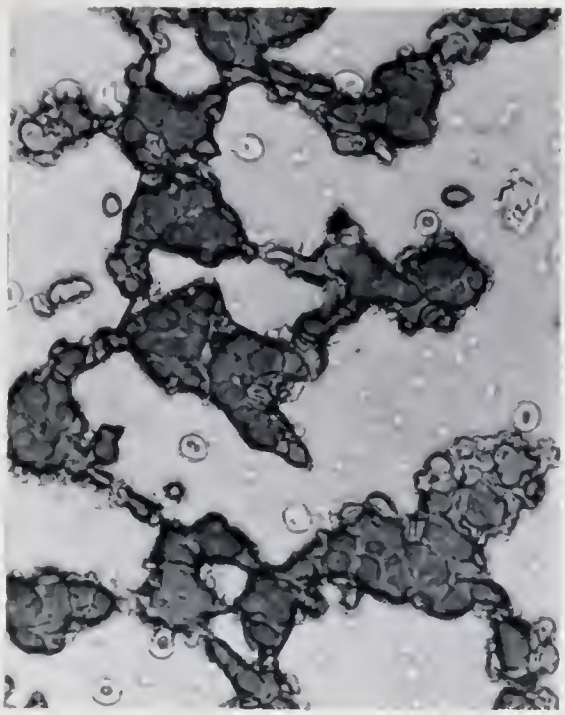


Location B

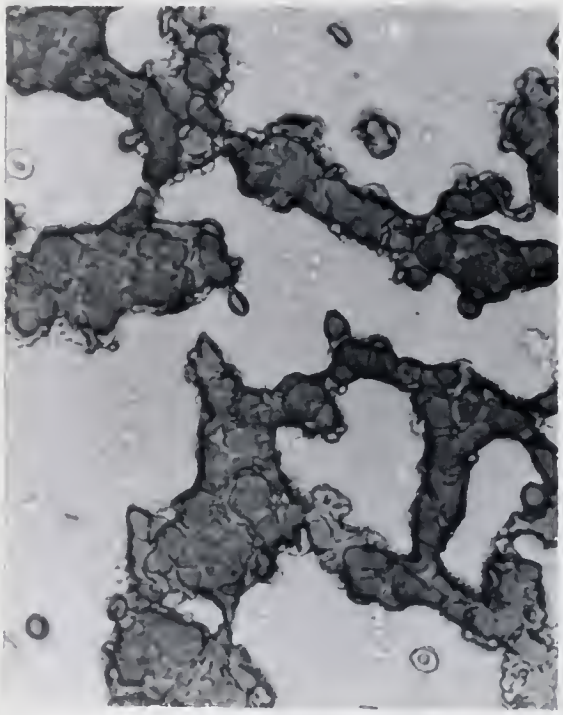
Blood Smear 2

After 50 Per Cent Normal Dose of HmDx: Blood Sludge Level, +3

Figure 13 - Continued

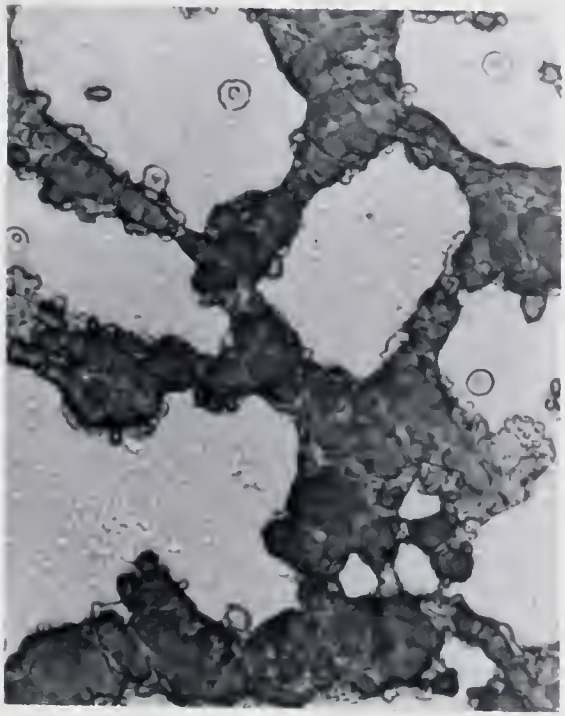


Location A

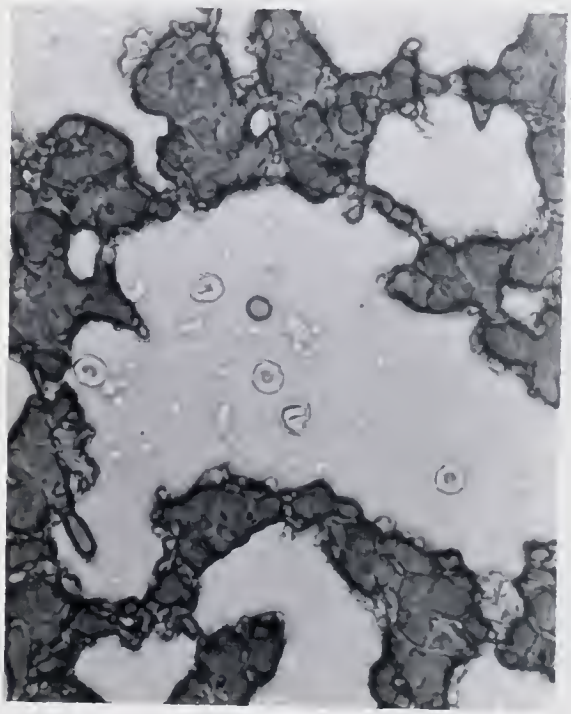


Location B

Blood Smear 1



Location A



Location B

Blood Smear 2

After 100 Per Cent Normal Dose of HmDx: Blood Sludge Level, +4

Figure 13 - Continued

Appendix B

Data From Experiment 1, Part III

Table 14

Food Intake (gms) per 12 Hour Interval and per Day for Each Animal and Group with Treatments Given at the End of Day 2

Normal Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	60	46	106	66	51	117	66	42	108	69	30	99	67	51	118	72	56	128
2	52	40	92	67	49	116	55	38	93	69	45	114	65	52	117	61	39	100
3	62	38	100	86	35	121	89	28	117	89	27	116	97	22	119	98	35	133
4	64	28	92	66	29	95	66	36	102	68	28	96	67	28	95	65	24	89
5	44	25	69	66	23	89	64	28	92	84	35	119	72	33	105	67	36	103
Total			459			538			512			544			554			553
Mean			92			108			102			109			111			111
SD			12.6			13.0			9.4			9.4			9.4			17.0

Saline Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	76	49	125	81	40	121	64	36	100	71	36	107	77	52	129	76	51	127
2	56	22	78	64	29	93	45	19	64	39	23	62	70	36	106	67	34	101
3	73	28	101	78	32	110	74	33	107	71	29	100	76	32	108	76	42	118
4	80	16	96	66	19	85	66	26	92	68	25	93	64	23	87	67	22	89
5	43	33	76	61	12	73	69	13	82	63	31	94	63	29	92	64	30	94
Total			476			482			445			456			522			529
Mean			95			96			89			91			104			106
SD			17.8			17.2			15.0			15.4			14.7			14.4

Table 14 - Continued

Dextran Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	78	48	126	95	34	129	80	77	157	73	29	102	67	34	101	64	49	113
2	53	35	88	51	34	85	57	30	87	54	30	84	53	29	82	57	31	88
3	66	32	98	64	29	93	56	21	77	65	31	96	70	44	114	86	31	117
4	72	27	99	71	26	97	60	24	84	68	26	94	66	24	90	82	23	105
5	57	19	76	64	34	98	71	26	97	61	18	79	51	32	83	52	35	87
Total			487			502			502			455			470			510
Mean			97			100			100			91			94			102
SD			16.5			15.0			29.0			8.4			12.1			12.4

HmDx (sludge) Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	96	42	138	95	34	129	86	4	90	57	18	75	72	50	122	76	60	136
2	52	32	84	65	29	94	49	19	68	66	31	97	62	45	107	64	36	100
3	58	32	90	78	34	112	70	38	108	66	26	92	66	32	98	74	37	111
4	51	26	77	67	27	94	24	34	58	58	34	92	56	31	87	60	29	89
5	52	50	102	57	42	99	62	40	102	67	36	103	68	38	106	70	37	107
Total			491			528			426			459			520			543
Mean			98			106			85			92			104			109
SD			21.5			13.4			19.3			9.3			11.5			15.6

Appendix B - Continued

Table 15

Water Intake (gms) per 12 Hour Interval and per Day for Each Animal and Group with Treatments Given at the End of Day 2

Normal Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	101	95	196	133	94	227	142	101	243	166	50	216	118	73	191	109	73	182
2	147	82	229	193	84	277	178	101	279	73	100	173	144	88	232	153	83	236
3	65	44	109	94	50	144	117	22	139	113	54	167	124	49	173	119	46	165
4	94	52	146	112	58	170	96	57	153	100	41	141	87	64	151	94	35	129
5	103	44	147	113	31	144	106	36	142	134	37	171	112	35	147	106	41	147
Total			827			962			956			868			894			859
Mean			165			192			191			174			179			172
SD			42.1			52.0			58.3			24.1			31.0			36.7

Saline Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	180	110	290	159	62	221	117	89	206	192	78	270	198	92	290	128	83	211
2	118	58	176	149	54	203	132	98	230	72	59	131	113	75	188	97	59	156
3	72	60	132	94	52	146	79	64	143	103	58	161	127	66	193	109	66	175
4	105	25	130	84	46	130	74	61	135	93	41	134	99	54	153	97	39	136
5	133	40	173	105	41	146	107	24	131	118	44	162	98	50	148	103	48	151
Total			901			846			845			858			972			829
Mean			180			169			169			172			194			166
SD			58.3			35.9			40.9			50.9			51.1			25.8

Table 15 - Continued

Dextran Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	336	223	559	385	159	544	285	93	378	399	271	670	323	182	505	345	161	506
2	92	31	123	98	59	157	106	61	167	119	72	191	102	75	177	124	53	177
3	133	71	204	126	68	194	92	79	171	149	81	230	130	83	213	134	71	205
4	49	37	86	87	46	133	63	35	98	81	41	122	98	35	133	102	35	137
5	152	17	169	125	62	187	119	44	163	103	54	157	84	47	131	86	46	132
Total			1141			1215			977			1370			1159			1157
Mean			228			243			195			274			232			231
SD			170.2			152.1			95.2			201.2			139.9			139.9

HmDx (sludge) Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	197	71	168	187	55	242	122	5	127	110	50	160	150	89	239	149	89	238
2	140	74	214	174	69	243	113	84	197	126	84	210	127	114	241	119	85	204
3	101	43	144	118	37	155	93	39	132	103	38	141	97	50	147	114	39	153
4	106	59	165	134	54	188	33	43	76	14	61	75	90	62	152	69	51	120
5	205	80	285	103	76	179	119	98	217	151	88	239	131	92	223	138	89	227
Total			1076			1007			749			825			1002			942
Mean			215			201			150			165			200			188
SD			54.1			35.3			42.5			56.9			42.1			45.0

Appendix B - Continued

Table 16

Mean Daily Body Weights (gms) for All Animals During 6 Days of Observations Made at 12 Hour Intervals with Treatments Given at the End of Day 2

Normal Control Group

Replication	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	1996	2022	2025	2049	2040	2061	2106
2	1970	2001	2007	2042	2091	2126	2132
3	2259	2233	2222	2230	2235	2266	2292
4	2215	2238	2270	2291	2309	2314	2329
5	1851	1878	1900	1917	1933	1962	2001
Total	10291	10372	10424	10529	10608	10729	10860
Mean	2058	2074	2085	2106	2122	2146	2172
SD	154.6	140.4	139.2	136.1	75.0	129.7	121.8

Saline Control Group

Replication	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	1878	1887	1939	1952	1978	2017	2070
2	2259	2170	2181	2189	2200	2221	2258
3	2513	2427	2437	2443	2437	2476	2502
4	2091	2081	2110	2140	2172	2199	2209
5	1864	1962	1973	2018	2056	2084	2100
Total	10605	10527	10640	10742	10843	10997	11139
Mean	2121	2105	2128	2148	2169	2199	2228
SD	244.4	187.8	177.9	169.7	156.2	157.2	153.3

Table 16 - Continued

Dextran Control Group

Replication	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	2185	2194	2243	2212	2225	2278	2339
2	1959	1928	1944	1954	1986	2012	2024
3	2495	2473	2456	2449	2459	2506	2540
4	2367	2342	2369	2383	2403	2437	2487
5	1747	1787	1784	1808	1835	1852	1900
Total	10753	10724	10796	10806	10908	11085	11290
Mean	2151	2145	2159	2161	2182	2217	2258
SD	270.6	251.6	255.4	245.8	239.2	249.3	253.6

HmDx (sludge) Group

Replication	Start	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	2273	2326	2322	2321	2298	2354	2418
2	2302	2321	2345	2385	2390	2399	2426
3	2299	2267	2282	2320	2313	2327	2357
4	2175	2144	2164	2164	2181	2189	2234
5	1716	1791	1830	1831	1928	1957	1987
Total	10765	10849	10943	11021	11110	11226	11422
Mean	2153	2169	2189	2204	2222	2245	2284
SD	223.3	200.4	189.8	200.3	161.5	159.7	163.8

Appendix B - Continued

Table 17

Home-cage Activity (number of round trips) for Each Animal and Group for Each 12 Hour and 24 Hour Interval Beginning 2 Days Before Treatments

Normal Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	47	45	92	30	40	70	34	57	91	36	53	89	26	34	60	34	27	61
2	51	19	70	37	14	51	39	20	59	37	8	45	40	13	53	39	18	57
3	94	38	132	49	44	93	82	46	128	51	36	87	46	36	82	40	48	88
4	51	32	83	12	12	24	15	20	35	25	10	35	27	15	42	27	7	34
5	68	20	88	24	5	29	32	2	34	23	5	28	23	2	25	11	3	14
Total			465			267			347			284			262			254
Mean			93			53			69			57			52			51
SD			20.8			25.7			35.9			26.0			18.9			25.1

Saline Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	125	54	179	45	31	76	18	37	55	26	50	76	37	32	69	27	22	49
2	38	20	58	11	12	23	10	15	25	10	10	20	17	17	34	15	16	31
3	89	19	108	41	28	69	44	42	86	28	27	55	33	17	50	25	27	52
4	63	44	107	19	36	55	39	20	59	35	15	50	28	22	50	28	11	39
5	65	29	94	43	18	61	44	15	59	22	18	40	38	14	52	30	12	42
Total			546			284			284			241			255			213
Mean			109			57			57			48			51			43
SD			39.3			18.3			19.4			18.3			11.1			7.5

Table 17 - Continued

Dextran Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	80	132	212	29	50	79	26	30	56	30	47	77	35	32	67	26	29	55
2	38	18	56	13	20	33	12	17	29	16	16	32	15	15	30	25	20	45
3	165	32	197	41	35	76	37	59	96	43	28	71	40	37	77	35	49	84
4	56	35	91	21	34	55	50	38	88	37	22	59	28	17	45	24	16	40
5	87	17	104	39	29	68	25	9	34	18	10	28	13	9	22	11	5	16
Total			660			311			303			267			241			240
Mean			132			62			61			53			48			58
SD			61.4			16.8			27.3			20.0			21.0			24.2

HmDx (sludge) Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T	pm	am	T
1	68	59	127	32	53	85	27	25	52	22	42	64	40	29	69	31	31	62
2	51	29	80	43	32	75	27	48	75	26	27	53	39	28	67	28	25	53
3	174	15	189	47	20	67	43	20	63	74	13	87	43	15	58	39	14	53
4	65	34	99	33	28	61	13	19	32	34	16	50	21	18	39	21	10	31
5	50	25	75	17	22	39	12	23	35	13	21	34	13	13	26	28	9	37
Total			570			327			257			288			259			236
Mean			114			65			51			58			52			47
SD			41.7			15.4			16.4			17.5			16.7			11.4

Appendix B - Continued

Table 18

Exploratory Activity (compartments entered) by Four Groups of Albino Rabbits During 12 Successive 1 Minute Exposures to a 4-Compartment "Square Maze" Over the 6 Day Observation Period Beginning at 2 Days Before Treatment

Normal Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T
1	4	3	7	5	0	5	6	8	14	7	7	14	4	6	10	3	3	6
2	6	9	15	7	8	15	5	4	9	2	3	5	2	2	4	4	2	6
3	6	7	13	7	2	9	3	0	3	2	1	3	3	1	4	0	0	0
4	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Total			37			29			26			22			19			12
Mean			7.4			5.8			5.2			4.4			3.8			2.4
SD			5.9			5.7			5.5			5.2			3.5			2.9

Saline Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T
1	3	5	8	8	3	11	3	3	6	4	4	8	5	3	8	3	3	6
2	5	5	10	4	5	9	5	3	8	2	2	4	0	0	0	1	0	1
3	2	2	4	6	3	9	7	5	12	7	5	12	2	0	2	2	2	4
4	0	1	1	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0
5	7	6	13	7	7	14	7	3	10	4	0	4	5	2	7	4	0	4
Total			36			46			36			28			17			15
Mean			7.2			9.2			7.2			5.6			3.4			3.0
SD			4.2			3.6			4.1			4.1			3.5			2.2

Table 18 - Continued

Dextran Control Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T
1	3	2	5	2	5	7	5	5	10	3	4	7	5	4	9	4	2	6
2	7	6	13	5	5	10	3	4	7	2	2	4	0	2	2	4	4	8
3	6	4	10	6	3	9	1	2	3	1	0	1	0	2	2	1	0	1
4	3	2	5	4	2	6	1	1	2	0	0	0	0	0	0	1	0	1
5	2	1	3	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Total			36			34			22			12			13			16
Mean			7.2			6.8			4.4			2.4			2.6			3.2
SD			3.7			2.8			3.6			2.7			3.3			3.2

HmDx (sludge) Group

Replication	Day 1			Day 2			Day 3			Day 4			Day 5			Day 6		
	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T	am	pm	T
1	2	2	4	3	2	5	4	2	6	6	4	10	6	1	7	4	2	6
2	3	6	9	9	11	20	12	8	20	11	7	18	7	4	11	6	3	9
3	6	3	9	6	7	13	4	2	6	0	0	0	2	3	5	2	0	2
4	4	6	10	5	1	6	2	0	2	0	0	0	0	0	0	0	0	0
5	1	1	2	1	1	2	0	0	0	0	0	0	1	0	1	0	0	0
Total			34			46			34			28			24			17
Mean			6.8			9.2			6.8			5.6			4.8			3.4
SD			3.2			6.5			7.0			7.3			4.0			3.6

Appendix B - Continued

Table 19

Blood Sludge Ratings, Based on Scale Described in Experiment 2, Part II, for Blood Smears Obtained From HmDx-Treated Animals at 12 Hour Intervals Beginning at 2 Days Before Treatments

(All control groups showed normal blood only)

Replication	Start	Day 1			Day 2			Day 3		
		am	pm	Mean	am	pm	Mean	am	pm	Mean
1	1	1	1	1	1	1	1	4	4	4
2	1	1	1	1	1	1	1	4	4	4
3	1	1	1	1	1	1	1	4	4	4
4	1	1	1	1	1	1	1	4	4	4
5	1	1	1	1	1	1	1	4	4	4
Mean	1.0			1.0			1.0			4.0

Replication	Day 4			Day 5			Day 6		
	am	pm	Mean	am	pm	Mean	am	pm	Mean
1	4	3	3.5	3	3	3	2	2	2
2	4	4	4	3	3	3	3	3	3
3	4	3	3.5	3	3	3	2	2	2
4	4	4	4	3	3	3	2	2	2
5	4	4	4	3	3	3	2	2	2
Mean			3.8			3.0			2.2

Appendix C

Data From Experiment 2, Part III

Table 20

Shock-escape Latencies (in sec.) for Three Successive 3-Trial Series for Six Replications Using Rabbits Tested at 1 Hour After Treatment

Normal Control Group

Replication	Series 1				Series 2				Series 3			
	1	2	3	total	1	2	3	total	1	2	3	total
1	36.8	63.7	9.8	110.3	5.7	5.7	5.3	16.7	5.3	5.8	9.3	20.4
2	14.8	6.9	7.5	29.2	5.0	7.7	19.2	31.9	5.0	7.5	13.3	25.8
3	15.3	13.9	11.3	40.5	9.2	7.3	41.1	57.6	11.9	23.1	5.4	40.4
4	16.0	8.7	11.7	36.4	10.6	6.1	8.6	25.3	7.3	8.7	7.8	23.8
5	127.3	4.6	63.5	195.4	9.1	6.0	5.0	20.1	10.2	6.5	5.0	21.7
6	166.5	24.0	8.3	198.8	25.8	4.6	6.5	36.9	22.7	16.4	7.7	46.8
Mean	62.8	20.3	18.7	101.8	10.9	6.2	14.3	31.4	10.4	11.3	8.1	29.8
SD	61.0	20.4	20.1	72.5	6.9	1.0	12.9	13.5	6.0	4.1	2.7	10.0

Saline Control Group

Replication	Series 1				Series 2				Series 3			
	1	2	3	total	1	2	3	total	1	2	3	total
1	69.4	19.6	18.6	107.6	7.7	4.5	3.3	15.5	3.1	3.1	4.3	10.5
2	65.3	10.5	8.0	83.8	5.2	5.7	12.2	23.1	4.7	15.8	9.5	30.0
3	266.3	9.7	11.8	287.8	8.7	18.6	15.7	43.0	10.0	5.7	14.5	30.2
4	20.0	19.4	9.3	48.7	9.6	13.9	14.3	37.8	11.1	16.2	18.2	45.5
5	33.5	14.9	6.5	54.9	4.6	4.6	3.8	13.0	5.1	3.1	5.0	13.2
6	143.3	31.7	26.1	201.1	203.6	29.4	184.4	417.4	175.6	100.0	254.2	529.8
Mean	99.6	17.6	13.4	130.6	39.9	12.8	38.9	91.6	34.9	24.0	50.9	109.9
SD	84.1	7.3	6.8	86.4	73.2	9.1	64.9	146.1	62.9	34.4	91.0	188.2

Table 20 - Continued

Dextran Control Group

Replication	Series 1				Series 2				Series 3			
	1	2	3	total	1	2	3	total	1	2	3	total
1	39.9	50.4	50.3	140.6	72.3	40.5	55.2	168.0	43.1	34.4	75.1	152.6
2	21.3	6.9	6.1	34.3	7.5	5.7	5.5	18.7	3.5	4.3	4.0	11.8
3	74.1	24.4	14.6	113.1	30.6	7.6	25.0	63.2	7.7	3.8	13.4	24.9
4	11.1	9.2	8.2	28.5	9.6	7.1	6.2	22.9	8.3	6.0	15.5	29.7
5	16.5	11.4	4.8	32.7	5.3	3.7	5.3	14.3	5.4	3.9	4.3	13.6
6	213.5	27.8	235.3	476.6	5.2	12.3	5.8	23.3	5.9	5.1	6.6	17.6
Mean	62.7	21.7	53.2	137.6	21.7	12.8	17.2	51.7	12.3	9.6	19.8	41.7
SD	70.6	15.0	82.9	145.7	25.6	35.5	18.3	54.5	13.8	11.1	25.1	50.0

HmDx (sludge) Group

Replication	Series 1				Series 2				Series 3			
	1	2	3	total	1	2	3	total	1	2	3	total
1	6.3	11.4	8.7	26.4	3.6	8.1	14.7	26.4	3.8	5.8	11.0	20.6
2	13.6	8.3	33.8	55.7	7.6	4.7	17.9	30.2	4.3	4.8	5.2	14.3
3	18.7	8.2	8.5	35.4	4.9	10.5	5.3	20.7	4.0	11.6	9.8	25.4
4	10.6	11.2	11.0	32.8	13.1	9.6	7.3	30.0	24.4	7.0	18.0	49.4
5	187.3	135.7	25.7	348.7	33.3	9.6	12.9	55.8	6.6	8.5	6.3	21.4
6	28.9	33.2	85.8	147.9	10.8	7.3	21.8	39.9	9.7	5.3	11.3	26.3
Mean	44.2	34.7	28.9	107.8	12.2	8.3	13.3	33.8	8.8	7.2	10.3	26.2
SD	64.4	46.0	27.1	115.3	15.8	1.9	5.7	11.3	7.3	2.3	4.1	11.0

Appendix C - Continued

Table 21

Blood Sludge Ratings for Blood Smears Taken Before (A) and After (B) Treatments and After Shock-escape Conditioning Trials (C)

Replication	Normal			Saline			Dextran			HmDx		
	A	B	C	A	B	C	A	B	C	A	B	C
1	1	1	1	1	1	1	1	1	1	1	4	4
2	1	1	1	1	1	1	2	2	2	1	4	4
3	1	1	1	1	1	1	1	1	1	1	4	4
4	1	1	1	1	1	1	1	1	1	1	4	4
5	1	1	1	1	1	1	1	2	1	1	4	4
6	1	1	1	1	1	1	1	1	1	1	4	4
Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.2	1.3	1.2	1.0	4.0	4.0

Appendix D

Data From Experiment 3, Part III

Table 22

Response latencies and Running Times (in sec.) for First Trials From Each of Three 5-Trial Food-Reinforced Test Series and One Extinction Series (4) Using Albino Rabbits Under Four Different Experimental Conditions

Normal Control Group

Replication	Response Latency				Running Time			
	1	2	3	4	1	2	3	4
1	41.4	6.8	4.1	18.2	50.3	13.9	29.8	3.8
2	56.5	1.8	1.3	3.0	45.2	4.5	7.1	3.6
3	97.1	12.3	5.4	4.1	42.7	14.7	7.0	9.0
4	64.8	17.4	23.5	9.1	103.9	42.5	8.8	16.7
5	46.7	20.9	18.1	19.4	249.2	3.8	87.1	12.5
6	99.9	2.8	-	-	60.0	359.4	-	-
Mean	66.1	10.3	10.5	10.8	91.9	73.1	28.0	9.1
SD	23.0	7.1	8.7	6.9	73.6	128.7	30.8	5.1

Saline Control Group

Replication	Response Latency				Running Time			
	1	2	3	4	1	2	3	4
1	114.7	3.8	13.6	15.5	98.1	20.1	208.9	22.2
2	47.3	8.5	2.6	4.9	39.5	6.0	7.4	4.0
3	63.1	15.6	8.4	6.8	27.4	145.8	11.9	4.6
4	37.6	3.7	4.3	1.8	22.0	3.0	2.5	1.6
5	27.9	16.7	15.4	29.4	102.2	8.5	20.6	100.0
6	44.6	4.1	4.8	4.1	102.5	9.5	5.0	6.7
Mean	59.2	8.7	8.2	10.4	65.3	32.2	42.7	23.2
SD	28.6	5.5	4.8	9.5	36.1	51.1	74.5	35.1

Table 22 - Continued

Dextran Control Group

Replication	Response Latency 1	2	3	4	Running Time 1	2	3	4
1	71.6	7.9	14.3	9.1	42.5	4.8	109.3	31.8
2	13.8	11.5	1.6	3.8	33.4	4.8	4.2	5.3
3	70.7	47.1	8.5	5.4	59.1	6.4	12.4	5.4
4	10.7	4.9	1.7	1.2	130.7	22.6	4.6	26.3
5	106.1	23.6	33.4	-	55.4	36.3	60.3	-
6	84.0	20.2	22.1	7.4	35.6	22.8	23.5	14.1
Mean	59.5	19.2	13.6	5.4	59.5	16.3	35.7	16.6
SD	35.2	14.1	11.4	2.8	33.2	11.9	38.0	10.8

HmDx (sludge) Group

Replication	Response Latency 1	2	3	4	Running Time 1	2	3	4
1	55.5	24.0	17.1	63.2	166.9	105.0	110.5	8.1
2	47.2	14.8	7.3	8.1	70.4	7.3	15.1	14.8
3	37.0	5.7	13.9	2.5	49.6	3.7	6.1	7.2
4	44.7	12.2	6.3	3.3	43.7	12.8	5.5	4.9
5	69.1	27.3	3.4	14.7	49.9	45.9	8.5	12.5
6	10.5	20.6	2.6	10.9	96.8	22.3	5.3	10.5
Mean	44.0	17.6	8.4	17.1	79.6	32.8	25.1	9.7
SD	18.0	5.6	5.3	21.0	42.9	35.1	37.7	3.3

Appendix D - Continued

Table 23

Total Response Latencies and Running Times (in sec.) for Three Successive Sets of Food Reinforced Learning Trials and One Extinction Series (4) in Four Groups of Albino Rabbits Tested in a 4-Compartment "Square Maze"

Normal Control Group

Replication	Response Latency				Running Time			
	1	2	3	4	1	2	3	4
1	71.5	25.0	33.9	47.9	123.6	49.6	183.1	107.4
2	81.4	9.8	11.1	11.5	76.5	25.5	24.3	22.5
3	138.2	34.9	60.0	55.3	192.4	99.8	99.0	224.4
4	106.0	51.2	65.1	57.4	286.7	82.9	106.9	96.6
5	76.3	51.7	40.5	65.0	294.6	124.6	257.2	90.0
6	210.9	-*	-	-	477.7	-	-	-
Mean	114.1	34.5	42.1	47.4	241.9	76.5	134.1	68.2
SD	48.8	16.0	19.4	18.8	131.8	35.3	79.5	76.7

Saline Control Group

Replication	Response Latency				Running Time			
	1	2	3	4	1	2	3	4
1	166.3	35.9	43.0	38.0	235.7	71.0	370.1	109.8
2	70.5	23.0	21.4	16.5	151.8	34.3	37.7	73.4
3	118.2	78.5	47.3	72.8	123.7	463.5	141.3	75.6
4	63.1	19.2	14.6	12.5	58.0	20.2	11.2	14.8
5	73.3	74.0	50.7	106.1	204.7	147.6	209.3	236.7
6	102.7	36.8	25.0	30.7	285.1	78.8	33.1	80.2
Mean	99.0	44.6	35.2	46.1	176.5	135.9	133.8	96.8
SD	35.8	23.3	13.9	33.2	74.8	152.0	126.5	59.3

Table 23 - Continued

Dextran Control Group

Replication	Response Latency 1	2	3	4	Running Time 1	2	3	4
1	107.6	56.5	65.3	57.3	91.0	137.0	260.3	79.4
2	37.9	23.9	18.9	24.3	87.2	41.2	32.2	34.0
3	103.7	94.9	40.7	43.5	150.6	63.7	139.8	385.4
4	37.2	35.4	19.8	20.6	308.6	93.0	27.1	173.0
5	151.2	54.7	99.4	-	469.2	219.6	323.9	-
6	111.0	56.8	63.6	22.3	149.1	170.2	134.6	54.3
Mean	91.6	53.7	51.3	33.6	209.3	120.8	103.0	145.2
SD	41.2	22.1	28.3	14.5	137.5	61.7	93.5	129.1

HmDx (sludge) Group

Replication	Response Latency 1	2	3	4	Running Time 1	2	3	4
1	921.1	145.1	101.3	-	114.4	221.2	246.0	-
2	102.7	53.2	43.4	42.5	200.2	103.1	63.1	173.8
3	71.0	25.2	34.8	12.6	94.6	40.8	43.2	57.7
4	85.2	42.0	23.2	27.2	120.8	50.5	39.8	33.0
5	179.0	77.7	47.1	91.0	153.3	233.8	265.8	-
6	29.2	57.7	34.8	30.6	192.5	77.6	57.1	85.7
Mean	231.4	66.8	47.4	40.8	146.0	121.2	119.2	87.6
SD	311.7	38.4	25.2	26.9	39.6	77.9	97.2	53.2

* Trials discontinued when either latency or running time exceeded 500 seconds

Appendix D - Continued

Table 24

Food Intake (Gms) During a 2 Hour Feeding Period Following Food Reinforced Learning Trials

Replication	Normal	Saline	Dextran	HmDx
1	16	30	23	25
2	21	24	22	26
3	19	16	30	18
4	26	15	17	39
5	27	20	21	29
6	19	24	20	21
Total	128	129	133	158
Mean	21.3	21.5	22.2	26.3
SD	4.0	5.2	4.0	6.7

Appendix D - Continued

Table 25

Blood Sludge Ratings for Blood Smears Taken Before (A) and After (B) Treatments and After Food Reinforced Learning Trials (C) for Four Groups of Rabbits

Replication	Normal			Saline			Dextran			HmDx		
	A	B	C	A	B	C	A	B	C	A	B	C
1	1	1	1	1	1	1	1	2	1	1	4	4
2	1	1	1	1	1	1	1	1	1	1	4	4
3	1	2	2	1	1	1	1	2	1	2	4	4
4	1	1	1	2	1	2	1	1	1	1	4	4
5	1	1	1	1	2	1	1	2	1	1	4	4
6	1	1	1	1	1	1	1	2	1	1	4	4
Total	6	7	7	7	7	7	6	10	6	7	24	24
Mean	1.0	1.2	1.2	1.2	1.2	1.2	1.0	1.7	1.0	1.2	4.0	4.0

Appendix E

Data From Experiment 1, Part III

Table 26

Total Responses (T) and Reinforced Responses (R) Before (A) and After (B) Treatment and During Extinction Series (C) 24 Hours After Treatments for Eight Rabbits Representing Two Replications of a 4 X 4 Latin Square

Latin Square #1

Animal Number		Normal			Saline			Dextran			HmDx		
		A	B	C	A	B	C	A	B	C	A	B	C
44	T	218	212	105	169	165	131	179	93	92	180	146	132
	R	85	76	63	93	68	61	93	61	41	101	86	54
40	T	200	186	48	187	150	111	167	168	82	168	105	152
	R	76	61	29	85	57	53	68	62	35	64	48	42
41	T	124	114	20	104	109	2	138	176	87	82	88	57
	R	81	55	15	62	43	2	65	80	59	56	47	32
33	T	142	171	68	149	128	77	163	185	98	176	164	112
	R	81	92	41	87	48	45	92	88	42	70	96	70
Mean	T	171	171	60	152	138	80	162	156	90	152	126	113
	R	81	71	37	82	54	40	79	73	44	73	69	50

Table 26 - Continued

Latin Square #2

Animal Number		Normal			Saline			Dextran			HmDx		
		A	B	C	A	B	C	A	B	C	A	B	C
32	T	274	259	201	244	274	162	295	260	193	189	109	16
	R	91	95	67	95	91	50	84	78	66	80	61	11
42	T	124	129	44	162	103	71	144	114	72	144	98	70
	R	85	88	36	86	79	48	90	74	45	96	55	53
30	T	177	159	93	105	133	40	159	121	104	114	91	46
	R	73	81	66	65	63	32	75	31	49	64	57	36
28	T	147	133	64	132	147	126	215	177	40	146	116	132
	R	104	95	35	103	102	68	93	89	28	100	82	66
Mean	T	180	170	101	161	164	100	201	168	102	148	104	66
	R	88	90	51	87	84	50	86	68	47	85	64	42

Means for Combined Scores

	A	B	C	A	B	C	A	B	C	A	B	C
T	176	170	80	157	151	90	181	162	96	150	115	90
R	84	80	44	85	69	45	83	70	46	79	67	46

Appendix E - Continued

Table 27

Analysis of Variance for the Data Obtained From Experiment 4. Separate Analyses are Shown for Total Responses, Reinforced Responses, Per Cent Reinforced Responses, and Number of Responses per Reinforcement. Difference Scores Were Used in Every Instance

Total Responses

	Latin Square #1				Latin Square #2			
Source	df	SS	MS	F	df	SS	MS	F
Treatments	3	530.56	176.85	1.83	3	2365.55	788.52	2.48
Subjects	3	2119.78	706.59	7.31*	3	379.05	126.35	.40
Days	3	2580.78	860.26	8.90*	3	519.55	173.18	.54
Error	6	580.27	96.71	-	6	1910.29	318.38	-
Total	15	5811.39	-	-	15	5174.44	-	-

	Combined Analysis, Squares 1 & 2			
Source	df	SS	MS	F
Treatments	3	1958.13	652.71	3.14
Between subjects in the same square	6	2498.83	416.47	2.01
Days	3	1543.00	514.33	2.48
Squares	1	319.41	319.41	1.54
Squares x days	3	1557.29	519.10	2.50
Squares x treatments	3	938.02	312.67	1.51
Error	12	2490.56	207.55	-
Total	31	11305.24	-	-

* $p < .05$

Table 27 - Continued

Reinforced Responses

	Latin Square #1				Latin Square #2			
Source	df	SS	MS	F	df	SS	MS	F
Treatments	3	2200.36	733.45	4.42	3	2065.69	688.56	2.04
Subjects	3	1315.82	438.61	2.64	3	253.67	84.56	.25
Days	3	3017.83	1005.94	6.06*	3	412.77	137.59	.41
Error	6	995.54	165.92	-	6	2024.29	337.38	-
Total	15	7529.55	-	-	15	4756.42	-	-

Combined Analysis, Squares 1 & 2

Source	df	SS	MS	F
Treatments	3	862.13	287.38	1.14
Between subjects in the same square	6	1569.49	261.58	1.04
Days	3	1042.14	347.38	1.38
Squares	1	47.77	47.77	.19
Squares x days	3	2388.46	796.15	3.16
Squares x treatments	3	3404.92	1134.64	4.51*
Error	12	3019.83	251.65	-
Total	31	12333.74	-	-

* $p < .05$

Table 27 - Continued

Per Cent Reinforced Responses

	Latin Square #1				Latin Square #2			
Source	df	SS	MS	F	df	SS	MS	F
Treatments	3	624.08	208.03	2.12	3	15.10	5.03	.03
Subjects	3	541.70	180.57	1.84	3	159.03	53.01	.30
Days	3	201.96	67.32	.69	3	120.70	40.23	.23
Error	6	587.79	97.97	-	6	1058.62	176.44	-
Total	15	1955.53	-	-	15	1353.45	-	-

Combined Analysis, Squares 1 & 2

Source	df	SS	MS	F
Treatments	3	319.17	106.39	.78
Between subjects in the same square	6	700.73	116.79	.85
Days	3	198.05	66.02	.48
Squares	1	391.30	391.30	2.85
Squares x days	3	124.61	41.54	.30
Squares x treatments	3	320.01	106.67	.77
Error	12	1646.41	137.20	-
Total	31	3700.28	-	-

Table 27 - Continued

Number of Responses per Reinforcement

	Latin Square #1				Latin Square #2			
Source	df	SS	MS	F	df	SS	MS	F
Treatments	3	1.93	.64	5.82*	3	.71	.24	.45
Subjects	3	.36	.12	1.09	3	.76	.25	.47
Days	3	.39	.13	1.18	3	.24	.08	.15
Error	6	.66	.11	-	6	3.15	.53	-
Total	15	3.34	-	-	15	4.86	-	-

Combined Analysis, Squares 1 & 2

Source	df	SS	MS	F
Treatments	3	1.53	.53	1.66
Between subjects in the same square	6	1.12	.37	1.16
Days	3	.12	.04	.13
Squares	1	.27	.27	.84
Squares x days	3	.51	.17	.53
Squares x treatments	3	1.11	.37	1.16
Error	12	3.81	.32	-
Total	31	8.47	-	-

* $p < .05$

Appendix E - Continued

Table 28

Blood Sludge Measures From Eight Rabbits Representing Two Latin Square Replications of a Test of Bar Press Performance on a DRL Schedule. Smears Were Taken Before (Smear A) and After (Smear B) Treatment, After the 1 Hour Test Session (Smear C), and 24 Hours Later Following the 1 Hour Extinction Series (Smear D)

Latin Square #1

Animal Number	Normal				Saline				Dextran				HmDx			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
33	1	1	1	1	1	1	1	1	1	3	2	1	1	4	4	4
40	1	1	1	1	1	1	1	1	1	2	2	1	1	4	4	4
41	1	1	1	1	1	1	1	1	1	2	2	1	1	4	4	4
44	1	1	1	1	1	1	1	1	1	1	1	1	1	4	4	4
Total	4	4	4	4	4	4	4	4	4	8	7	4	4	16	16	16
Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.8	1.0	1.0	4.0	4.0	4.0

Latin Square #2

Animal Number	Normal				Saline				Dextran				HmDx			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
28	1	1	1	1	1	1	1	1	1	2	1	1	1	4	4	4
30	1	1	1	1	1	1	1	1	1	1	1	1	1	4	4	4
32	1	1	1	1	1	1	1	1	1	2	2	2	1	4	4	4
42	1	1	1	1	1	1	1	1	1	2	2	2	1	4	4	4
Total	4	4	4	4	4	4	4	4	4	7	6	6	4	16	16	16
Mean	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.8	1.5	1.5	1.0	4.0	4.0	4.0

Appendix E - Continued

Table 29

Food Intake (Gms) by Albino Rabbits During Each 2 Hour Posttreatment Feeding Period Following Testing on a Bar Press Task Using a DRL Reinforcement Schedule

Animal #	Normal	Saline	77K Dextran	HmDx
		Latin Square 1		
#33	83	76	-	88
#40	-	78	70	67
#41	73	-	100	69
#44	96	73	77	-
Total	252	227	247	224
Mean	84	76	82	75
		Latin Square 2		
#28	110	113	95	74
#30	84	78	68	77
#32	76	73	76	50
#42	65	61	77	61
Total	335	325	316	262
Mean	84	81	79	66
		Latin Squares 1 and 2		
Mean	84	79	80	69

Appendix E - Continued

Table 30

Performance on a Bar Press Task Under a DRL Schedule by Albino Rabbits During 5 Days Before and for 5 Days After Treatments

Animal #	31			37			38			39		
Treatment	Normal			Saline			Dextran			HmDx		
	T	C	%	T	C	%	T	C	%	T	C	%
Before Treatment												
Day 5	200	50	25.0	293	78	26.6	177	89	50.3	216	76	35.2
Day 4	181	66	36.4	223	90	40.3	194	108	55.7	169	73	43.2
Day 3	135	54	40.0	226	96	42.5	158	96	60.6	137	72	52.5
Day 2	159	59	37.1	207	99	47.9	229	88	38.4	202	99	49.0
Day 1	159	51	32.1	200	87	43.5	171	99	57.9	189	94	49.7
Total	834	280	170.6	1149	450	200.8	929	480	262.9	913	414	229.6
Mean	167	54	34.1	230	90	40.2	186	96	52.6	183	83	45.9
After Treatment												
Day 1	158	66	41.7	169	94	55.5	159	63	39.6	132	54	40.9
Day 2	187	42	22.4	186	95	50.2	185	95	51.4	181	89	49.1
Day 3	220	63	28.6	184	106	57.6	129	61	47.3	142	58	40.8
Day 4	254	42	16.5	194	97	50.0	141	74	52.5	176	87	49.4
Day 5	209	49	23.4	191	99	51.8	203	89	43.7	174	89	51.1

REFERENCES

Adamkiewicz, V. W. and Adamkiewicz, Lidia. Glucose and the dextran "anaphylactoid" inflammation. Amer. J. Physiol., 1960, 198, 51-53.

Balke, B. Klin. Wschr., 1944, 23, 223. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963, p. 178.

Barcroft, J. Presidential address (abridged) on anoxaemia. Delivered before the Physiological section of the British Association for the Advancement of Science. Lancet, 1920, 2, 485-489.

Bartlett, R. G., Jr. and Phillips, N. E. Restraint adaptation and altitude tolerance in the rat. USN Sch. Aviat. Med. Res. Rep., 1960, 2, 10 (Psychol. Abstr.).

Bauer, L. H. Aviation Medicine. Baltimore: Williams and Wilkins, 1928. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963, p. 314.

Bergen, J. R., Hunt, Charlotte and Hoagland, H. Blood flow and oxygen consumption of brain in relation to adrenal cortical action. Fed. Proc., 1952, 11, 12.

Bigelow, W. G., Heimbecker, R. O. and Harrison, R. C. Intravascular agglutination of erythrocytes (sludged blood), vascular stasis and the sedimentation rate of blood in trauma. Arch. Surg., 1949, 59, 667-690.

Blalock, A. A consideration of the present status of the shock problem: Problems on shocks. Surg., 1943, 14, 487-508.

Bloch, E. H. The in vivo microscopic intravascular and vascular reactions in acute poliomyelitis. Amer. J. Med. Sci., 1953, 226, 24-37.

Bloch, E. H. Microscopic observations of the circulating blood in the bulbar conjunctiva in man in health and disease. Ergebn. Anat., 1956, 35, 1-98.

Boyd, R. J. and Connolly, J. E. Tolerance of anoxia of the dog's brain at various temperatures. Surg. Forum, 1961, 12, 408-401.

Brooks, F. H., Dragstedt, L. R., Warner, Louise and Knisely, M. H. The sequence of circulatory changes following severe thermal burns. Arch. Surg., 1950, 61, 387-418.

Bullard, R. W. and Synder, J. L. Hypoxic alteration of righting ability and pain threshold in two mammalian species. Proc. Soc. Exp. Biol. Med., 1961, 106, 341-343.

Clowes, G. H. A., Jr. Extracorporeal maintenance of circulation and respiration. Physiol. Rev., 1960, 40, 826-919.

Cope, O., Nardi, G. L., Quijano, R. L., Rovit, R. L., Stanbury, J. B. and Wight, A. Metabolic rate and thryoid function following acute thermal trauma in man. Ann. Surg., 1953, 137, 165-174.

Crowell, J. W. and Houston, B. Effect of acidity on blood coagulation. Amer. J. Physiol., 1961, 201, 379-382.

Cullen, C. F. and Swank, R. L. Intravascular aggregation and adhesiveness of the blood elements associated with alimentary lipemia and injections of large molecular substances. Effect on blood-brain barrier. Circulation, 1954, 9, 335-346.

Cyrus, E., Close, A. S., Foster, L. L., Brown, D. H. and Ellison, E. H. Effect of low molecular weight dextran on infarction after expermental occlusion of the middle cerebral artery. Surg., 1962, 52, 25-31.

DeBaron, L. Instrumental reward discrimination in rabbits. Electroencephalographic and behavioral effects of a series of lysergic acid derivatives. In 2nd International Pharmacological Meeting Prague, 1962. Biochem. Pharmacol., 1963, 12, Supp. p. 269 (Abstr.).

Deutsch, J. A. Nest building behavior in domestic rabbits under seminatural condition. Brit. J. Animal Behav., 1957, 5, 53-54.

Ditzel, J. The nature of the intravascular erythrocyte aggregation in diseases with particular reference to Diabetes Mellitus. Acta. Med. Scand., 1955, 152, 371-378.

Ditzel, J. Relationships of blood protein composition to intravascular erythrocyte aggregation (sludged blood). Acta Med. Scand., 1959, 164 (Suppl. 343).

Ditzel, J. (Univer. of Copenhagen) Microvascular changes in diabetes. Seminar at Univer. of Florida Medical School, 5/20/64.

Ditzel, J. and Sagild, U. Morphologic and hemodynamic changes in the smaller blood vessels in Diabetes Mellitus. II. The degenerative and hemodynamic changes in the bulbar conjunctiva of normotensive diabetic patients. Cited in: Ditzel, 1959. New England J. Med., 1954, 250, 587-594.

Edwards, A. L. Experimental Design in Psychological Research. Rev. ed. New York: Rinehart & Co., Inc., 1960.

Evans, J. R. and Hughes, R. E. Respiratory quotient of scorbutic guinea pigs. Life Sci., 1964, 3, 15-17.

Fahraeus, R. Suspension stability of the blood. Physiol. Rev., 1929, 9, 241-274.

Fahraeus, R. The influence of the rouleaux formation of the erythrocytes on the rheology of blood. Acta Med. Scand., 1958, 161, 151-165.

Fajers, C. M. and Gelin, L. E. Kidney, liver, and heart damage from trauma and from induced intravascular aggregation of blood cells: An experimental study. Acta Path. et Microbol. Scand., 1959, 46, 97-104.

Ferster, C. B. and Skinner, B. F. Schedules of Reinforcement. New York: Appleton-Century-Crofts, 1957.

Fink, H. K. Mind and Performance. A comparative study of learning in mammals, birds, and reptiles. New York: Vantage Press, 1954.

Finsterbusch, W., Long, D. M., Sellers, R. D., Amplatz, K., and Lillehei, C. W. Renal arteriography during extracorporeal circulation in dogs, with a preliminary report upon the effects of low molecular weight dextran. J. Thorac. Cardio. Surg., 1961, 41, 252-262.

Forbes, H. S. Study of blood vessels on cortex of living mammalian brain-description of technique. Anat. Rec., 1954, 120, 309-315.

Fowler, E. P. Capillary circulation with changes in sympathetic activity. I. Blood sludge from sympathetic stimulation. Proc. Soc. Exp. Biol. Med., 1949, 72, 592-594.

Fromer, R. Conditioned vasomotor responses in the rabbit. J. comp. physiol. Psychol., 1963, 56, 1050-1055.

Frunder, H. Der Stoffevechsel des entzündeten und geschädigten Gewebes. In: Jasmin, G. and Robert, A. (Eds) Mechanism of Inflammation. Montreal, Acta, 1953. p. 175. Cited in: Ungar, G. Excitation. Springfield, Ill. Charles C. Thomas, 1963, p. 307.

Geiger, A. Correlation of brain metabolism and function by the use of a brain perfusion method in situ. Physiol. Rev., 1958, 38, 1-20.

Gelin, L. E. Studies in anemia of injury. Acta Chir. Scand., 1956, Suppl. 210.

Gelin, L. E. The significance of intravascular aggregation of blood cells following injury. Bull. Soc. Int. Chir., 1959, 18, 4-19.

Gelin, L. E. Rheologic disturbances and the use of low viscosity dextran in surgery. Rev. Surg., 1962, 19, 385-400.

Gelin, L. E. A method for studies of aggregation of blood cells, erythrostasis and plasma skimming in branching tubes. Biorheology, 1963, 1, 119-127.

Gelin, L. E., Korsan-Bengtsen, K., Ygge, J. and Zederfeldt, B. Influence of low viscous dextran on the hemostatic mechanism. Acta Chir. Scand., 1961, 122, 324-328.

Gelin, L. E., Persson, E. and Zederfeldt, B. Influence of low viscous dextran on the electrolyte balance in healthy subjects. Acta Chir. Scand., 1961, 122, 329-332.

Gelin, L. E. and Shoemaker, W. C. Hepatic blood flow and microcirculatory alterations induced by dextran of high and low viscosity. Surg., 1961, 49, 713-718.

Gelin, L. E., Sölvell, L. and Zederfeldt, B. The plasma volume expanding effect of low viscous dextran and Macrodex. Acta Chir. Scand., 1961, 122, 309-323.

Gelin, L. E. and Zederfeldt, B. Experimental evidence of the significance of disturbances in the flow properties of blood. Acta Chir. Scand., 1961, 122, 336-342.

Gelin, L. E. and Thoren, O. K. A. Influence of low viscous dextran on peripheral circulation in man. Acta Chir. Scand., 1961, 122, 303-308.

Gellhorn, E., Ingraham, R. C. and Moldavsky, L. The influence of hypoglycemia on the sensitivity of the central nervous system to oxygen want. J. Neurophysiol, 1938, 1, 301-312.

Gellhorn, E. and Kessler, M. Interaction of electroshock and insulin hypoglycemia. Arch. Neurol. and Psychiat., 1943, 49, 808-819.

Gessler, H. Untersuchungen über Entzündung. Arch. Exp. Path. Pharmakol., 1932, 163, 456-477. In: Ungar, G. Excitation. Springfield, Ill.: Charles C. Thomas, 1963, p. 307.

Gilmore, J. P. and Fozzard, H. A. Liver function following thermal injury. Amer. J. Physiol., 1960, 198, 491-495.

Gregersen, M. I., Peric, B., Usami, S., Chien, S., Chang, C. and Sinclair, D. G. Studies on blood viscosity at low shear rates: Effects of low and high molecular dextrans. Fed. Proc., 1963, 22, 641 (Abstr.).

Grollman, S. A study of oxygen debt in the albino rat. J. Exp. Zool., 1955, 128, 511-523.

Grotte, G. Passage of dextran molecules across the blood-lymph barrier. Acta Chir. Scand., 1956 (Suppl. 211).

Gwinn, G. T. The effects of punishment on acts motivated by fear. J. exp. Psychol., 1949, 39, 260-269.

Hamberger, A. and Hyden, H. Inverse enzymatic changes in neurous and glia during increased function and hypoxia. J. Cell. Biol., 1963, 58 (Suppl. 203).

Harris, J. M. and West, G. B. Rats resistant to the dextran anaphylactoid reaction. Brit. Jour. Pharmacol. and Chemother., 1963, 20, 550-562.

Hegedus, S. A. and Shackelford, R. T. Effect of cervical sympathectomy on obstructed cerebral blood flow. J. Amer. Med. Assoc., 1963, 183, 1091-1093.

Heimbecher, R. O. and Bigelow, W. G. Intravascular agglutination of erythrocytes (sludged blood) and traumatic shock. Surg., 1950, 28, 461-473.

Himwich, H. E. Effect of shock therapies on the brain. In: The Biology of Mental Health and Disease. New York: Paul B. Hoeber, Inc., 1952, pp. 548-561.

Hinshaw, J. R., Pories, W. J., Harris, P. D., Davis, T. P. and Schwartz, S. I. Effect of the molecular size of dextran on liver and kidney oxygen tension. Surg. Forum, 1960, 11, 360.

Hirsch, H., Bolte, H., Schandig, A. and Tonnis, D. Uber die weiderbelebung des gehirns bei hypothermie. Pflugers Archiv., 1957, 265, 328. Cited in: Wright, R. L. Experimental cerebral ischemia. Angiol., 1965, 16, 397-404.

Hirschboeck, J. S. and Woo, M. A clinical evaluation of the blood "sludge" phenomenon. Amer. J. Med. Sci., 1950, 219, 538-541.

Hoyt, J. W. and Soli, Giorgio. Algal cultures: ability to reduce turbulent friction in flow. Sci., 1965, 149, 1509-1511.

Hunter, J. Works. Palmer, I. (Ed.) Hunter's Lectures, 1786. London, 1835. Cited in: Fahraeus, R. Suspension stability of the blood. Physiol. Rev., 1929, 9, 241-274.

Hurder, W. P. Quantitative cortical changes produced by anoxia in rats. J. comp. physiol. Psychol., 1952, 45, 362-367.

Jackson, J. Personal communication.

Jones. Edinburgh Med. Surg. J., 1843, 60. Cited in: Fahraeus, R. Suspension stability of the blood. Physiol. Rev., 1929, 9, 241-274.

Jongbloed, J. Klin. Wschr., 1935, 14, 1564. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963.

Kety, S. S. Blood flow and metabolism of the human brain in health and disease. In: Elliott, K. A. C., Page, I. H. and Quastel, J. H. (Eds.) Neurochemistry: The chemical dynamics of brain and nerve. Springfield, Ill.: Charles C. Thomas, 1955, pp. 294-310.

King, B. D., Sokoloff, L. and Wechsler, R. L. The effects of l-epinephrine and l-nor-epinephrine upon cerebral circulation and metabolism in man. J. Clin. Invest., 1952, 31, 273-279.

Klein, S. J., Mendelson, E. S. and Gallagher, T. J. The effects of reduced oxygen intake on auditory threshold shifts in a quiet environment. J. comp. physiol. Psychol., 1961, 54, 401-404.

Klosovskii, B. N. Blood circulation in the brain. (Translated from the Russian by Behmoaram, A. D.) Jerusalem: Israel program of scientific translations Ltd., 1963 (Published through NSF, Washington, D. C.).

Knisely, M. H. A method of illuminating living structures for microscopic study. Anat. Rec., 1936, 64, 499-524.

Knisely, M. H. An improved fused quartz rod illuminator. Anat. Rec., 1938, 71, 503.

Knisely, M. H. The histophysiology of peripheral vascular beds. Amer. Assoc. Advancement Sci., Washington, D. C.: The Science Press, 1940, No. 13. pp 303-307.

Knisely, M. H. An annotated bibliography on sludged blood. Postgrad. Med., 1951, 10, 69-93.

Knisely, M. H. The settling of sludge during life. Acta Anat., 1961 (Suppl. 41).

Knisely, M. H. and Bloch, E. H. Microscopic observations of intravascular agglutination of red cells and consequent sludging of the blood in human diseases. Anat. Rec., 1942, 82, 426.

Knisely, M. H., Bloch, E. H., Brooks, F. and Warner, L. Microscopic observations of the circulating blood of nine healthy normal horses all of which had unagglutinated circulating blood cells and high in vitro erythrocyte sedimentation rates. Amer. J. Med. Sci., 1950, 219, 249-267.

Knisely, M. H., Bloch, E. H., Eliot, T. S. and Warner, Louise. Sludged blood. Sci., 1947, 106, 431-440.

Knisely, M. H., Bloch, E. H., Eliot, T. S. and Warner, Louise. Sludged blood. Transactions of the American Therapeutic Society, 1950, 48-49, 95-112.

Knisely, M. H., Bloch, E. H. and Warner, Louise. Selective phagocytosis. I. Microscopic observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and the rate of phagocytic removal of particles from the blood . K. Danske Videnskabernes Selskab, Biologiske Skrifter, Copenhagen, vol. 4, No. 7, 1948. Cited in: Knisely, M. H., Bloch, E. H., Eliot, S. and Warner, Louise. Sludged blood. Sci., 1947, 106, 431-440.

Knisely, M. H., Eliot, T. S. and Bloch, E. H. Sludged blood in traumatic shock. I. Microscopic observations of the precipitation and agglutination of blood flowing through vessels in crushed tissues. Arch. Surg., 1945, 51, 220-236.

Knisely, M. H., Stratman-Thomas, Wl K. and Eliot, T. S. In vivo studies of the progressive pathologic changes in experimental malaria of monkeys, microscopic observation of the changes in the blood and circulation in the small vessels of internal organs in living animals (motion picture). South. Med. J., 1940, 33, 1221.

Knisely, M. H., Stratman-Thomas, W. K. and Eliot, T. S. Capillary circulation in the malaria-infected monkey; a cinematographic study. Abstract of report before 13th annual meeting Central Soc. Clinical Res., Chicago, Nov. 2, 1940. J. Amer. Med. Assoc., 1941, 116, 2430-2431.

Knisely, M. H., Stratman-Thomas, W. K., Eliot, T. S. and Bloch, E. H. Knowlesi malaria in monkeys. I. Microscopic pathological circulatory physiology of rhesus monkeys during acute Plasmodium knowlesi malaria. J. Nat. Mal. Soc., 1945, 4, 285-300.

Knisely, M. H., Stratman-Thomas, W. K., Eliot, T. S. and Bloch, E. H. Knowlesi malaria in monkeys. II. A first step in the separation of the mechanical pathologic circulatory factors of one sludge disease from possible toxic factors of that disease. Amer. J. Physiol., 1949, 159, 575 (Abstr.).

Knisely, M. H., Stratman-Thomas, W. K., Eliot, T. S. and Bloch, E. H. Knowlesi malaria. II. A first step in separation of mechanical pathological circulatory factors of one sludge disease from possible specific toxic factors of that disease. Anat. Rec., 1951, 109, 53 (Abstr.).

Knisely, M. H., Warner, Louise and Harding, F. Anti-mortem settling. Microscopic observations and analyses of the settling of agglutinated blood cell masses to the lower side of vessels during life: a contribution to the biophysics of disease. Angiol., 1960, 11, 535-588.

Korner, P. I. Circulatory adaptations in hypoxia. Physiol. Rev., 1959, 39, 687-730.

Krogh, A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J. Physiol., 1919, 52, 409-415.

Lack A. R. The occurrence of intravascular agglutinations in avian malaria. Sci., 1942, 96, 520-521.

Lack, A., Adolph, W., Ralston, W., Leiby, C., Winsor, T. and Griffith, G. Biomicrography of the conjunctival vessels in hypertension: A capillary "hypertension pattern" and the occurrence of intravascular clumping (sludged blood) are described. Amer. Heart J., 1949, 38, 654-664.

Landis, E. M. Microinjection studies of capillary permeability. III. The effect of lack of oxygen on the permeability of the capillary wall to fluid and to plasma proteins. Amer. J. Physiol., 1928, 83, 528-542.

Lassen, N. A. Cerebral blood flow and oxygen consumption in man. Physiol. Rev., 1959, 39, 183-238.

Laufman, H. Sludged blood. A critique. Arch. Surg., 1951, 62, 486-492.

Levy, R. L. Advanced heart failure. General symptoms. Nelson's Loose-Leaf Medicine, 1920, 4, 294. Cited in: Peters, J. P. and Van Slyke, D. Hemoglobin and Oxygen. Carbonic acid and acid-base balance. Baltimore: Wms. and Wilkin, 1931, p. 585.

Licklider, J. C. R. A gridless, wireless rat-shocker. J. comp. physiol. Psychol., 1951, 44, 334-337.

Liebesny, P. Med. Wschr., 1922, vol. 18, p. 228.. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia, Chicago: Univer. of Chicago Press, 1963.

Lister, J. On early stages of inflammation. Phil. Trans., London, 1858, pp. 645-702.

Livesey, P. J. A note on double alternation by rabbits. J. comp. physiol. Psychol., 1964, 57, 104-107.

Livesey, P. J. Comparisons of double alternation performance of white rats, rabbits, and cats. J. comp. physiol. Psychol., 1965, 59, 155-158.

Lofstrom, B. Intravascular aggregation and oxygen consumption. Aggregation of red blood cells produced by high molecular weight dextran or by hypothermia. Acta Anaesth. Scand., 1959, 3, 41-51.

Lofstrom, B. and Zederfeldt, B. Wound healing after induced hypothermia. II. An experimental investigation of the importance of intravascular aggregation of blood cells. Acta Chir. Scand., 1957, 113, 272-281.

Long, D. M., Jr. Status of plasma expanders in open heart surgery. Diseases of the Chest, 1962, 41, 578-581.

Long, D. M., Jr., Sanchez, L., Varco, R. L. and Lillehei, C. W. The use of low molecular dextran and serum albumin as plasma expanders in extracorporeal circulation. Surg., 1961, 50, 12-28.

Luft, U. C. In: Armstrong, H. G. (Ed.) Aerospace Medicine. Baltimore: Wms. and Wilkins, 1961, p. 138. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963, p. 359.

Lutz, B. R. Intravascular agglutination of formed elements of blood. Physiol. Rev., 1951, 31, 107-130.

Lutz, B. R., Fulton, G. P. and Akers, R. P. White thromoembolism in the hamster cheek pouch after trauma, infection and neoplasia. Circulation, 1951, 3, 339-352.

Mayerson, H. S., Wolfram, C. G., Shirley, H. H., Jr. and Wasserman, K. Regional differences in capillary permeability. Amer. J. Physiol., 1960, 198, 155-160.

McFarland, R. A. Arch. Psychol., 1932, No. 145. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963, p. 314.

McFarland, R. A. and Forbes, W. H. The effects of variations in the concentration of oxygen and of glucose on dark adaptation. J. Gen. Physiol., 1940, 24, 69-78.

Neely, W. A. and Youmans, J. R. Anoxia of canine brain without damage. J. Amer. Med Assoc., 1963, 183, 1085-1087.

Nielson, H. C., Zimmerman, J. M. and Cooliver, J. C. Effect of complete arrest of cerebral circulation on learning. J. comp. physiol. Psychol., 1963, 56, 974-978.

Norris, R. On the causes of various phenomena of attraction and adhesion, as exhibited in solid bodies, films, vesicles, liquid globulin, and blood corpuscles. Trans. Roy. Soc. London, 1869, vol. 17, 429-436.

Odell, L, Aragon, G. and Pottinger, R. E. Relationship between erythrocyte sedimentation rate, sludged blood and plasma proteins during pregnancy. Amer. J. Obst. and Gynec., 1947, 54, 596-610.

Osol, A. and Farrar, G. E. The Dispensatory of the United States. (25th edition) Philadelphia: J. B. Lippincott Co., 1955, p. 1813.

Peters, J. P. and Van Slyke, D. D. Hemoglobin and oxygen. Carbonic acid and acid-base balance. Baltimore: Williams and Wilkins, 1931, p. 585.

Ploman, G. Hygiea, 1920, 82, 363. Cited in: Ditzel, J. The nature of intravascular erythrocyte aggregation in diseases with particular reference to Diabetes Mellitus. Acta Med. Scand., 1955, 152, 371-378.

Pritchard, W. H., Moir, T. W. and MacIntyre, W. J. Measurement of the early disappearance of iodinated (I^{131}) serum albumin from circulating blood by a continuous recording method. Circulation Res., 1955, 3, 19.

Rand, P. W. and Lacombe, Eleanor, Hemodilution, tonicity, and blood viscosity. J. Clin. Invest., 1964, 43, 2214-2226.

Robbins, R. C. The relationship of an unidentified volatile substance to the intravascular agglutination of erythrocytes. Univer. of Florida: Florida Agricultural Experiment Stations Report of Progress. State Project No. 1948, June, 1963 to June, 1964.

Robbins, R. C. Effect of vitamin C and flavonoids on blood cell aggre- and capillary resistance. Int. J. Vit. Res., to be published.

Robertson, H. S., Wolf, S. and Wolff, H. G. Blood "sludge" phenomenon in human subjects. Notes on its significance and on the effects of vasomotor drugs. Amer. J. Med. Sci., 1950, 219, 534-537.

Sawin, P. B., Denenberg, V. H., Ross, S., Hafter, E. and Zarrow, M. X. Maternal behavior in the rabbit: hair loosening during gestation. Amer. J. Physiol., 1960, 198, 1099-1102.

Schneiderman, N. and Gormezano, I. Conditioning of the nictitating membrane of the rabbit as a function of CS-UCS interval. J. comp. physiol. Psychol., 1964, 57, 188-195.

Small, H. S., Weitzner, S. W. and Nahas, G. G. Cerebrospinal fluid pressured during hypercapnia and hypoxia in dogs. Amer. J. Physiol., 1960, 198, 704-708.

Sokoloff, L. Local blood flow in neural tissue. In: W. F. Windle (Ed.) New research techniques of neuroanatomy. Springfield, Ill.: Charles C. Thomas, 1957, pp. 51-61.

Stainsby, W. N. and Otis, A. B. Blood flow, blood oxygen uptake, and oxygen transport in skeletal muscle. Amer. J. Physiol., 1964, 206, 858-866.

Stewart, C. N. and Warren, J. M. The behavior of cats on a double alternation problem. J. comp. physiol. Psychol., 1957, 50, 26-28.

Stokes, J. III, Chapman, W. P. and Smith, L. H. Effects of hypoxia and hypercapnia on perception of thermal cutaneous pain. J. Clin. Invest., 1948, 27, 299-304.

Sugar, O. and Gerard, R. W. Anoxia and brain potentials. J. Neurophysiol., 1938, 1, 558-572.

Swank, R. L. Changes in blood produced by fat meal and by intravenous heparin. Amer. J. Physiol., 1951, 164, 798-811.

Swank, R. L. Effect of fat on blood viscosity in dogs. Circulation Res., 1956, 4, 579-585.

Swank, R. L. and Cullen, C. F. Circulatory changes in the hamster's cheek pouch associated with alimentary lipemia. Proc. Soc. Exp. Biol. Med., 1953, 82, 381-384.

Swank, R. L. and Escobar, A. Effects of dextran injections on blood viscosity in dogs. J. Appl. Physiol., 1957, 10, 45-50.

Swank, R. L. and Hain, R. F. The effect of different sized emboli on the vascular system and the parenchyma of the brain. J. Neuropath. Exptl. Neurol., 1952, 11, 280-299.

Swank, R. L. and Hissen, W. Influence of serotonin on cerebral circulation. Arch. Neurol., 1964, 10, 468-472.

Swank, R. L. and Nakamura, H. Convulsions in hamsters after lipid meals. Electroencephalograms and available oxygen. Arch. Neurol., 1960, 3, 594-600.

Tanaka, H. Rept. Aero Research Inst. (Tokyo Imperial Univer.) 1928, 3, 128. Cited in: Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963.

Thorn, G. W., Clinton, M., Jr., Farber, S. and Edmonds, H. W. Studies on altitude tolerance. I. Studies on normal rats - effect of repeated short exposures to reduced atmospheric pressure. Bull. Johns Hopkins Hosp., 1946, 79, 59-69.

Thorsen, G. and Hint, H. Aggregation, sedimentation and intravascular sludging of erythrocytes. Acta Chir. Scand., 1950 (Suppl. 154).

Thygesen, J. E. The mechanism of blood sedimentation. Acta Med. Scand., 1942 (Suppl. 134).

Tower, D. B. The neurochemical substrates of cerebral function and activity. In: Harlow, H. F. and Woolsey, C. N. Biological and biochemical bases of behavior. Madison, Wis.: The Univer. of Wisconsin Press, 1958, pp. 285-366.

Tschirgi, R. D. Chemical environment of the central nervous system. In: Field, J., Magoun, H. W. and Hall, V. E. (Eds.) Handbook of Physiology, Sec. I. Neurophysiology, Vol. III. Washington, D. C.: American Physiological Society, 1960, pp. 1865-1890.

Van Harreveld, A. The effect of asphyxia on reflex inhibition. Amer. J. Physiol., 1939, 128, 13-18.

Van Harreveld, A. and Marmont, G. The course of recovery of the spinal cord from asphyxia. J. Neurophysiol., 1939, 2, 101-111.

Van Liere, E. J. and Stickney, J. C. Hypoxia. Chicago: Univer. of Chicago Press, 1963.

Voronin, L. G. and Napalkov, A. V. Zhurn. Vyss. Nerv. Deyat. (Pavlov), 1959, 2, 5. Cited in: Voronin, L. G. and Napalkov, A. V. Complex systems of conditioned reflexes in the analysis of drug effects. In: Votava, Z., Horvath, M. and Vinar, O. Psychopharmacological Methods. New York: Macmillan Co., 1963, pp. 182-190.

Weinberger, L. M., Gibbon, Mary H. and Gibbon, J. H. Temporary arrest of the circulation to the central nervous system. II. Pathologic effects. Arch. Neurol. Psychiat., 1940, 43, 961-986.

Wright, R. L. Experimental cerebral ischemia. Angiol., 1965, 16, 397-403.

Wright, R. L. and Ames, A. III. Measurement of maximal permissible cerebral ischemia and a study of its pharmacologic prolongation. J. Neurosurg., 1964, 21, 567-574.

Wyke, B. Brain function and behavior disorders. London: Butterworth's, 1963.

Zarrow, M. X., Sawin, P. B., Ross, S. and Denenberg, V. H. Maternal behavior and its endocrine basis in the rabbit. In: Bliss, E. L. (Ed.) Roots of Behavior. New York: Harper and Bros., 1962, pp 187-197.

Zederfeldt, B. Studies on wound healing and trauma with special reference to intravascular aggregation of erythrocytes. Acta Chir. Scand., 1957 (Suppl. 224), 1-85.

Zweifach, B. W. Functional Behavior of the Microcirculation. Springfield, Ill.: Charles C. Thomas, 1961.

BIOGRAPHICAL SKETCH

Karl Michael Brooks was born August 17, 1926, at Rockland, Maine. He served in the United States Navy from April, 1944, until April, 1946. Following graduation from Thomaston High School in June, 1946, he became employed in a family owned lumber business. In September, 1949, he entered the University of Maine. During the final two years he worked as an undergraduate teaching assistant, and received the degree of Bachelor of Arts in February, 1954. Following graduation he became self-employed as a boat captain and partner in a boat building business. In September, 1959, he returned to the University of Maine and entered the Graduate School there in February, 1960, where he was a graduate teaching assistant and a National Science Foundation Cooperative Graduate fellow until August, 1961, when he received the degree of Master of Arts. From September, 1961, he has pursued his work toward the degree of Doctor of Philosophy.

Karl Michael Brooks is married to the former Sally Mabelle Gillchrest and is the father of one child. He is a member of Psi Chi, Phi Kappa Phi, Phi Beta Kappa and Sigma Xi.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 23, 1966

Dean, College of Arts and Sciences

Dean, Graduate School

Supervisory Committee:

Chairman