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Studies on the action of sublethal percentages of illuminating gas

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STUDIES ON THE ACTION OF SUBLETHAL PERCENTAGES OF ILLUMINATING GAS

By

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INTRODUCTION

Carbon monoxide poisoning is a danger to which everyone may be exposed. Industrial workers, especially men working in mines and in iron and steel mills, frequently face this hazard. Garages in which there are running automobiles are places of especial danger. The carbon monoxide content of the air in a garage repair shop was found to be as high as 0.3 per cent as long as six hours after all engines had been turned off (Egdahl, 1923). Recently there have been two cases reported in Iowa in which the occupants of enclosed cars parked with the engines running have met their death. Buildings, especially old ones with leaky gas fixtures are dangerous, and when these are living quarters, the persons in gravest danger are the mothers and young children who do not get away from home frequently (Stevens, 1926). Carbon monoxide may be formed in dangerous amounts from open fires, and from furnace or stove fires. City streets, hemmed in as they are by tall buildings, contain appreciable amounts of carbon monoxide (Wilson et al, 1926).

A source of carbon monoxide poisoning that has recently been noted is tobacco smoking. The carbon monoxide content of the blood of smokers was found to be higher than that of non-smokers (Hamon and Hastings, 1933).

Most of these sources of carbon monoxide poisoning result

in a chronic, subacute poisoning. The degree of carbon monoxide in the blood remains low because of a low percentage of the gas in the air breathed, or because the individual frequently breathes air containing no carbon monoxide, during which time he loses some or all of that held in the blood. Under these circumstances the individual does not ordinarily become acutely ill owing to the poison, and he is in most cases unaware of it. Either he does not become conscious of the symptoms of carbon monoxide poisoning, or he attributes them to other causes.

Because of these considerations, it seemed valuable to undertake a study of the effects of a long continued, subacute condition of carbon monoxide poisoning, as nearly as possible achieving in the laboratory the type of carbon monoxide poisoning most commonly found in man. As a matter of expediency, and also because to most individuals the greatest ever-present source of danger from carbon monoxide poisoning is illuminating gas (Henderson, 1930), illuminating gas was used as the source of the carbon monoxide in the work reported.

All of the toxic effects of illuminating gas may not be attributable to carbon monoxide. Other constituents of illuminating gas appear to be toxic. Henderson and Haggard (1920) found that in their experiments using pure carbon monoxide in air as the asphyxiant, the animals died with a carbon monoxide hemoglobin content in the blood of 85 per cent, but using illuminating gas as the asphyxiant, the animals died with a blood

carbon monoxide hemoglobin content of as low as 65 per cent. In experiments reported subsequently (Henderson and Haggard, 1922), the average carbon monoxide hemoglobin of the dogs at the time of death was found to be 84 per cent when death was caused by breathing a pure carbon monoxide and air mixture, and 72 per cent when death was caused by breathing an illuminating gas-air mixture. These investigators, on the basis of their observations on the animals asphyxiated in these different ways, consider illuminating gas to be a greater respiratory stimulant than pure carbon monoxide. If benzene is a constituent present in the illuminating gas, as it may be, it has a very toxic effect (Haggard and Henderson, 1921a).

As the work on the experiment to be reported progressed, it was found that the experimental procedure had a depressing effect upon the reproductive powers of the experimental animals. The purpose of this study, was to discover whether this effect was brought about through the action of the experimental procedure on the male or on the female animals, or on both, and to learn as much as possible concerning the mechanism of this action.

REVIEW OF LITERATURE

Effects of Chronic Carbon Monoxide Poisoning

Certain effects of chronic poisoning with carbon monoxide have been noted. One is the effect on the hemoglobin. Curiously, both polycythemia and anemia have been found, apparently resulting from carbon monoxide poisoning. Sayers and Davenport (1930) cite a paper of Koren (1891) in which he attributes pernicious anemia in three children to chronic carbon monoxide poisoning. Strassman (1919) states that the chronic action of carbon monoxide taken into the blood over a period of years may result in a condition of pernicious anemia. Rossiter (1928) lists anemia among the symptoms of chronic poisoning from carbon monoxide. During the war (Macpherson et al. 1923) it was found that while in some individuals anemia resulted from carbon monoxide poisoning, in others a polycythemia developed. This polycythemia might be accompanied by (a) no change in hemoglobin; (b) increased hemoglobin; (c) decreased hemoglobin.

Because of these reports in the literature, there have been attempts to bring about a condition of anemia by subjecting animals to chronic carbon monoxide poisoning. However the results reported indicate the development of a condition of polycythemia and a higher than normal blood hemoglobin, rather than one of

of anemia. Nasmith and Graham (1906) kept guinea pigs in closed chambers with a carbon monoxide content sufficiently high to keep the blood of the experimental animals saturated with carbon monoxide to the extent of 25 per cent, and found that there was an increase in the erythrocytes, and an increase in the hemoglobin content of the blood as the animals became acclimated to the experimental conditions. Sayers et al.(1929) found that men exposed daily to gasoline engine exhaust gas-air mixtures showed a distinct increase in hemoglobin accompanied by an increase in the red blood cells, but no significant change in the leucocytes.

When the response to continued exposure to carbon monoxide results in increased hemoglobin, a certain toleration is established: compensation has resulted. It was found during the war that men working where the air contained small percentages of carbon monoxide (about 0.02 to 0.03 per cent) established a toleration to the poison such that these men could be exposed for longer periods to carbon monoxide than could men who had not developed a tolerance. This tolerance did not persist after the men had spent some time in pure air (Macpherson et al. 1923).

Nasmith and Graham (1906) also report an increase in the white cells of the guinea pigs subjected to experimental conditions. This was in part at least caused by an increase in eosinophils and pseudo-eosinophils, until the toxemic condition induced by the carbon monoxide grew more severe. Sayers et al (1929) find that no significant change in the number of leuco-

cytes or in the differential count occurred in the men who breathed the automobile exhaust gas-air mixture for sixty-eight days.

That there is no change in the erythrocytes or leucocytes themselves is to be concluded from work done by Strassman (1919) on the blood of individuals killed by carbon monoxide poisoning. Nicloux (1921) affirms this. Boedikor (1932) found in his experimental animals subjected to daily poisoning with pure carbon monoxide an increased fragility of the erythrocytes to the upper limits of the normal.

Bianchini and Fabborni (1922) found that in rabbits poisoned with carbon monoxide, the number of platelets was temporarily increased to twice the normal number, but that the increase became successively less as the rabbits were poisoned with carbon monoxide on successive days.

Blood in which a large portion of the hemoglobin is united with carbon monoxide to form carbon monoxide hemoglobin does not coagulate as readily as normal blood, according to Stewart (1920). Forbes and Hompe (1921) could not verify this experimentally.

Repeated poisoning by carbon monoxide may affect the heart: in the World War it was found by the British medical officers that in the more susceptible men who had been gassed several times, heart trouble often ensued (Macpherson et al. 1923).

Exposures to carbon monoxide have a depressing effect upon body metabolism and temperature. Walter (1927) determined the

rate of metabolism of white rats exposed and not exposed to carbon monoxide. He found that concentrations above 0.05 per cent had a markedly depressing effect upon the metabolism. He considered that the rats became acclimated to carbon monoxide, as the same exposure repeated over a period of days decreased metabolism and body temperature less on the last day than on the first.

The blood sugar may be higher than normal following exposure to carbon monoxide, and urinary sugar may be present (Straub, 1897; Hikami, 1926; Rossiter, 1928).

Growth may be retarded, and weight kept below normal. Sayers (1928) found that very young rats and guinea pigs exposed continuously to small concentrations of carbon monoxide do not gain weight as rapidly as do the control animals. Rossiter (1928) mentions diminished muscular power, indigestion, loss of appetite, change of weight, and muscular weakness as symptoms of chronic carbon monoxide poisoning.

The observation has been repeatedly made, both on men and on animals under experimental conditions, that the younger individuals are more seriously affected by the poison (Henderson, 1916; Macpherson et al. 1923; Stevens, 1926; Sayers, 1928). The explanation of this lies in part in the fact that the young child or young individual has a greater relative respiratory exchange for its size, and the formation of carbon monoxide hemoglobin occurs at a greater rate than in an older individual (Henderson, 1916). The rate of metabolism of the individual is a

factor (Henderson and Haggard, 1927; Mack, 1933). Paradoxically, it has been found that rats just born do not succumb to carbon monoxide poisoning as quickly as do older rats (Avery and Johlin, 1932).

Individual susceptibility to carbon monoxide poisoning other than that due to age (rate of metabolism) and size has been observed. One factor is undoubtedly general well-being. Any heart trouble, or anemia, or fatigue is predisposing toward greater susceptibility. So also are alcoholism, digestive disturbances, arterio-sclerosis (Rossiter, 1928). Exertion either during or following exposure to carbon monoxide increases the rate of development and the severity of the symptoms (Sayers et al, 1929). There seems to be a sexual difference, women being found to be less susceptible to the poison than men (Harbitz, 1917; Webster, 1930). Female rats seem to be less affected by the poison than are male rats (McMillin, 1932).

A fundamental cause for this variation may be based on the differences in the affinity of the hemoglobin of different individuals for carbon monoxide. That this difference exists between men as well as among species was shown by the English investigators (Douglas, Haldane, and Haldane, 1912). Henderson and Haggard (1922) do not believe that this difference is great enough to be a factor in explaining individual susceptibility to the poison. They consider that much more important factors are the individual differences in susceptibility of the body to

the ill effects of oxygen want, and variations in the volume of breathing among individuals.

Löwy (1925) claims that carbon monoxide poisoning reduces the immunization of the body to disease. He bases this judgment on the fact that three infectious illnesses followed a rather light case of carbon monoxide poisoning. Pneumonia may develop, especially following prolonged exposure to the gas (Macpherson et al, 1923). Egdahl (1923) reports an experiment in which he compared the degree of spread of infection of Bacillus tuberculosis and Staphylococcus aureus in guinea pigs being exposed for one hour daily to 0.1 per cent carbon monoxide with that in control guinea pigs. The results with the staphylococcus were unconclusive, but the gassed animals definitely showed a more widespread tuberculous infection than did the controls.

There have been some comments in the literature on the effect of carbon monoxide poisoning on sexual activity, and on reproduction. Rossiter (1928) comments that "loss of sexual desire" is given as a symptom by the men who are exposed to small amounts of carbon monoxide daily. He adds that it is hard to determine whether this symptom, together with others given by the same men, is the result of the gas or of some other factor. McCombs (1912) reports, among other sequelae to carbon monoxide poisoning, the loss of sexual power. As he makes the statement that all of these sequelae cleared up within six months, this condition was evidently transient.

McCombs (1912) also reports that he has observed a number of pregnant women who have survived the asphyxiation of carbon monoxide poisoning. In no case was the subsequent delivery abnormal, nor was the child affected. A recent query (May 20, 1933) in the Journal of the American Medical Association as to whether poisoning from illuminating gas could cause abortion was answered in the affirmative.

McMillin (1932) reports the death of one of her experimental animals subjected to illuminating gas-air mixture, with an inability to deliver the litter. On autopsy, the uterus of the female appeared black and congested, and contained four well-developed foeti. There had been a bloody vaginal discharge during the three days preceeding death. A second female among the experimental animals was pregnant, and bore a litter, none of which survived beyond the first day.

Wells (1933) administered an air-gas mixture containing 1.5 per cent carbon monoxide daily to female rats during the gestation period, the gassing time averaging from five to eight minutes, and being terminated when unconsciousness was reached. He reports abnormal prenatal development, and small, abnormal litters. The female rats showed a slight edema of the nerve cells of the cortex, but no change in the nervous system of the offspring could be demonstrated.

The effect of chronic carbon monoxide poisoning that have been enumerated above cause the following symptoms, according to Webster (1930):

"progressive fatigue, muscular weakness, headache, dullness and mental depression, disturbed vision, a peculiar transient ashy pallor which may change to a more or less greenish pallor, red patches on the cheek bones, palpitation of the heart, nausea, vomiting, vertigo, slowing of the pulse, and dyspnea."

Luden (1921) gives an account of an individual suffering from what was found later to be chronic carbon monoxide poisoning:

"Intense abdominal pains, visible spasms of lumbar and abdominal muscles, sudden attacks of syncope, and chills without rise of temperature, alternated with periods of comparative well being.....Minor symptoms developed: headaches located behind the eyes, but the patient had suffered from headaches for years; occasional, if rare attacks of nausea; obstinate constipation; irritation of the bladder with difficulties in micturition; the bladder symptoms were ascribed to the elimination of the camphor, but were also observed when no camphor had been given. The temperature was normal or subnormal, with an occasional evening rise; this too, had been frequently observed in previous years. The heart sounds were normal. The pulse was of a good quality, on the whole regular, 68 to 72 beats to the minute. At intervals, however, the pulse showed irregularities of a puzzling character: series of good strong beats alternated with series of small, 'flat' beats that were scarcely perceptible, but without acceleration. The electro-cardiograph failed to reveal these abnormalities, although they could be felt at the wrists. Urinalysis and blood counts showed nothing abnormal; the erythrocytes were 4,500,000 to 5,000,000 which seemed high considering the general debility of the patient; the hemoglobin was 70 to 75 per cent; the leukocytes 8,000 to 10,000. The blood pressure was low: systolic 70 to 80, diastolic 60 to 70; the pulse pressure often did not exceed 10 mm., observations being made daily at the same hour and frequently repeated at different times of the day with the Tycos sphygmomanometer. The blood sugar was very low, 0.05 to 0.06 gm. per 100 cc. of blood; the blood cholesterol values were high on the whole, 130 to 155 mg. per 100 cc. of whole blood."

Pathological Changes Following Carbon Monoxide Poisoning

The anoxemia which is induced as a result of the specific effect of carbon monoxide upon hemoglobin will become serious, if the carbon monoxide poisoning is severe or prolonged. Henderson and Haggard (1922) say that a severe but short exposure to carbon monoxide is less dangerous than a lesser degree of the poisoning, prolonged. The disastrous results of the anoxemia may continue after the individual has been removed from the source of the poisoning, unless adequate treatment is instituted immediately. In cases of prolonged severe poisoning, irreparable damage may have been done, and if tissue degeneration has begun there is no remedy known to cause tissue recovery.

Lewin (1920) found cases showing inflammation in the epiglottis, pharynx, esophagus, and stomach; and cases showing bloody effusions in the mucous membrane of the intestines and inflammation with hemorrhages in the large intestines. He found an even greater number of cases where inflammation was present in the air passages.

Rossiter (1928) records that the stomach, intestines, and peritoneum show congestion, petechial spots, effusions, and hemorrhages at times; indeed that all the organs of the body, and particularly the brain, may show signs of congestion and petechial hemorrhages on the surface and throughout their structures. Hill and Semerak (1918), in their study of thirty-two individuals killed by carbon monoxide, found in fifteen of the

cases petechial hemorrhages in numerous mucous and serous membranes throughout the body, as in the stomach, bowels, peritoneum, renal pelves, urinary bladder, pleura, pericardium, pharynx, larynx, and skin. They found hyperplasia of the lymphoid tissues of the body, including the spleen, thymus, tonsils, and lymph glands.

Poisachowitch (Tscherkess and Melnikowa, 1928) found changes in the suprarenal capsule and in the thyroid gland of animals killed by carbon monoxide. If the death resulted from a short acute poisoning with carbon monoxide, exhaustion of the chromaffin substances of the suprarenal was found, while if death followed prolonged exposure to carbon monoxide, a hyperfunction of the medullary layer of the suprarenal capsule was found. Similarly, after acute carbon monoxide poisoning, a histo-chemical change in the thyroid follicle was observed, and increased colloid formation over normal after prolonged poisoning.

No change has been observed in muscle tissue (Ramsay and Eilmann, 1932).

The most noteworthy change is found in nervous tissue, which is exceedingly sensitive to oxygen deficiency. Hill and Somerak (1918) report the following pathological changes observed in their study of individuals killed by carbon monoxide: Hyperemia of the brain and the leptomeninges were observed in twenty-nine of the cases studied, and edema and internal hydrocephalus in twenty-one. In 14 cases a bilateral softening of the lentic-

ular nucleus and internal capsule was observed grossly, while in all the other cases necrosis of the lenticular nucleus was observed microscopically. The globus pallidus was invariably affected, and the putamen and internal capsule to a lesser degree. Thrombosis of the veins, and even of the arteries was observed in certain cases in the lenticular nucleus, with evidence of stasis of circulation in others. Other factors, such as syphilis, tuberculosis, age, which may have been responsible for the conditions found were ruled out in so far as possible.

Hiller (1924) says that softening of the pallidum is the peculiar and characteristic change in the central nervous system caused by carbon monoxide. Wiemann (1926) states that the brain injury is not due to direct injury of the nerve tissues by carbon monoxide or to alteration in the vascular walls, or to thrombotic closure of the blood vessels, but to disturbances in the circulation.

Further evidence in support of the fact that the brain injury resulting in carbon monoxide poisoning is not due to the specific action of carbon monoxide on nervous tissue was presented by Haggard (1922) in an experiment in which he grew chick neuroblast in an atmosphere consisting of as much as 79 per cent carbon monoxide. No ill effect was noted. However, Haggard found that as little as 0.1 per cent of illuminating gas in the chamber with the growing chick neuroblast had an inhibiting effect. This points to other toxic properties found in illuminating gas than the carbon monoxide, but Haggard concludes that

his experiment is not to be interpreted to indicate that carbon monoxide is not the chief poisonous constituent of illuminating gas.

Cause of Death in Carbon Monoxide Poisoning

Campbell (1929) made a study comparing the pathological effects of prolonged exposure to carbon monoxide with those produced by very low oxygen pressure. (The two conditions are not strictly comparable (Haldane, 1922).) As the oxygen tension was reduced, either by carbon monoxide in the air breathed, or by lowering the oxygen pressure, signs of circulatory failure developed: conditions of congestion in the liver, lungs, and brain particularly, of edema, of degeneration, and of atrophy were observed. Capillary and venous engorgement was noted. As he found the same conditions developing in both sets of experimental animals, Campbell believes that his experiment indicates that the cause of death in cases of carbon monoxide poisoning is the same as that in cases of anoxemia due to lowered oxygen pressure, and he concludes that the ability to withstand carbon monoxide poisoning depends upon the ability of the vital organs to function and survive under conditions of low oxygen tension.

The cause of death was studied by Haggard (1921) on dogs asphyxiated with carbon monoxide. He found that upon inducing a rather rapid asphyxiation with carbon monoxide, the course of events was as follows: An excitement stage was followed by

depression and unconsciousness. Respiration became augmented soon after gassing was started, and developed into dyspnea which reached its height shortly before unconsciousness developed; subsequently, as respiratory failure developed, respiration became diminished and irregular, and ceased. The heart, which had beat more rapidly during the hyperpnea, and then more slowly as the pulmonary ventilation became reduced, continued to beat for several minutes after cessation of inspirations. During this time impairment in auriculo-ventricular conduction developed, and in some cases partial and complete block ensued. In other cases, ventricular extra-systoles occurred with increasing frequency, until the heart finally passed into a state of ventricular fibrillation. Haggard states that the respiratory failure is caused by the excessive loss of carbon dioxide induced by the hyperpnea existing for a time. Any stimulus of oxygen deficiency for breathing he considers to be lost because of decreased sensitivity of the respiratory center to the condition of anoxemia. The anoxemic condition of the heart is responsible for the cardiac activities observed following cessation of respiration.

When death results from a prolonged period of carbon monoxide poisoning, Haggard found the same sequence of events to take place, except that heart block and ventricular fibrillation developed somewhat more quickly after respiratory failure. Before respiratory failure, no evidence of impairment of cardiac conduction developed.

Mechanism of Carbon Monoxide Poisoning

The most commonly accepted view-point is that carbon monoxide is physiologically an inert gas, in contradistinction to a gas such as carbon dioxide, and one that would be classified as a simple asphyxiant if it were not for the great affinity of carbon monoxide for hemoglobin (Henderson and Haggard, 1927). (The evidence that carbon monoxide is a specific tissue poison will be presented later.) Haldane (1895) placed mice in chambers containing sufficient carbon monoxide to saturate entirely the hemoglobin of the mice, and also containing oxygen to the extent of two atmospheres of oxygen pressure. The mice survived, under conditions in which no oxygen was transported to the body tissues by the hemoglobin, but only in solution in the plasma. (Three to four volumes per cent were carried in solution in the plasma, under these conditions.) This experiment was interpreted as proof that if the tissue anoxia could be prevented, carbon monoxide itself was non-toxic.

The toxic effect of carbon monoxide is therefore ascribed to the anoxia resulting when so much of the hemoglobin has united with carbon monoxide to form carbon monoxide hemoglobin that insufficient amounts of oxygen are available to the tissues for normal tissue respiration and metabolism. Stadie and Martin (1925) claim that there is an alteration from the normal oxygen dissociation in conditions of carbon monoxide poisoning, with the result that oxygen is not as readily dissociated from hemo-

globin in the capillaries, as in the normal; and that an individual who has half of his available hemoglobin tied up in the form of carbon monoxide hemoglobin is in a much more serious state of anoxemia than an individual who, through severe anemia, has only an equal amount of hemoglobin (i.e. half the normal) available to carry oxygen to the tissue.

Carbon monoxide unites with hemoglobin according to the law of mass action, the amount of carbon monoxide hemoglobin being formed depending upon the partial pressure of carbon monoxide and of oxygen in the alveolar air. The same is true of the dissociation of carbon monoxide hemoglobin, the reaction being reversible, and being practically instantaneous in either direction (Hartridge and Roughton, 1922).

The relative attraction of carbon monoxide and oxygen for hemoglobin in man are in the ratio of approximately 300:1. This being true, then if the oxygen content of the air breathed were 300 times as great as the carbon monoxide content (a carbon monoxide content of 0.07 per cent), the distribution of the two gases in the blood should be about equal (Henderson, 1927). But equilibrium at this point would be obtained slowly. Henderson says: "The rate of absorption of carbon monoxide by the lungs cannot exceed the amount of the gas drawn in by breathing. Thus the volume of respiration is a limiting factor in the rate at which carbon monoxide hemoglobin can be formed." And the volume of respiration in comparison to the size of the body and the volume of blood in the body is greater in small individuals.

Sayers and Yant (1923) found that with a concentration of carbon monoxide in the air of from 0.07 to 0.10 per cent, inclusive, it required from three to four hours to saturate the blood 47 to 53 per cent. Presumably, as the pH of the blood increases with the loss of carbon dioxide attendant on over-breathing during poisoning with carbon monoxide, the affinity of the hemoglobin for carbon monoxide will increase (Stadie and Martin, 1925).

The elimination of carbon monoxide from the hemoglobin is slow, because the alveolar air continues to have high carbon monoxide content, the carbon monoxide liberated from the blood being eliminated from the alveoli only slowly. Clinically it has been found that administering an oxygen mixture containing seven per cent carbon dioxide to the victim of carbon monoxide poisoning speeds up the recovery (Henderson, 1916; 1930). (This is the treatment of choice in all cases of asphyxia, but has been objected to on the grounds of possible damage to the heart (Rossiter, 1928).)

Stadie and Martin (1925) demonstrated in experiments on animals in which the rate of breathing was controlled by tracheal cannulation and an artificial respiration apparatus that the hyperventilation induced by the carbon dioxide is of minor importance in the elimination of carbon monoxide, as compared to the effect of decreased blood pH and reduced affinity for carbon monoxide. Carbon dioxide therapy would seem to be ideal in that it brings about both hyperventilation and increased

blood hydrogen-ion content.

Haggard and Henderson (1921) find that carbon monoxide poisoning causes an alkalosis: that the loss of carbon dioxide from the blood causes a decrease in the blood bicarbonates. Hence the rational treatment of the condition is to administer carbon dioxide to the victim of the poisoning. These investigators state also that during the recovery period an acidosis may be present.

Carbon Monoxide as a Specific Tissue Poison

Some evidence has been forthcoming that carbon monoxide has a specific toxic effect on tissues: Hott raised the question (1907). Mathews (1930) suggested that carbon monoxide might act by uniting with oxygen receptors other than hemoglobin. Warburg (1926) found that carbon monoxide depressed the rate of oxygen consumption of yeast cells, and also that if the oxygen pressure were increased, it required correspondingly more carbon monoxide to obtain the same depression.

J.B.S. Haldane (1927) found that large amounts of carbon monoxide affected the activity of moths and the germination rate of seeds. He also performed an interesting experiment using a rat as the test animal. He placed a rat in a chamber in which the oxygen pressure was three atmospheres, and the carbon monoxide pressure one atmosphere. The rat showed almost normal behavior. If the amount of oxygen were reduced to only two atmospheres of

pressure, the rat showed increased rate of breathing, but no other symptoms of distress. But he found that raising the carbon monoxide pressure in the chamber to two atmospheres killed the rat, whereas in the control experiment, the adding of one atmosphere of nitrogen to the gases already in the chamber apparently caused the rat no distress. For this reason, Haldane claims that the added carbon monoxide was toxic. He considered that it was toxic because of its effect as a tissue poison, since by the conditions of the experiment the hemoglobin of the rat was already saturated with carbon monoxide to the extent of 98.3 per cent, and the rat was being maintained on oxygen carried in solution in the plasma. (To arrive at this figure Haldane assumed that the temperature coefficient of the affinity of carbon monoxide for the hemoglobin of the rat is the same as that for the hemoglobin of man. Anson, Barcroft, Mirsky, and Oinuma (1924) found in the human the affinity of carbon monoxide as compared to that of oxygen to be 400 to 1 at 15°C. and to be 250 to 1 at 37°C. For the rat they found the affinity to be 380 to 1 at 15°C.)

Keilin (1929) reported that carbon monoxide has a definite inhibitory effect upon the activity of indophenol oxidase in yeast cells and in mammalian muscle tissue.

Recently the administration of methylene blue as a therapeutic measure in the treatment of carbon monoxide poisoning has been advocated (Brooks, 1932; 1932a). A number of cases so

treated are on record (Geiger, 1933; Bell, 1933; Nass, 1933; Christoferson, 1933). The basis for such a suggestion lies in the work of Warburg on isolated tissues. Warburg found (1930) that the oxygen uptake of red blood cell suspensions was practically unaffected by carbon monoxide in the presence of sufficiently large amounts of methylene blue. Haggard and Greenberg (1933) and Henderson (1933) do not believe that the results obtained on isolated tissues can be applied to the living mammal. They state that methylene blue is a synergist, rather than an antidote for carbon monoxide poisoning in the mammal. They support their statement with experimental evidence, and on the theoretical basis that the action of methylene blue is to convert hemoglobin into methemoglobin, a change which would not reduce the amount of carbon monoxide united with the hemoglobin. They point out that the conversion of hemoglobin into methemoglobin would increase an already existing anoxemia caused by the presence of carbon monoxide. Brooks (1934) disputes the idea that methemoglobin is formed in the presence of methylene blue <u>in vivo</u> in the presence of glucose. Deutsch and Weiss (1934) report favorable results in treating victims of carbon dioxide poisoning, using intravenous injections of methylene blue and glucose.

MATERIALS AND METHODS

Experimental Animals

The experimental animals used chiefly were white rats (Mus norvegicus albinus) of the Wistar strain. They were descendents of rats obtained from the Wistar colony in October 1931 and in March 1932. Twelve of the rats used for the study of the oestrus cycle were obtained from the chemistry department of Iowa State College, three of these rats being pied females, three gray females, and the other six albino rats.

The animals were kept in one of the animal laboratories of the zoology department, in Science building. This laboratory consists of two corner basement rooms of cement construction, that are adequately lighted and ventilated. The rats were caged in hanging metal cages of coarse screening, in each of which were kept two small beakers, the one filled with water, the other with the diet fed. Sexes were kept segregated, except when data on reproduction was sought.

Animals on experiment were given the following diet (from McCollum: Lee, 1926) ad libitum:

Whole wheat flour	625 parts
Powdered skim milk	150 "
Casein	150 "
Calcium carbonate	15 "
Sodium chloride	10 "
Cotton seed oil	50 "

This was supplemented by cod liver oil, given individually once or twice a week during the winter months; by lettuce twice a week; by raw liver twice a week; and by day old bread, whole wheat and white, which was fed daily to the rats.

The experimental rats were subjected each day for a given interval to a 1.43 per cent air-illuminating gas mixture. This interval was increased to one hour each day as soon as the rats could tolerate so long a period, and a few of the rats were gassed for as much as two hours each day.

Respiratory Apparatus and Gas Analyses

The closed circuit respiratory apparatus in which the rats were subjected to the air-illuminating gas mixture consists of an animal chamber connected by tubes with the two openings of a rebuilt basal metabolism apparatus of the Benedict-Roth type. The animal chamber is an eight and one-half liter glass jar with two openings, each closed with a one-hole rubber stopper. (See Fig. 1.) A blower is inserted between the animal chamber and the Sansolime container to insure constant circulation of the enclosed gas. A layer of No. 14 and a layer of No. 8 Sansolime filter the enclosed gas, and keep the air-illuminating gas mixture breathed by the rats from becoming excessively moist. The volume of gas in the apparatus is controlled by the height of the spirometer bell which floats on a water seal.

The total volume of the apparatus as set up was calculated

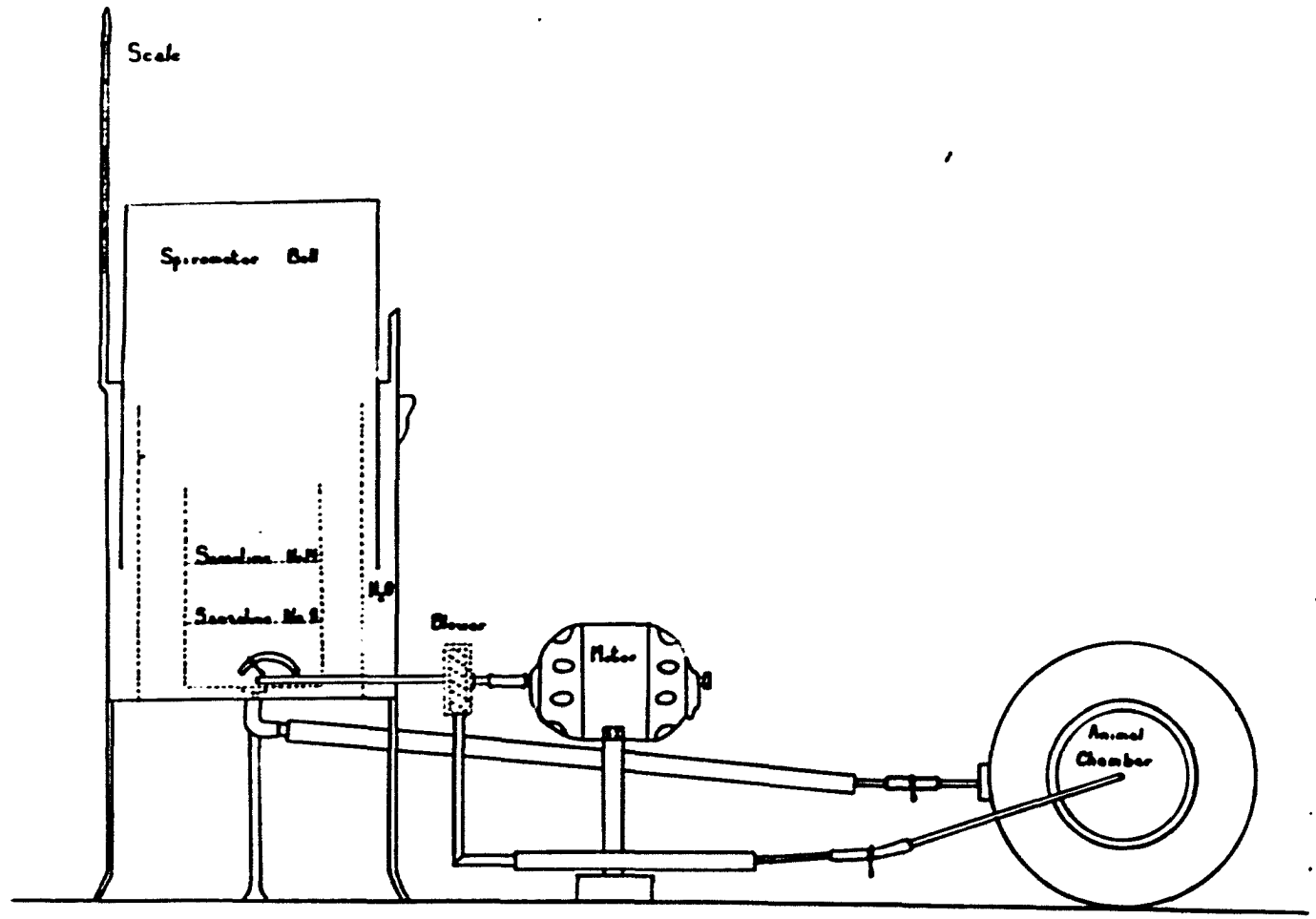


Figure 1. Closed Circuit Respiratory Apparatus

to be 20,639 cc. To this volume of air was added 300 cc. of illuminating gas, making an air-illuminating gas mixture of 1.43 per cent. Gas analyses obtained from the Iowa Railway and Light Corporation at Boone, Iowa, for the twelve days from May 23 to June 6, 1933, inclusive, may be considered representative. During this time, the average percentage by volume of carbon monoxide in the illuminating gas was 23.5 per cent, an amount such that the approximate percentage of carbon monoxide in the air-illuminating gas mixture in the apparatus was 0.34, or 34 parts per 10,000. Sayers and Yant (1923) found that persons breathing air containing 0.30 to 0.50 per cent carbon monoxide developed a carbon monoxide hemoglobin of 68 to 73 per cent in twenty to thirty minutes.

The complete analysis of the volume percentage composition of the illuminating gas used from May 23 to June 6, 1933, as provided by the company may be summarized as follows: Carbon dioxide, 6.9 ± 0.04; Illuminants, 8.8 ± 0.02; Oxygen, 1.14 ± 0.06; Carbon monoxide, 23.5 ± 0.086; Hydrogen, 27.9 ± 0.06; Methane, 12.4 ± 0.09; Nitrogen, 1937 ± 0.1.

Method of Procedure

A routine procedure in preparing the apparatus for subjecting the experimental animals to a gassing period was followed: The apparatus was first flushed out thoroughly with air, by

allowing the spirometer bell to sink in position while the tubes and animal chamber were open. This was repeated several times. Then the spirometer bell was adjusted to a height reading 3,700 on the scale, and the tubes clamped. The animals were placed in the chamber, the chamber closed, and illuminating gas from a wall outlet forced into the apparatus through an outlet near the blower. Enough gas was let in to cause the spirometer bell to rise from 3,700 to 4,000 on the scale. The tubes leading to the animal chamber were subsequently unclamped, and the motor turning the blower started. Frequent observations of the rats were made during the experimental period.

Determinations Made on Experimental and Control Animals

Determinations of the carbon monoxide content of the blood of the rats was made by the Pyrotannic Acid method for the quantitative determination of carbon monoxide in blood and in air (Sayers and Yant, 1925). This method is accurate but rough. With it, carbon monoxide hemoglobin percentages may be determined within five or ten per cent, according to the number of standards made. The determinations were made under the following conditions: (1) On the control rats kept in the room with the experimental animals and with the gassing apparatus. (2) On the experimental animals twenty-four hours after the last period of gassing. (3) On the experimental animals immediately following the last period of gassing, and for various intervals thereafter. (4) On

animals killed by illuminating gas.

Weight records were kept of the rats, and hemoglobin determinations were made at intervals. The hemoglobin determinations were made according to the Improved Newcomer technic (Newcomer, 1919); the results are expressed in grams of hemoglobin in 100 cubic centimeters of blood. Red cell volumes were obtained by the Van Allen Hematocrit method (Van Allen, 1925). Cell fragility was obtained by the Hypotonic Sodium Chloride Hemolysis method described by Lamb (1930).

Motility of spermatozoa was determined by observing the spermatozoa in a hanging drop of marmalian Ringer's solution, to which had been added a very small amount of semen obtained from the cut epididymis of the rat.

Normal testis weight of the rat under consideration was determined by the following formula, given by Donaldson (1924) for rats with a body weight greater than 80 grams: 2.810 log body weight - 4.520.

The oestrus cycle of the female rats subjected to the experimental conditions, and of the control female rats was determined. Vaginal smears were obtained daily by means of the dry smear method used by Haterius (Yeager and Haterius, 1930). The smears were identified according to the classification of Long and Evans (1922). The length of one cycle was taken to be the number of days from the initiation of one oestrus period to the initiation of the following oestrus period. Oestrus periods

were recognized by the presence of cornified cells in the smears and the absence of other types of cells. The length of the cycles found was statistically analyzed.

Serial sections were made of the ovary tissues of the rats whose oestrus cycles had been determined. The tissue was cut ten microns thick, and stained with Delafield's hematoxylin and eosin. Comparison was made of the sizes of the Graafian follicles of the females subjected to the experimental procedure with those of the control females, in five rats killed while in the oestrus period.

RESULTS

The results of the matings of the control and experimental animals are given on the pages accompanying the graphs of the respective animals. The data given include the date the litter was born; the date of the death of the last of the young, if death occurred before weaning age, or if the young were weaned, their survival is noted. The data include the size of the litter, and when the experimental rat under consideration is a female, any comments which may have been recorded concerning the apparent condition of health of the litter, and the care given the litter by the mother. The experimental procedure to which to parent under consideration was subjected may be determined by examining the accompanying graph; that for the other parent of the litter is recorded.

Many of the litters born are recorded twice, the hemoglobin, weight, and gassing time records having been kept for both parents. In other cases, healthy rats from the stock, which had already reproduced, and, if they were females, had successfully raised litters, were mated with the experimental animals. Such animals have been designated as "normal" or "stock," in contradistinction to the litter-mate control animals about which more information has been acquired.

It will be noted that all rats numbered with even numbers

are litter-mate control animals, not subjected to the illuminating gas-air mixture. All experimental gassed animals, are numbered with uneven numbers.

Certain of the rats were fed a milk diet from November 5 (date of weaning) till November 28. On the latter date, the rats were placed on an adequate diet (that fed the experimental animals) and a period of gassing was begun. The purpose of this was to determine whether the hemoglobins of anemic rats fed an adequate diet could be caused to rise at a more rapid rate than those of their litter-mate controls which had also been made anemic, but which were not being subjected daily to the illuminating gas-air mixture. No conclusions were drawn from the results obtained, as pregnancies occurred among the females, confusing the issue. (In regard to this experiment, compare the hemoglobins of male rats 3 and 5 with that of male rat 2. Compare the hemoglobin of female rat 3 with that of female rat 2.)

At this time the observation was made that the experimental treatment the animals were receiving seemed to be a factor in their ability to reproduce. As this seemed to be a question of some importance, further study on the question of hemoglobin regeneration was not made, but instead investigation of the effect of the experimental procedure on reproduction was begun.

All rats subsequently studied, were of course, fed an adequate diet. Nevertheless, the records of those rats, which in

addition to being gassed were made anemic and allowed to recover, and their controls, have been included as being significant in a study of reproduction. All such cases are separately indicated.

Figure 2. Female rats: Control rats on the left; experimental rats on the right. Experimental period 154 days.

Figure 3. Male rats: Control rat on the right; experimental rat (♂7) on the left. Experimental period 153 days.

Figure 2.

Figure 3.

Case Histories

Group 1. Control Female Rats.

Figure 4.

Female rat 2 was fed a milk diet from November 5 till November 28.

The following litters were borne by this female:

Birth	Death	Litter size	Condition of male
1-31-33	Soon	8	Milk diet Nov. 5-28; not gassed (♂2)
3- 7-33	3-10-33	12	Milk diet Nov. 5-28; not gassed (♂2)

Female rat 4 bore the following litters:

Birth	Death	Litter size	Condition of male
6- 6-33	6- 9-33	6	Not gassed (♂4)
7-10-33	Survived	7	Not gassed (♂6)

The young born June 6 were never cared for.

Figure 4.

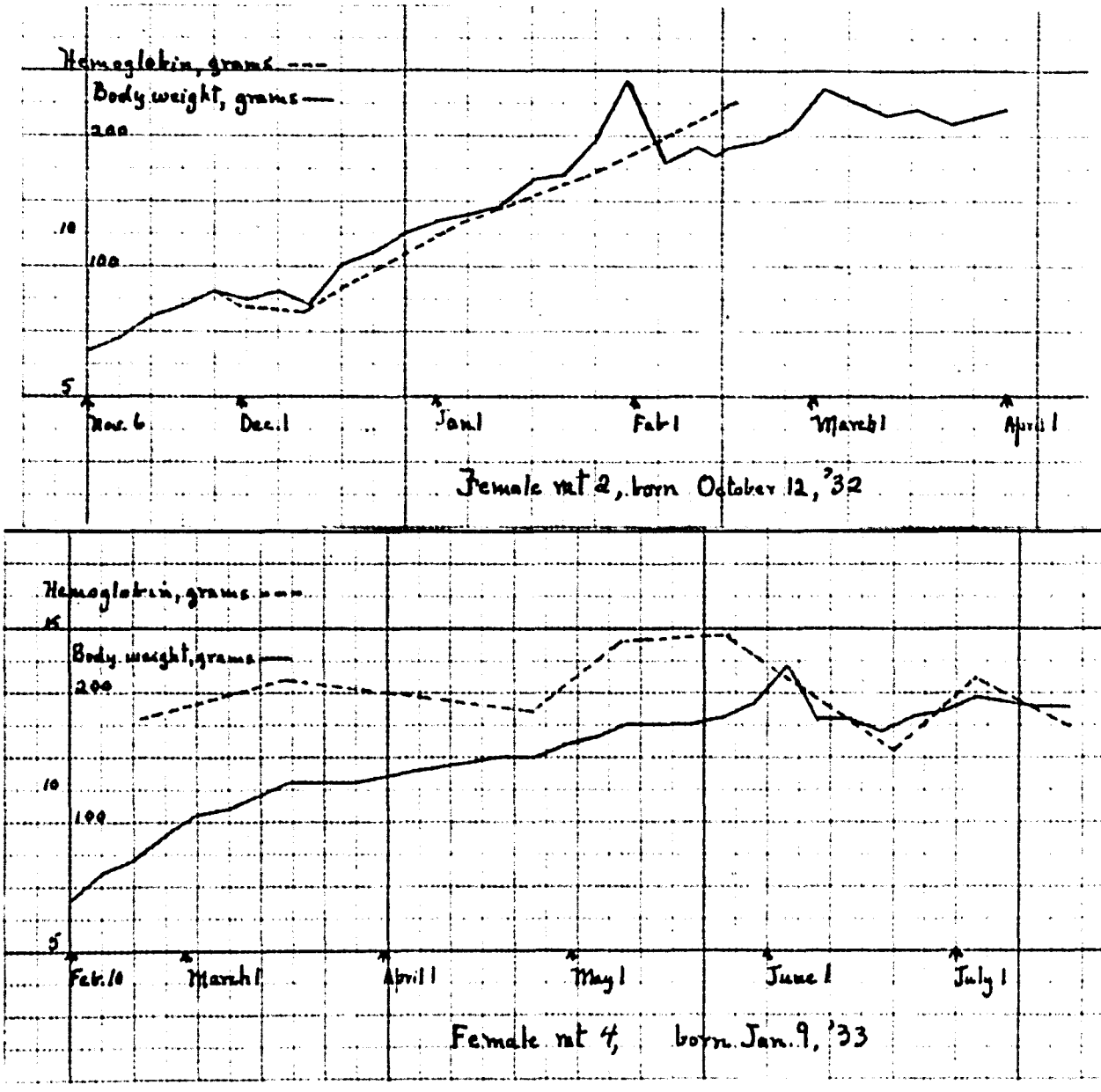

Hemoglobin, grams ---
Body weight, grams —
200
100
5
Nov. 6
Dec. 1
Jan. 1
Feb. 1
March 1
April 1
Female rat 2, born October 12, '32
Hemoglobin, grams ---
15
Body weight, grams —
200
10
100
5
Feb. 10
March 1
April 1
May 1
June 1
July 1
Female rat 4, born Jan. 9, '33

Figure 5.

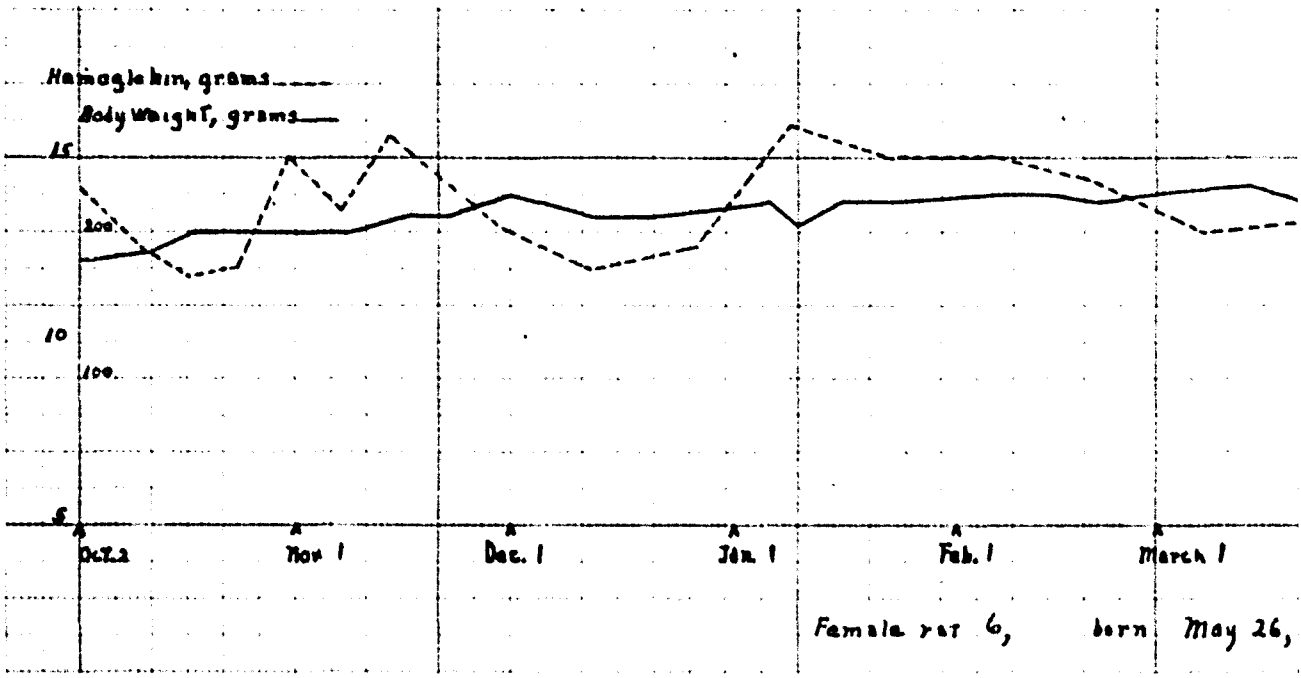
Female rat 6 was placed with a normal male rat from July 9 to July 13. On July 9 the female was in the di-oestrus interval.

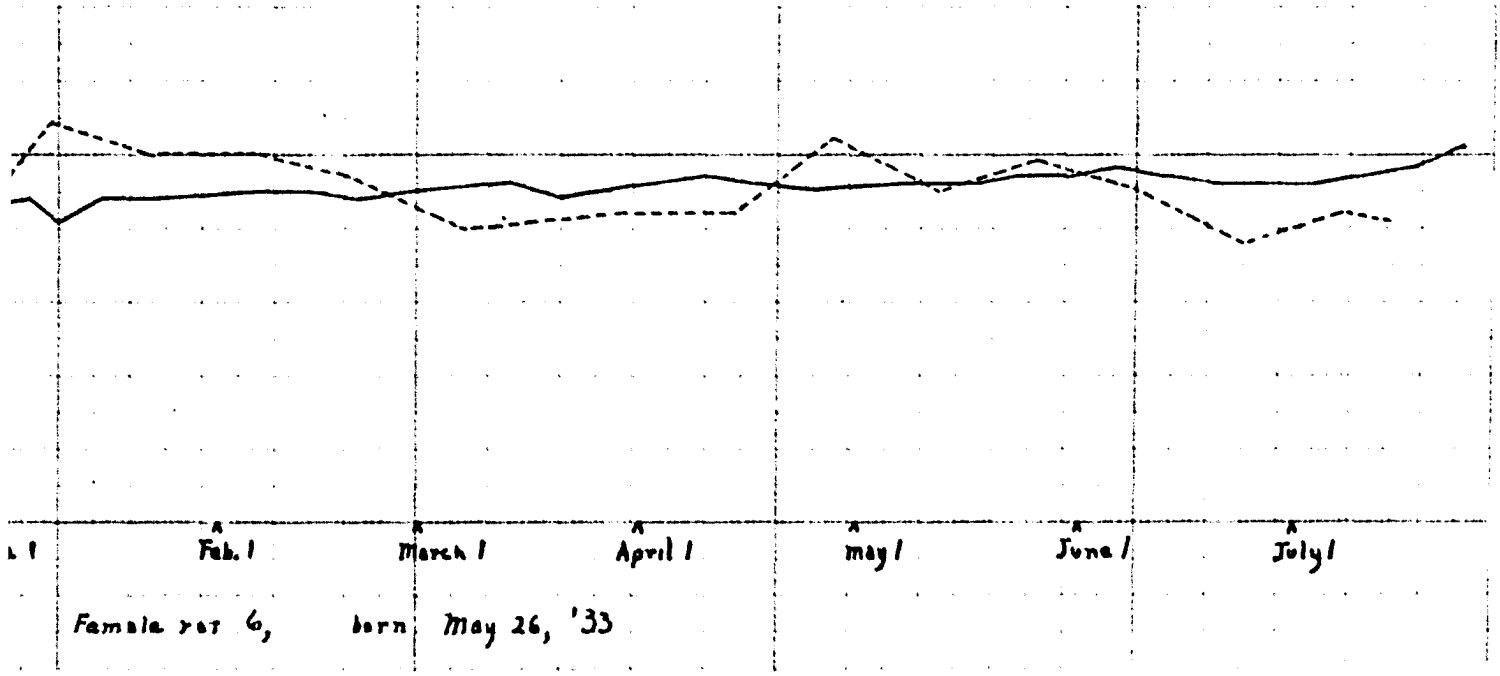
The following litter was born:

Birth	Death	Litter size	Condition of male
8- 5-34	8- 6-34	7	Normal

Six of the young were dead when first observed. They were normal in size.

Figure 5.


Hemoglobin, grams
Body weight, grams
15
200
10
100
5
Oct. 2
Nov 1
Dec. 1
Jan. 1
Feb. 1
March 1
Female rat 6, born May 26,



Female rat 6, born May 26, '33

Group 2. Gassed Female Rats.

Figure 6.

Female rat 1 has the following record:

Birth	Death	Litter size	Condition of male
1- 5-33	1- 7-33	7	Gassed (♂1)
3-24-33	3-24-33	6	Milk diet Nov. 5-28; not gassed (♂2)
4-29-33	5- 2-33	4	Milk diet Nov. 5-28; not gassed (♂2)
6- 5-33	6- 8-33	4	Not gassed (♂6)
7-25-33	Soon	?	Not gassed (♂6)

*Of these, 5 were born dead, four being in birth sacs when seen. The female had been having a bloody discharge since March 21.

The hemoglobin of this rat was 12.7 grams on July 18.

The weight of the rat ranged from 242 gm. to 334 gm. between May 15 and July 18.

Female rat 3 was kept on a milk diet from November 5 to November 28.

The rat bore the following litters:

Birth	Death	Litter size	Condition of male
2-13-33	2-14-33	3	Milk diet; not gassed (♂2)
3-23-33	3-25-33	5	Milk diet; not gassed (♂2)

The female did not seem to be aware of the first litter born, and gave it no care.

Figure 6.

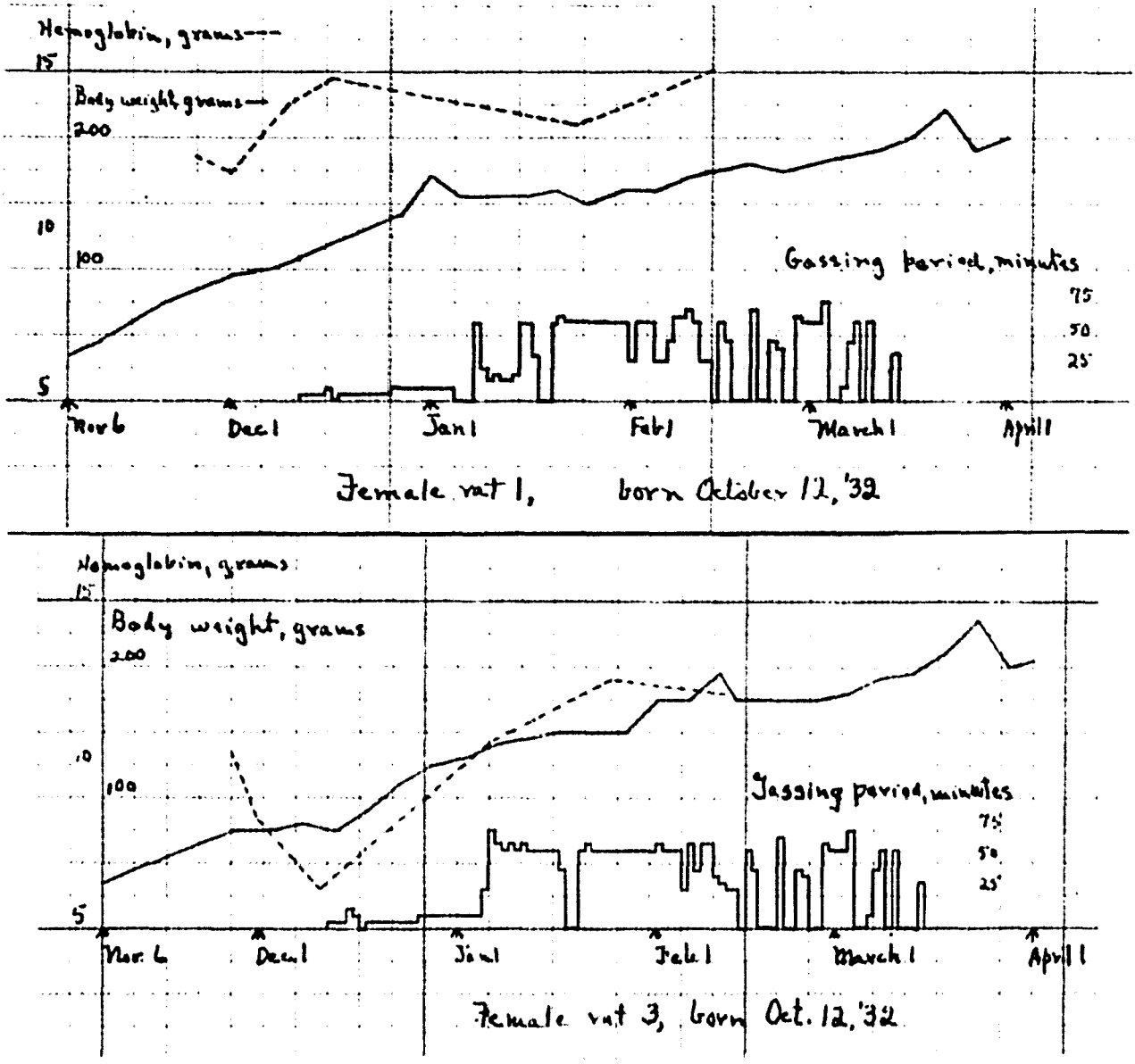

Hemoglobin, grams
15
Body weight, grams
200
10
100
5
Gassing period, minutes
75
50
25
Nov 6
Dec 1
Jan 1
Feb 1
March 1
April 1
Female rat 1, born October 12, '32
Hemoglobin, grams
15
Body weight, grams
200
10
100
5
Gassing period, minutes
75
50
25
Nov 6
Dec 1
Jan 1
Feb 1
March 1
April 1
Female rat 3, born Oct. 12, '32

Figure 7.

Female rat 5 has the following record:

Birth	Death	Litter size	Condition of male
5-30-33	6- 2-33	7	Normal
7-12-33	7-12-33	2?	Normal

The female killed three young of the first litter, and did not care for the others. The two young of the second litter that were seen had been killed by the mother.

As far as could be judged by gross examination, the female appeared normal when killed July 20.

Female rat 7 bore one litter:

Birth	Death	Litter size	Condition of male
6- 3-33	1 survived*	10	Normal

*8 dead June 4, and 1 dead June 20; survivor killed June 27.

Subsequent history of surviving young:

June 8	Each weighs 5 grams
June 13	Each weighs 7 grams
June 14	Caudal necrosis observed in each
June 15,16	Eyes open
June 18	Weights 9 and 11 grams, respectively
June 20	Smaller of two dies; severe caudal necrosis
June 23	Weight of surviving rat 20 grams
June 27	Weight of surviving rat 24 grams
	Rat killed: normal appearing, except for almost total absence of tail.

Figure 7.

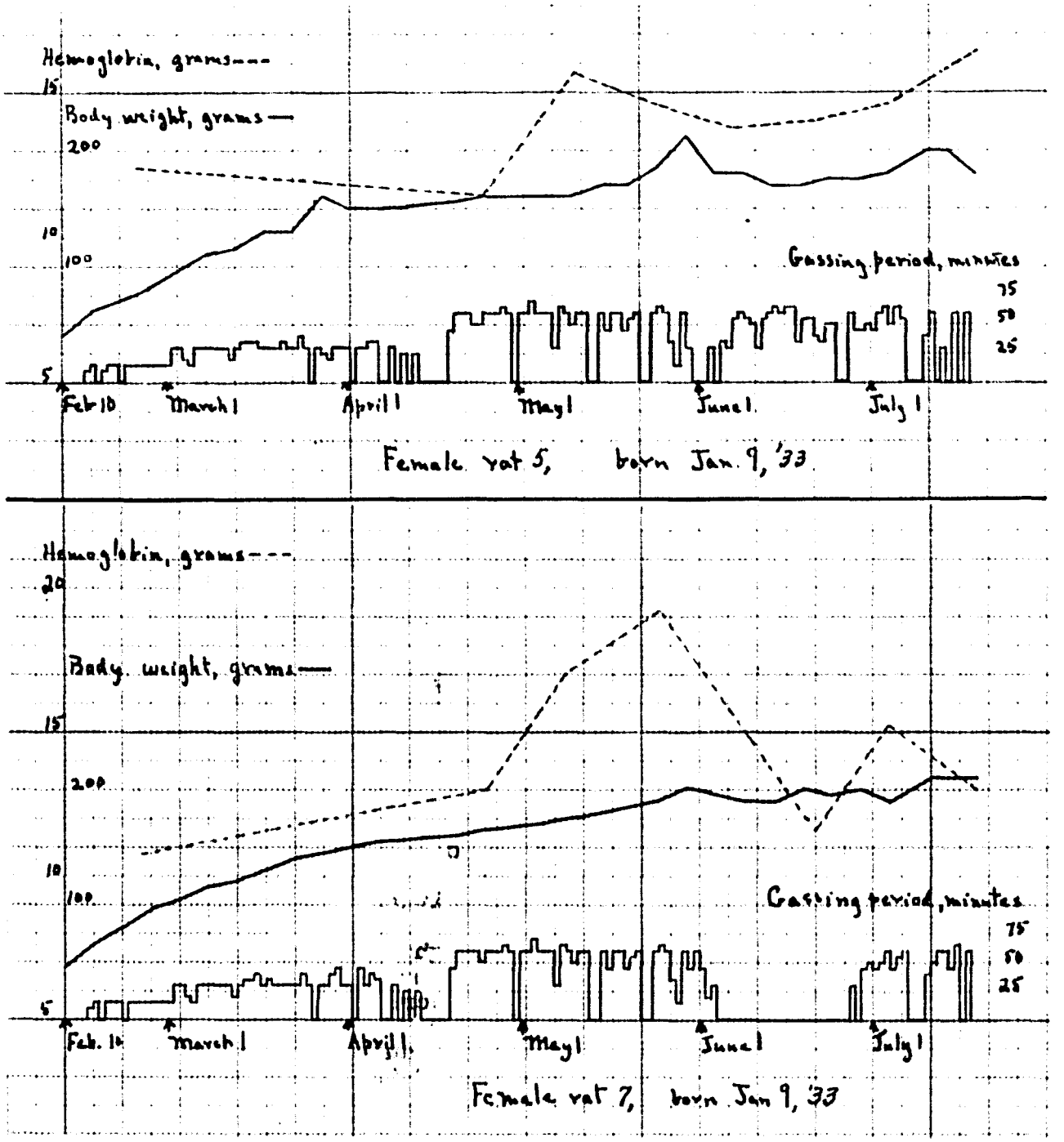

Hemoglobin, grams---
Body weight, grams——
Gassing period, minutes
Feb 10
March 1
April 1
May 1
June 1
July 1
Female rat 5, born Jan. 9, '33
Hemoglobin, grams---
Body weight, grams——
Gassing period, minutes
Feb. 1
March 1
April 1
May 1
June 1
July 1
Female rat 7, born Jan 9, '33

Figure 8.

Female rat 9 bore one litter:

Birth	Death	Litter size	Condition of male
6- 3-33	6- 5-33	8	Normal

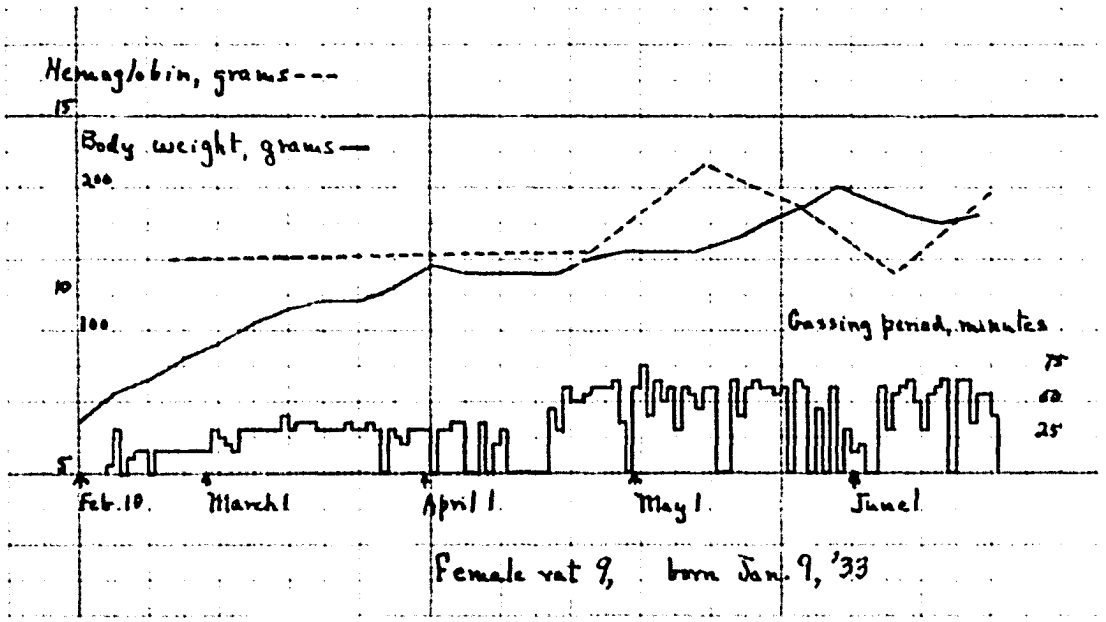
The young were of an abnormal, yellow color, and were not cared for by the mother.

Female rat 11 bore one litter:

Birth	Death	Litter size	Condition of male
6- 3-33	6- 6-33	6	Normal

The young appeared to be normal at birth, and were seen nursing.

Figure 8.


Hemaglobin, grams---
15
Body weight, grams—
200
10
100
Gassing period, minutes
75
50
25
5
Feb. 10.
March 1
April 1.
May 1.
June 1
Female rat 9, born Jan. 9, '33

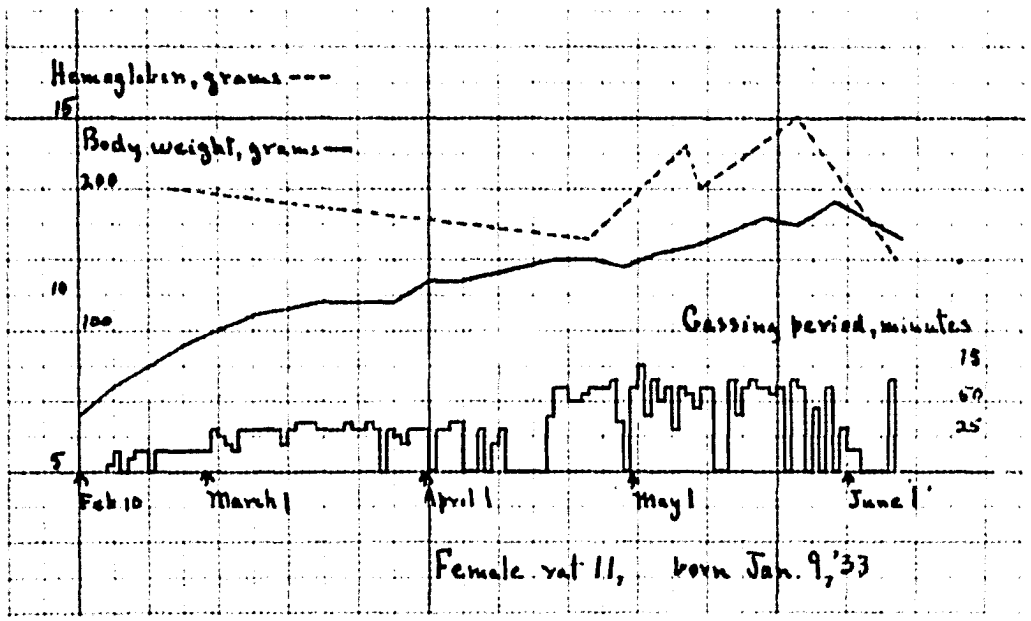

Hemaglobin, grams---
15
Body weight, grams—
200
10
100
Gassing period, minutes
75
50
25
5
Feb 10
March 1
April 1
May 1
June 1
Female rat 11, born Jan. 9, '33

Figure 9.

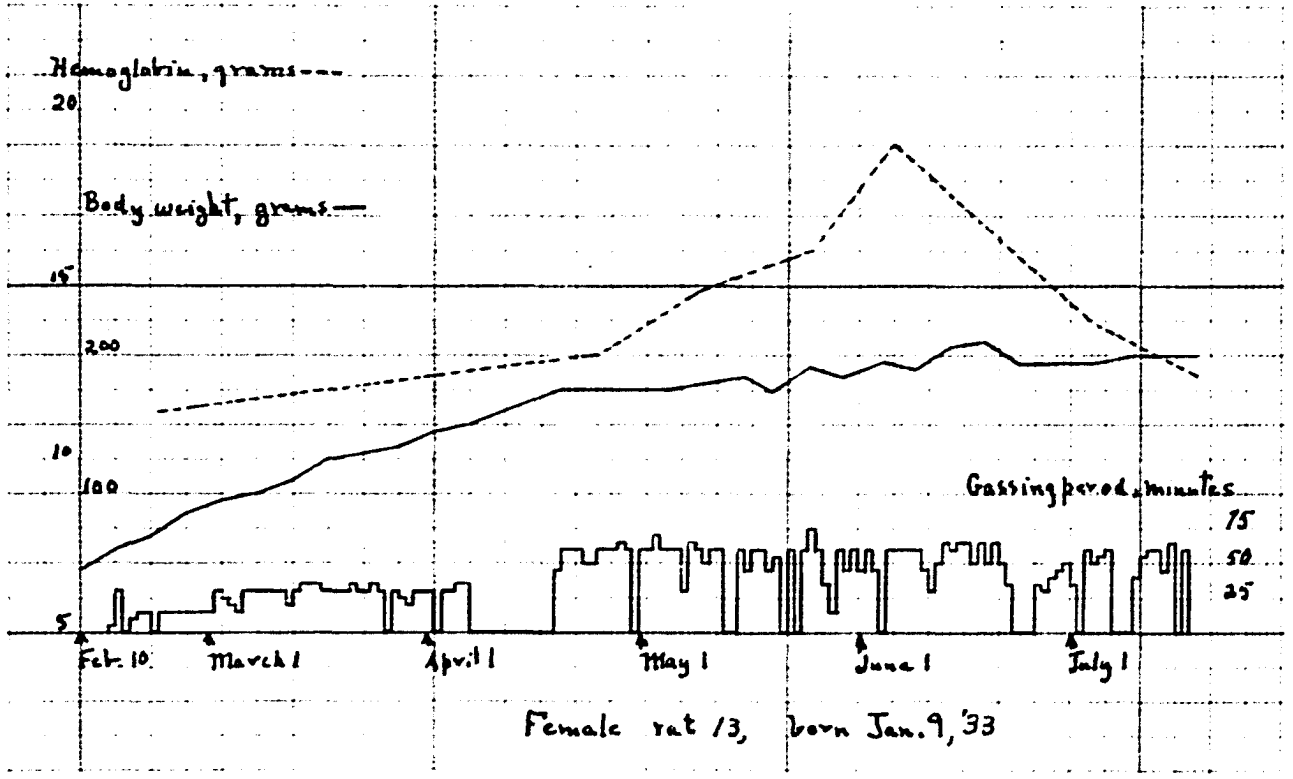

Hemoglobin, grams---
20
Body weight, grams—
15
200
10
100
Gassing period, minutes
75
50
25
5
Feb. 10
March 1
April 1
May 1
June 1
July 1
Female rat 13, born Jan. 9, '33

Figure 9.

Three normal males were placed with female rat 13 on May 5, May 30, and June 5, respectively.

One litter was born:

Birth	Death	Litter size	Condition of male
6-22-33	6-23-33	1	Normal

The young was small, yellow, abnormal looking.

The female was placed again with a normal male, and found to be pregnant when killed July 20. Nine foeti were found in the uterus. A bloody discharge in the vagina indicated the rat to be in about the 14th day of gestation.

Figure 10.

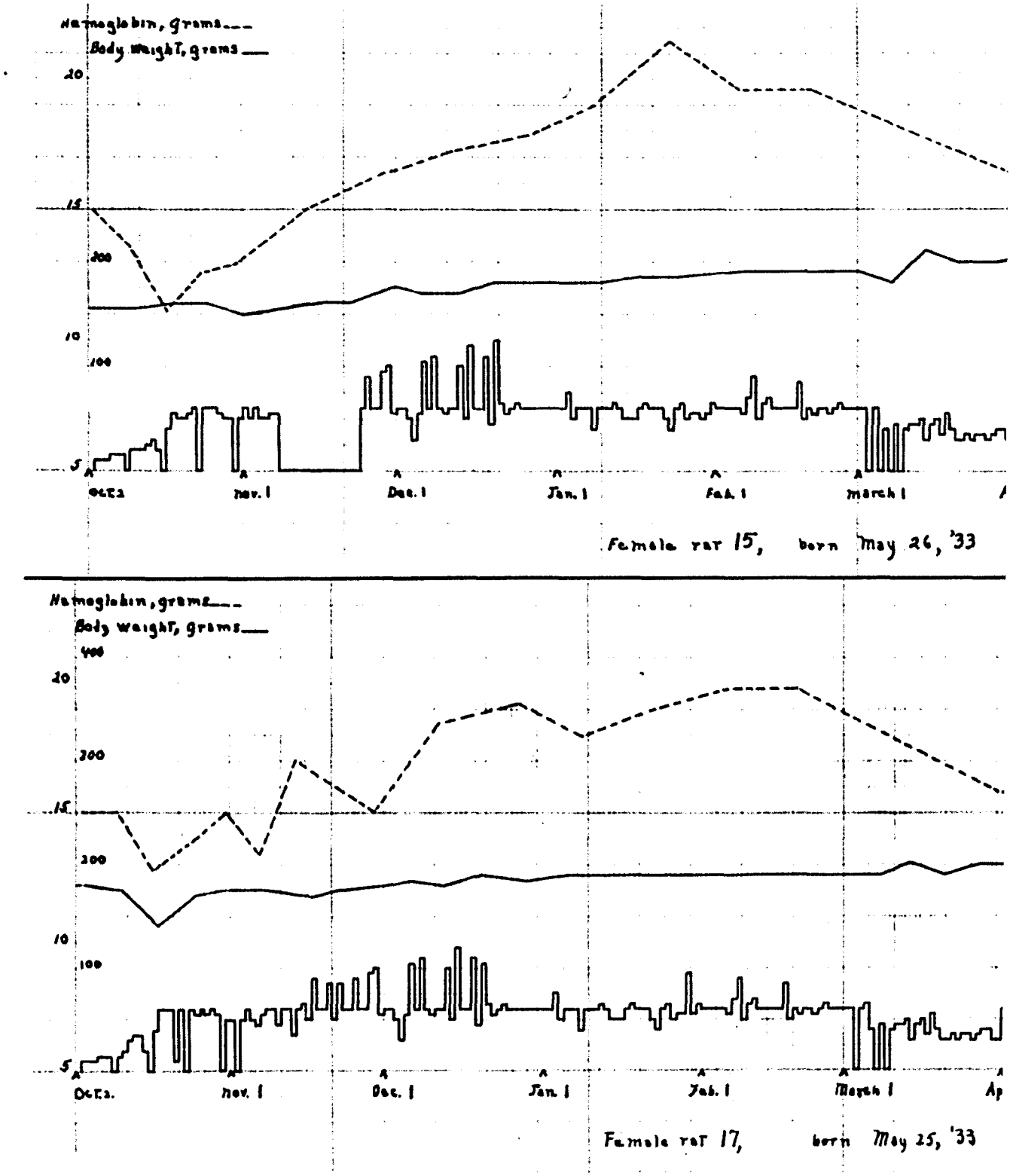
Female rat 15 was placed with a normal male from July 9 till July 13. The rat was in a post-oestrus stage of the reproductive cyle when placed with the male.

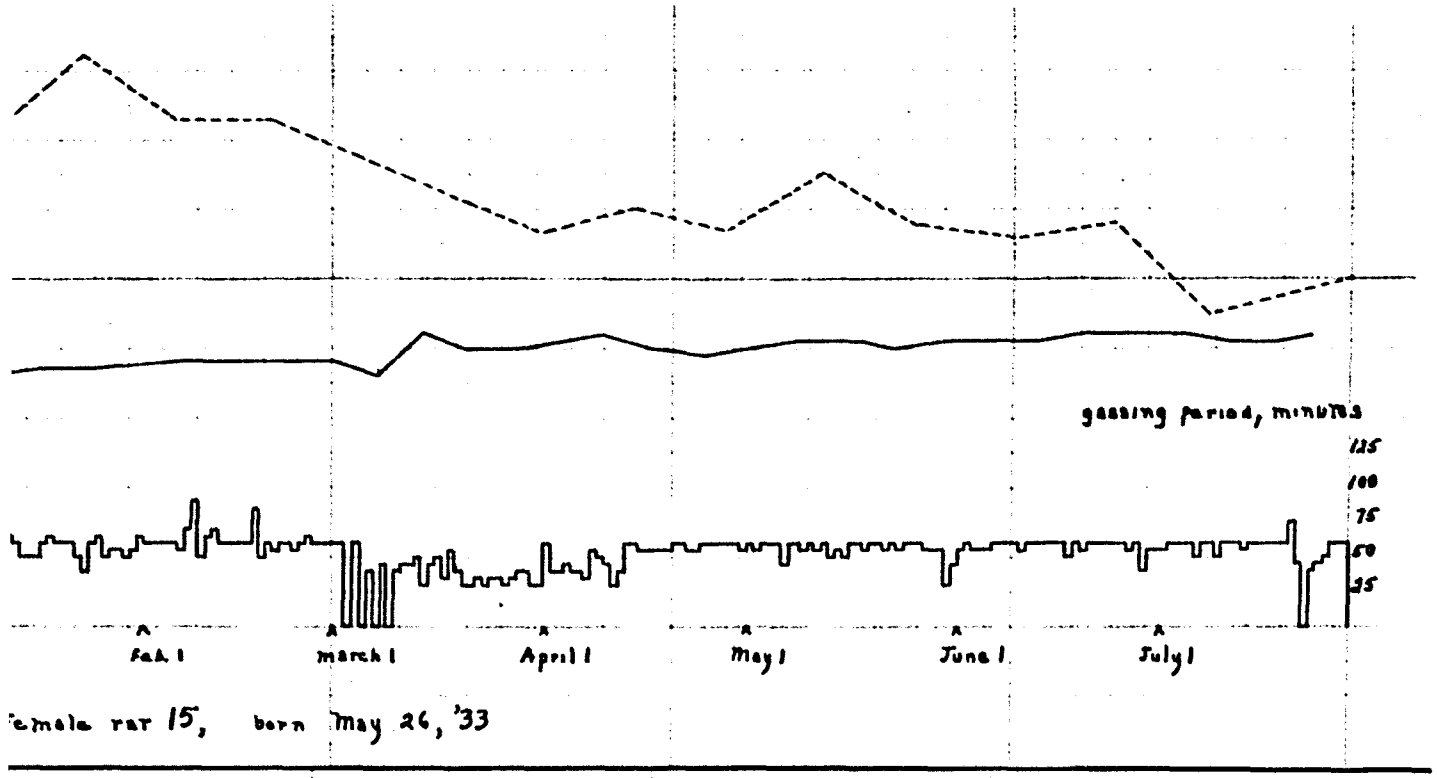
No litter was produced.

Female rat 17 was placed with a normal male July 9-13. The rat was in the pro-oestrus stage of the reproductive cycle when placed with the male.

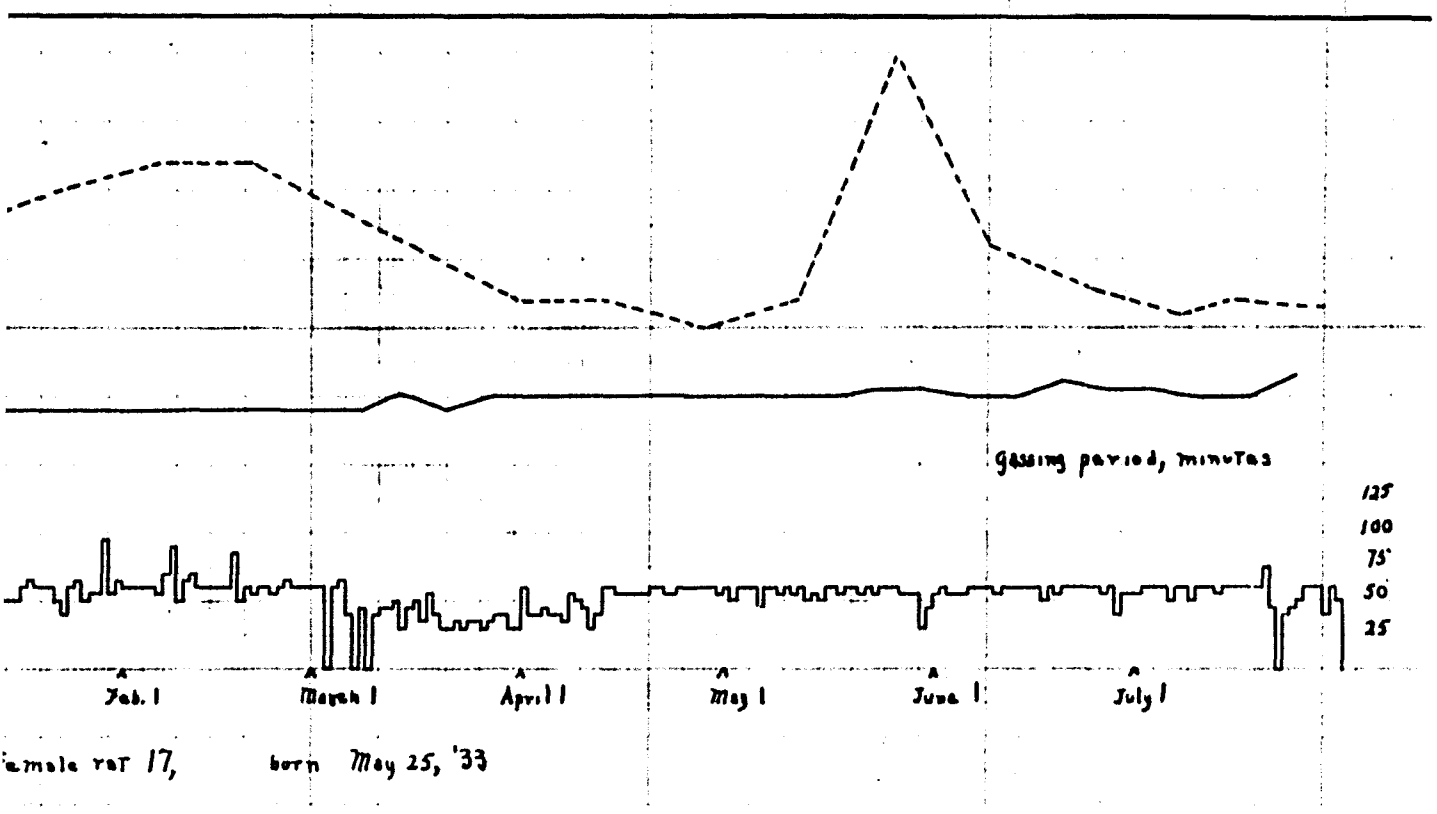
No litter was produced.

Figure 10.


Hemoglobin, grams
Body weight, grams
20
15
10
5
200
100
Oct.1
Nov. 1
Dec. 1
Jan. 1
Feb. 1
March 1
Female rat 15, born May 26, '33
Hemoglobin, grams
Body weight, grams
400
20
300
15
200
10
100
5
Oct.1.
Nov. 1
Dec. 1
Jan. 1
Feb. 1
March 1
Female rat 17, born May 25, '33



female rat 15, born May 26, '33



female rat 17, born May 25, '33

Group 3. Control Male Rats.

Figure 11.

Male rat 2 was kept on a milk diet from November 5 to November 28.

The following litters were produced:

Birth	Death	Litter size	Condition of female
1-31-33	Soon	8	Milk diet Nov. 5-28; not gassed (♀2)
2-13-33	2-14-33	3	Gassed (♀3)
3- 7-33	3-11-33	12	Milk diet Nov. 5-28; not gassed (♀2)
3-23-33	3-25-33	5	Gassed (♀3)
3-24-33	3-24-33	6	Gassed Dec. 1-March 14 (♀1)
4-20-33	5- 2-33	4	Gassed Dec. 1-March 14 (♀1)

Male rat 4 gave rise to the following litters:

Birth	Death	Litter size	Condition of female
6- 6-33	6- 9-33	6	Not gassed (♀4)
7- 6-33	Survived	6	Normal

The female from which this rat was removed on July 6 was pregnant on July 18, but record of the birth was not kept.

On autopsy, a copulation plug and motile spermatozoa were observed.

Figure 11.

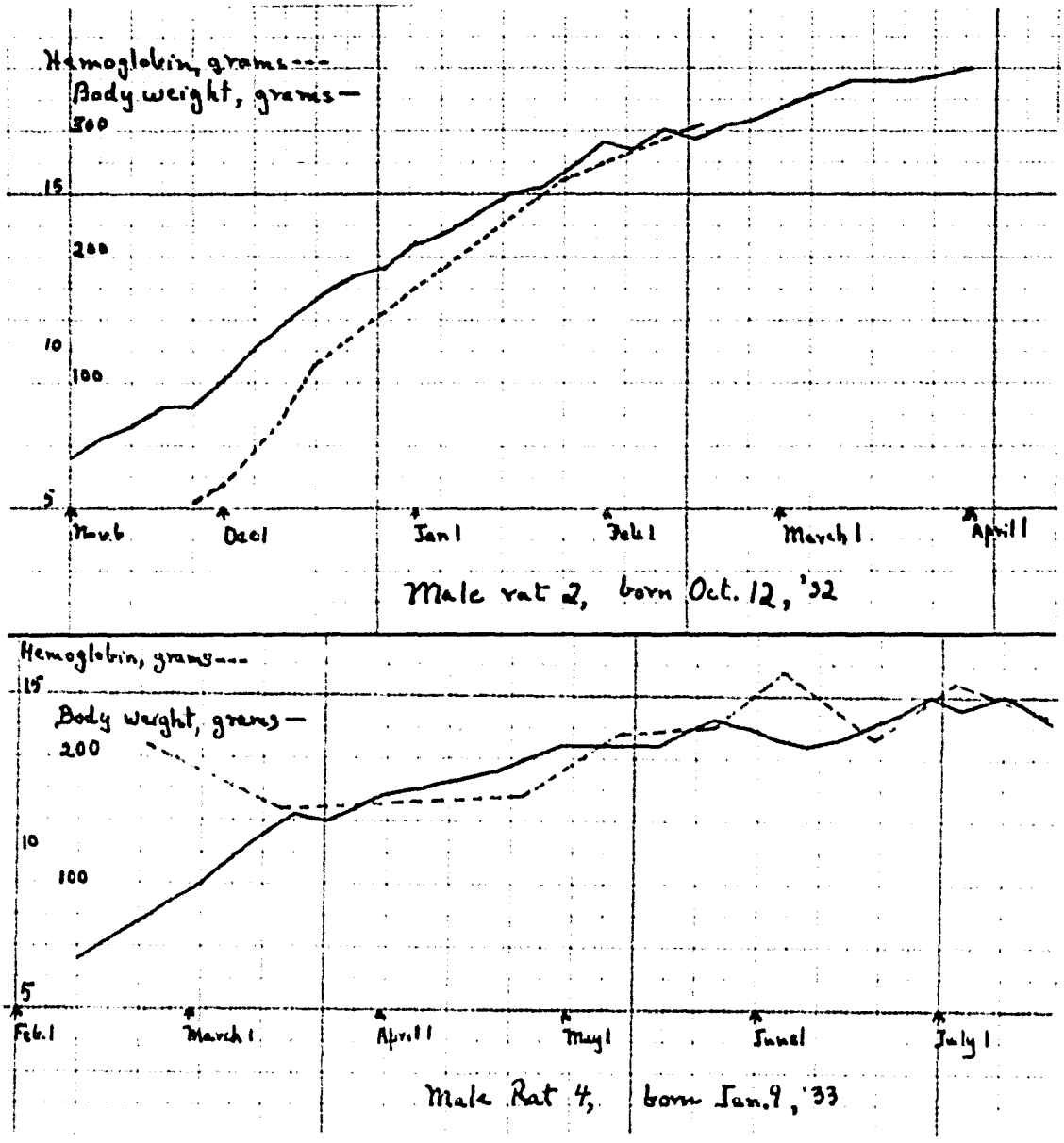

Hemoglobin, grams---
Body weight, grams—
300
200
100
15
10
5
Nov.1
Dec.1
Jan.1
Feb.1
March 1
April 1
Male rat 2, born Oct. 12, '32
Hemoglobin, grams---
Body weight, grams—
15
200
100
10
5
Feb.1
March 1
April 1
May 1
June 1
July 1
Male Rat 4, born Jan. 9, '33

Figure 12.

Male rat 6 gave rise to the following litters:

Birth	Death	Litter size	Condition of female
6- 5-33	6- 8-33	4?	Gassed Dec. 1-March 14 (♀ 1)
7-10-33	Survived	7	Not gassed (♀ 4)
7-25-33	Soon	?	Gassed Dec. 1-March 14 (♀ 1)

Male rat 8 was kept with females, but never gave rise to progeny. There is no record of its ever having been placed with normal females.

Figure 12.

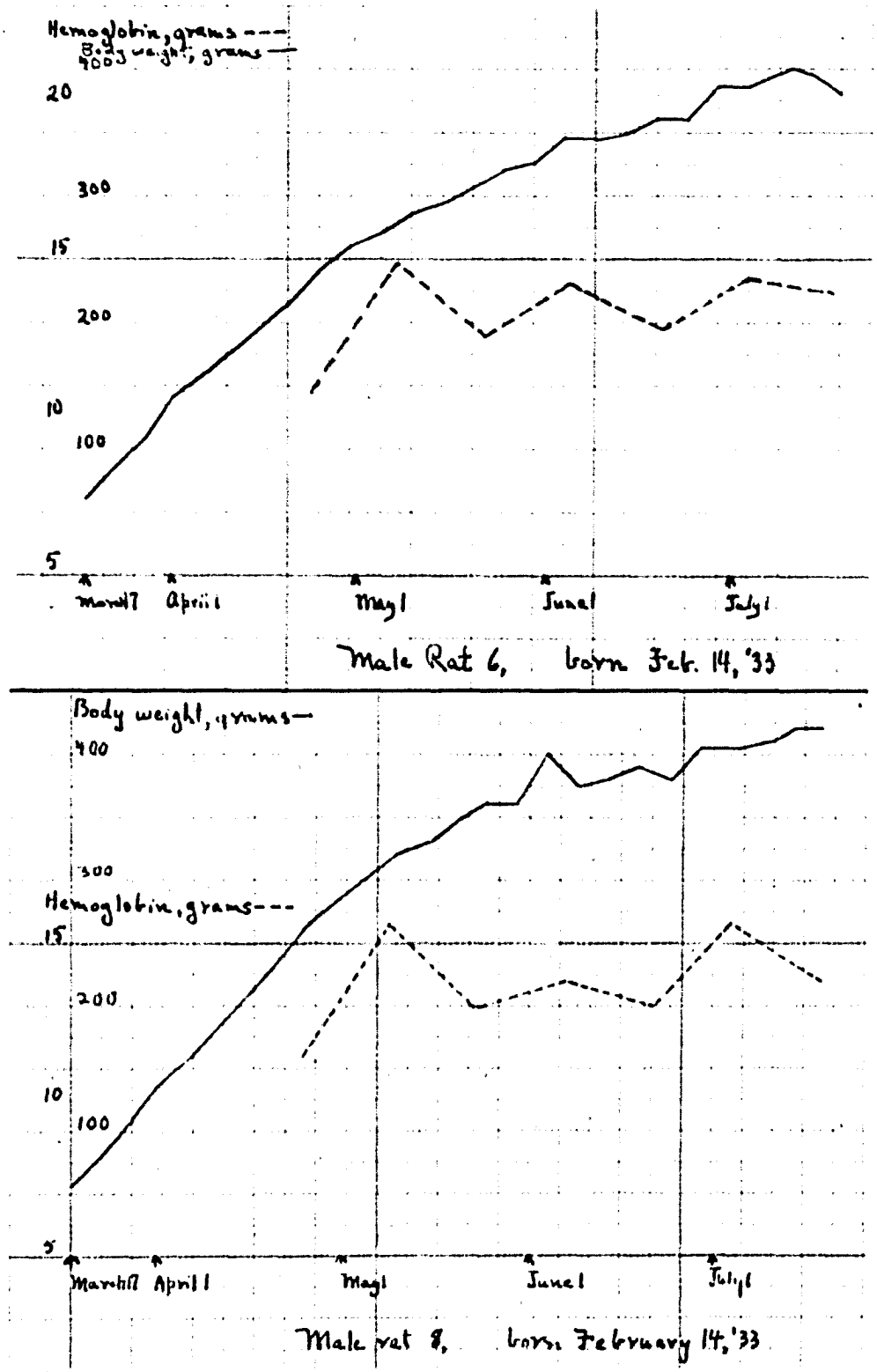

Hemoglobin, grams ---
Body weight, grams —
20
300
15
200
10
100
5
March 7
April 1
May 1
June 1
July 1
Male Rat 6, born Feb. 14, '33
Body weight, grams—
400
300
Hemoglobin, grams---
15
200
10
100
5
March 7
April 1
May 1
June 1
July 1
Male rat 8, born February 14, '33

Figure 13.

Male rat 10 was placed with normal female rats November 23-28 and December 18-23.

One litter resulted:

Birth	Death	Litter size	Condition of female
1-11-34	Survived	Average	Normal

Male rat 12 was placed with female rats November 23-28 and December 18-23.

The following litters resulted:

Birth	Death	Litter size	Condition of female
12-18-33	Survived	Average	Normal
1-13-34	Survived	12	Normal

The rat was autopsied February 20. Motile spermatozoa were observed. Testis weight was found to be 2.6 gm. Donaldson (1924) gives 2.66 gm. to be normal testis weight for a rat of this size.

Figure 13.

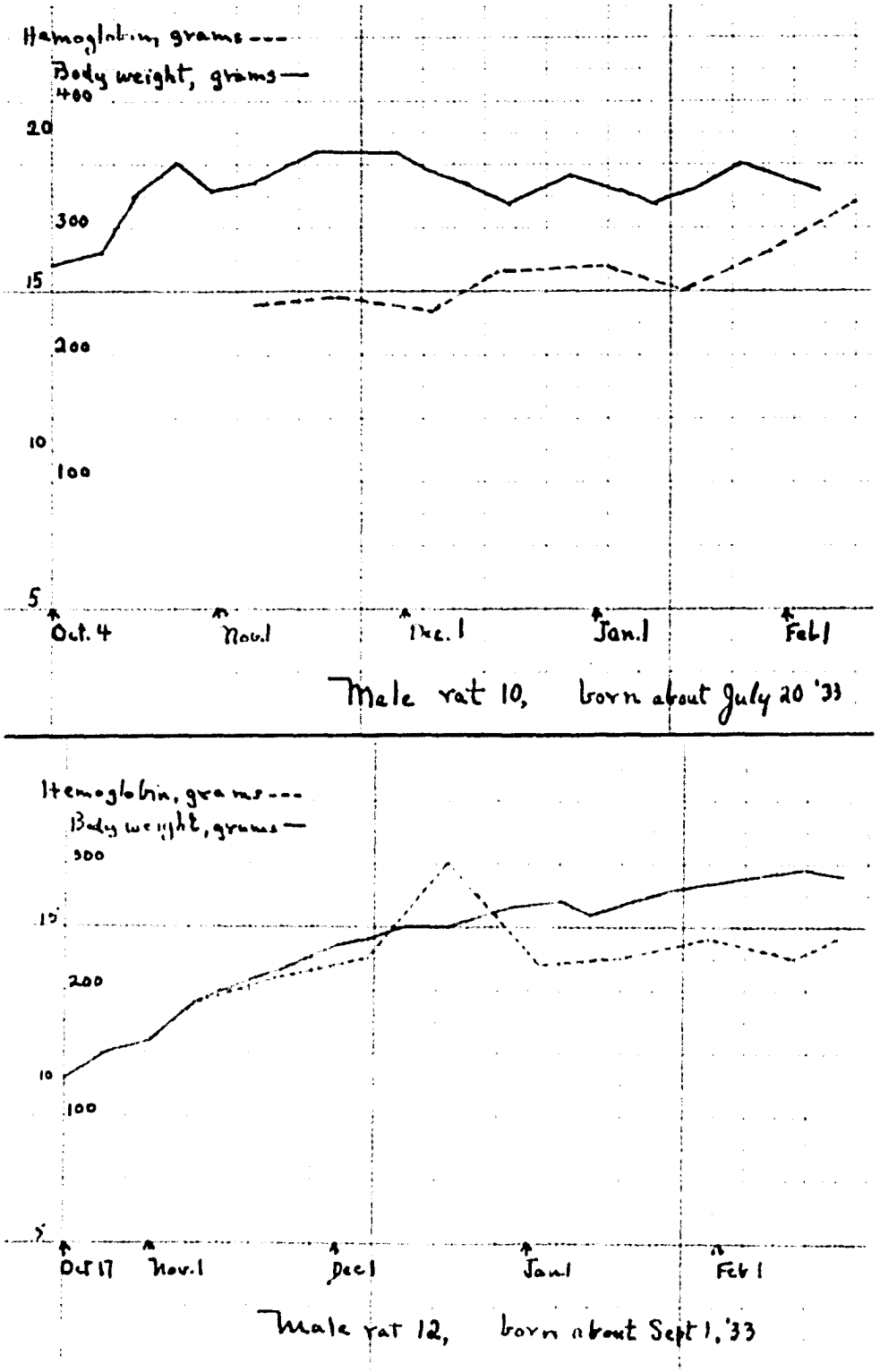

Hemoglobin, grams ---
Body weight, grams —
400
300
200
100
20
15
10
5
Oct. 4
Nov. 1
Dec. 1
Jan. 1
Feb 1
Male rat 10, born about July 20 '33
Hemoglobin, grams ---
Body weight, grams —
300
200
100
15
10
5
Oct 17
Nov. 1
Dec 1
Jan. 1
Feb 1
Male rat 12, born about Sept 1, '33

Figure14.

Male rat 14 was placed with females November 23-28 and December 18-23.

One litter resulted:

Birth	Death	Litter size	Condition of female
1-13-34	Survived	Average	Normal

At autopsy actively motile spermatozoa were observed. Testis weight was found to be 2.2 gm., the normal for a rat of this weight being 2.6 gm.

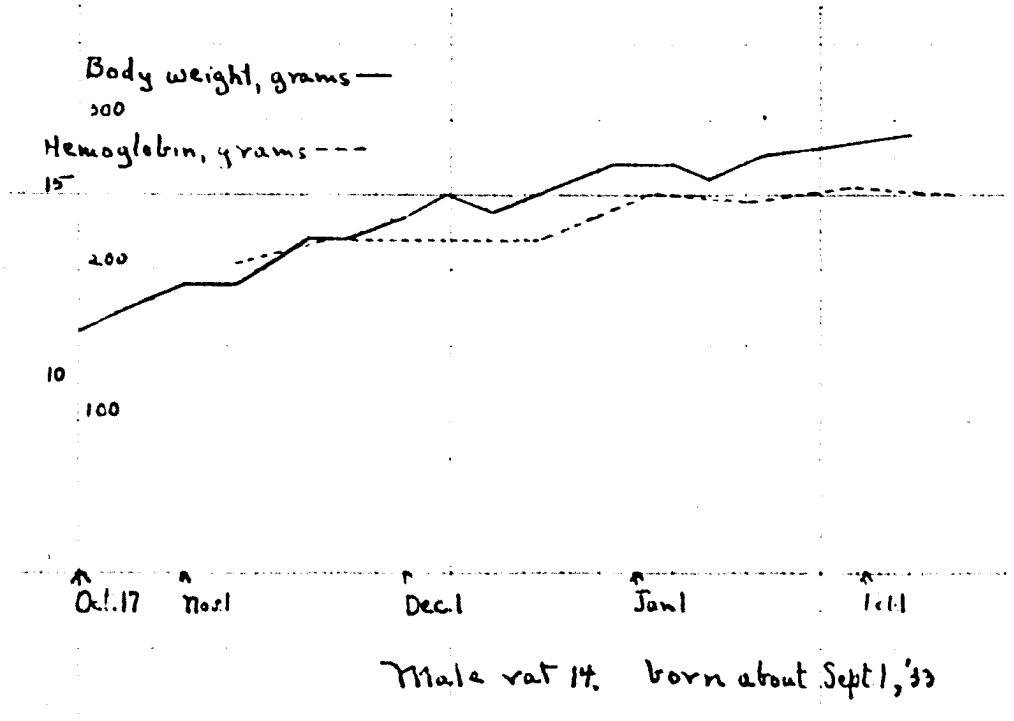
Male rat 16 was placed with normal females May 27-30. On June 20 it was placed with a female in pro-oestrus, and on June 23, with a second female in pro-oestrus. These females were removed June 25.

The following litters resulted:

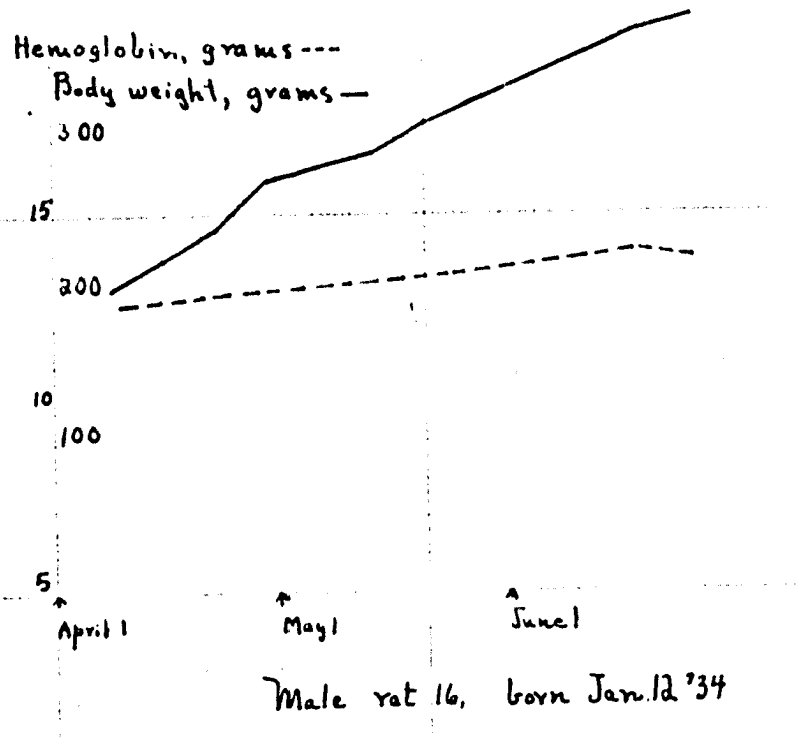
Birth	Death	Litter size	Condition of female
6-19-34	Survived	7	Normal
7-17-34	6 survived	7	Normal

Motile spermatozoa were observed on autopsy. Testis weight was 2.1 gm., the expected normal for this rat being 2.96 gm.

Figure 14.



Male rat 14. born about Sept 1, '33



Male rat 16, born Jan. 12 '34

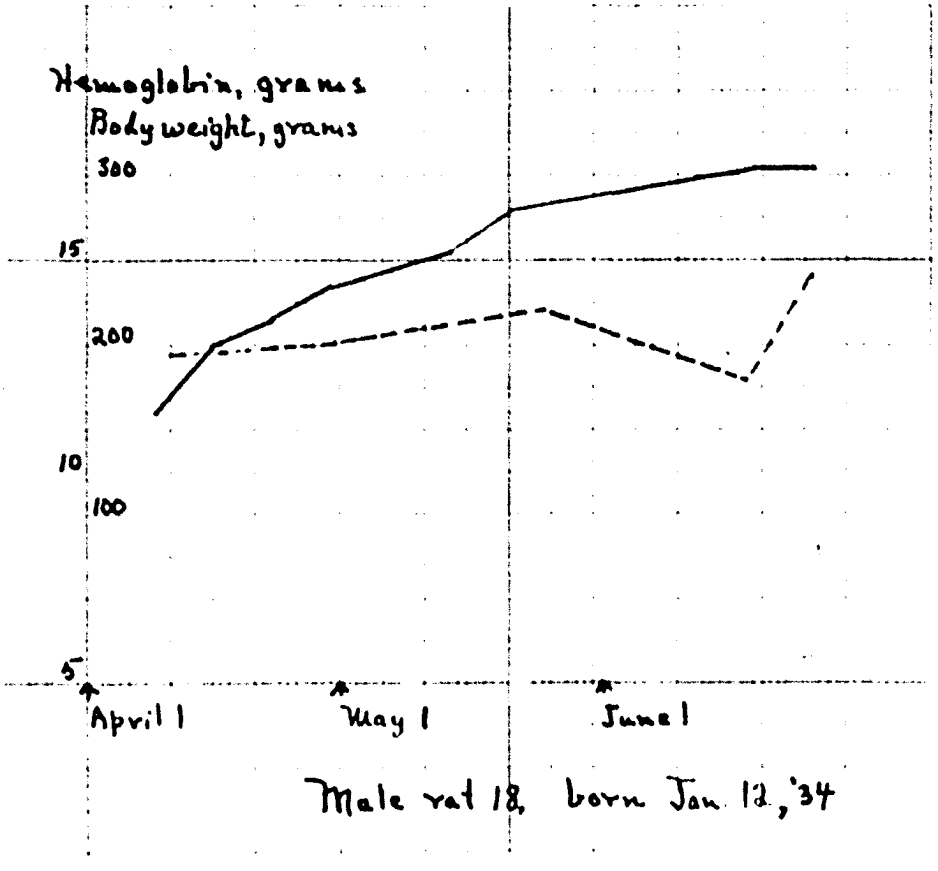
Figure 15.

Male rat 18 was placed with normal females May 27-30 and on June 20 and June 23. On the latter dates the females placed with the male were in pro-oestrus. They were removed June 25.

The following litters were produced:

Birth	Death	Litter size	Condition of female
7-14-34	Survived	9	Normal
7-15-34	Survived	9	Normal

Figure 15.


Hemoglobin, grams
Body weight, grams
300
15
200
10
100
5
April 1
May 1
June 1
Male rat 18, born Jan 12, '34

Group 4. Gassed Male Rats

Figure 16.

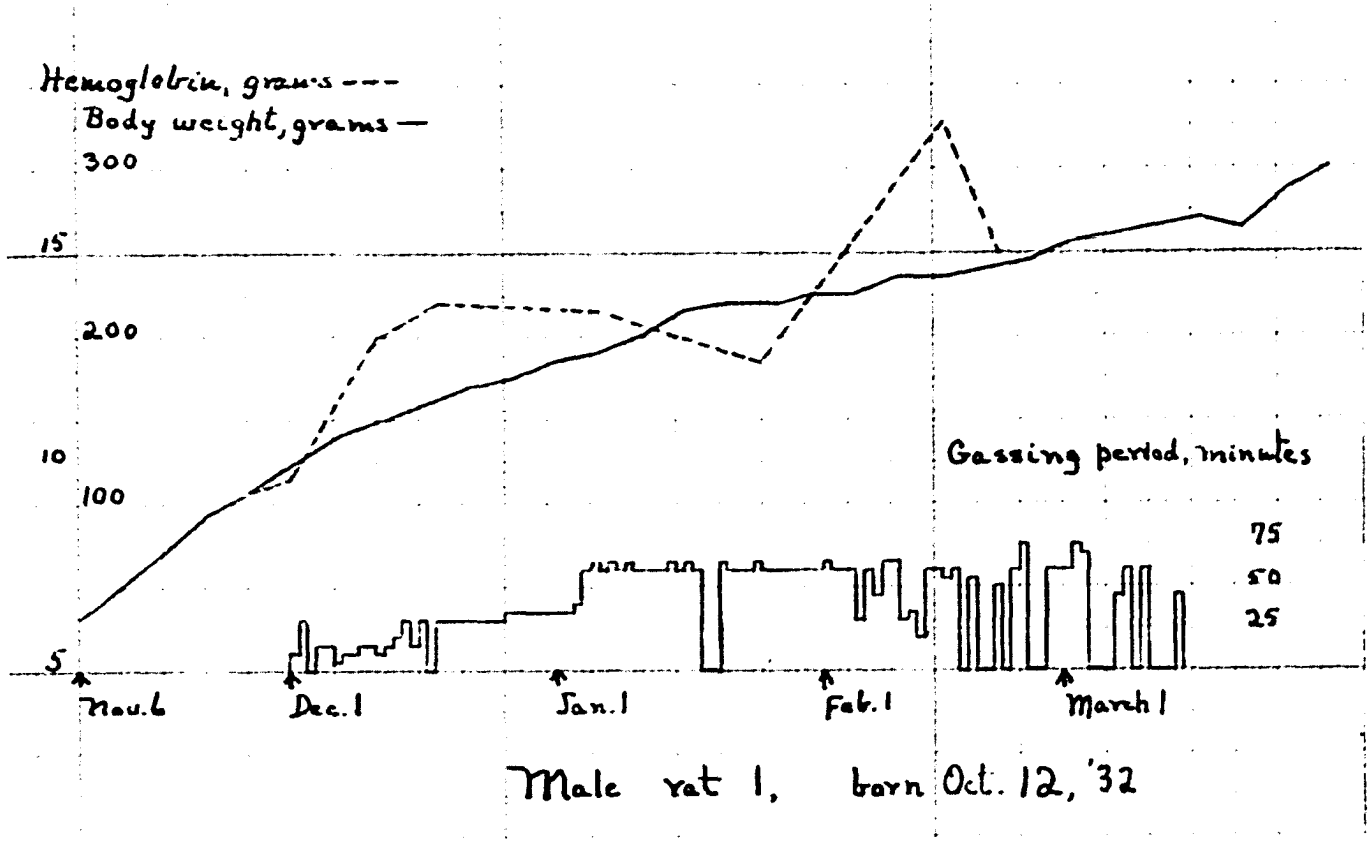

Hemoglobin, grams ---
Body weight, grams —
300
15
200
10
100
5
Gassing period, minutes
75
50
25
Nov. 6
Dec. 1
Jan. 1
Feb. 1
March 1
Male rat 1, born Oct. 12, '32

Figure 16.

Male rat 1 was kept with female rats during the most of its life. Two pregnancies resulted during the early part of the experiment, when the total gassing time to which the rat had been subjected was between three and five hours.

Record of the litters:

Birth	Death	Litter size	Condition of female
1- 5-33	1- 7-33	7	Gassed (♀1)
1- 7-33	1-15-33	3	Normal (the control rat of an earlier experiment)

Figure 17.

Male rat 3 was fed a milk diet from November 5 to November 28.

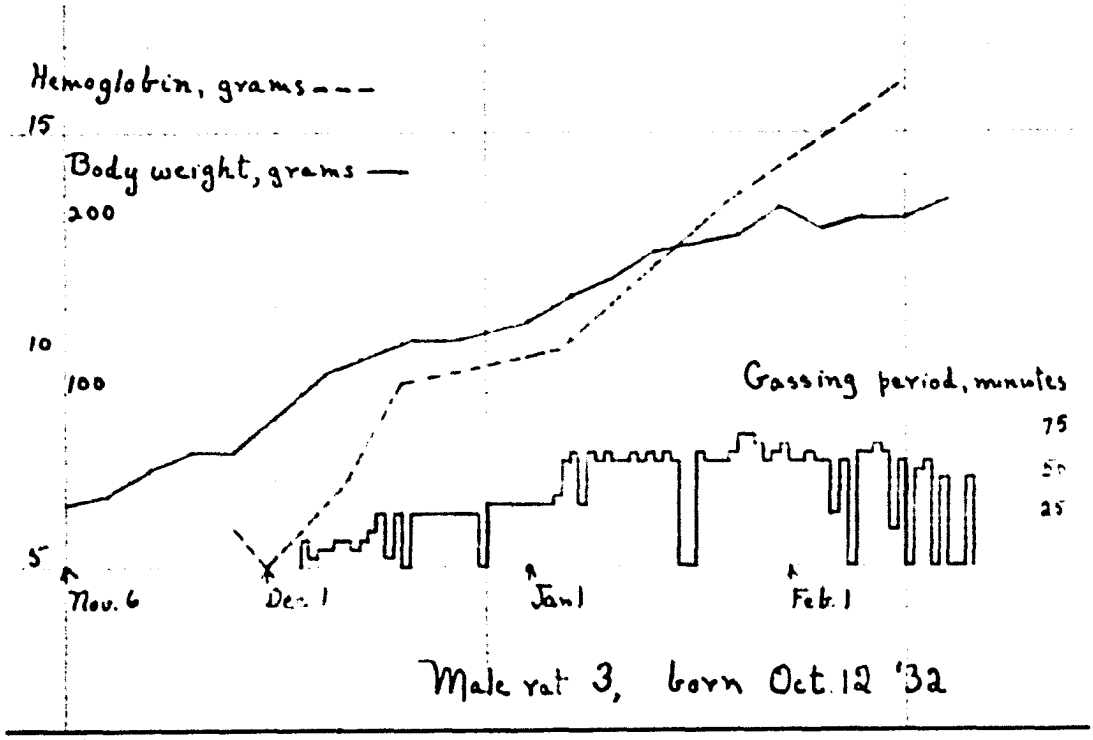
Though it was kept with a normal female (the control rat of an earlier experiment) which bore young, it never gave rise to progeny.

When examined after death, one testis was found to be only one-third normal size.

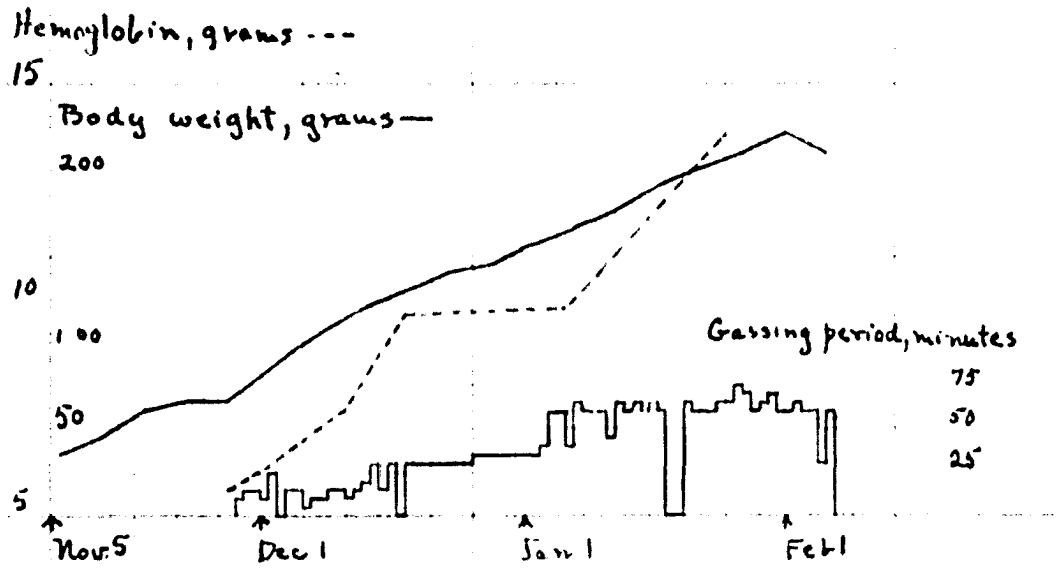
Male rat 5 was fed a milk diet from November 5 to November 28.

It was kept with two female rats (♀2 and ♀3), both of which reproduced, during its entire life, but it never gave rise to young.

Figure 17.



Male rat 3, born Oct. 12 '32



Male rat 5, born Oct. 12, '32

Figure 18.

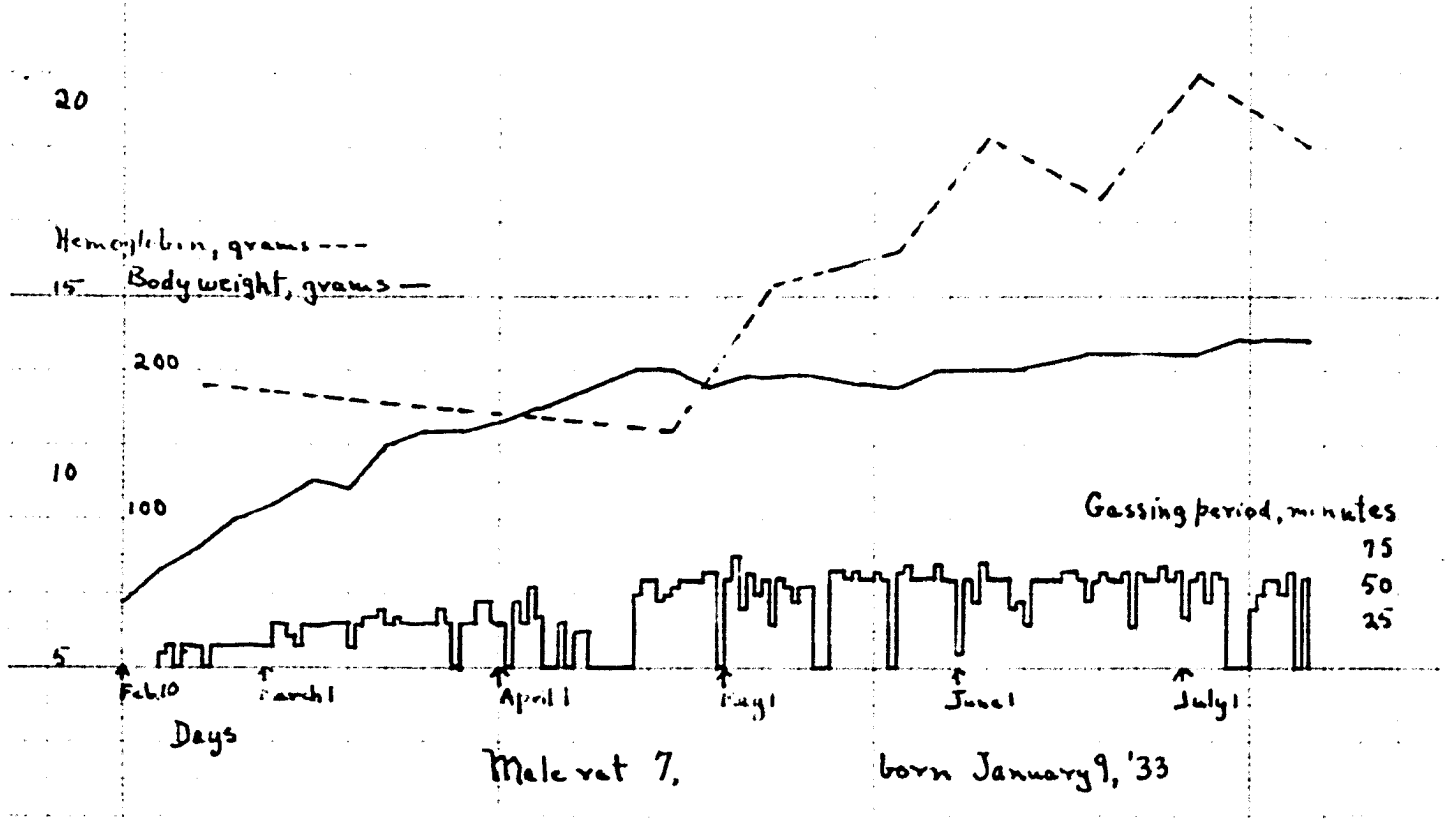

20
Hemoglobin, grams ---
15 Body weight, grams —
200
10
100
Gassing period, minutes
75
50
25
5
Feb 10
March 1
April 1
May 1
June 1
July 1
Days
Male rat 7, born January 9, '33

Figure 18.

Male rat 7 was placed with two normal female rats, each of which had produced litters, on May 15. A third female was placed in the cage on June 20. None of these females gave rise to young.

The following wieght record of the females was kept, in an effort to determine the possibility of impregnation and subsequent resorption taking place:

Date	Weight grams	Weight grams	Weight grams
5-15-33	180	220	
5-19-33	176	236	
5-25-33	198	242	
5-29-33	196	244	
6- 3-33	196	246	
6- 8-33	194	250	
6-13-33	198	240	
6-18-33	202	252	
6-23-33	210	252	210
6-28-33	206	260	218
7- 3-33	208	252	228
7-10-33			220

The male rat constantly showed a priapism.

Figure 19.

Male rat 9 was kept with normal females that had raised litters from May 27 till its death. None of these females had litters or were pregnant when killed.

The weights of the females were recorded in an attempt to ascertain if resorption were taking place. The weight record is given:

Date	Weight grams	Weight grams	Weight grams	Weight grams
5-29-33	240?			
6- 3-33	276			
6- 8-33	270			
6-13-33	270			
6-18-33	278	184		
6-23-33	294	194	192	
6-28-33	294	206	198	238
7- 3-33	288	196	206	246
7-10-33	294	200	214	254
7-13-33	298	206	218	256
7-18-33	294	200	208	252

Male rat 11 was kept with two normal females which had raised litters from May 27 and June 3 respectively, till its death. Neither female bore young.

The following weight record of the females was obtained:

Date	Weight grams	Weight grams
5-29-33	250	
6- 3-33	260	212
6- 8-33	260	210
6-13-33	266	220
6-18-33	276	222
6-23-33	290	218
6-28-33	294	212
7- 3-33	280	214
7-10-33	286	216
7-13-33	284	218

Figure 19.

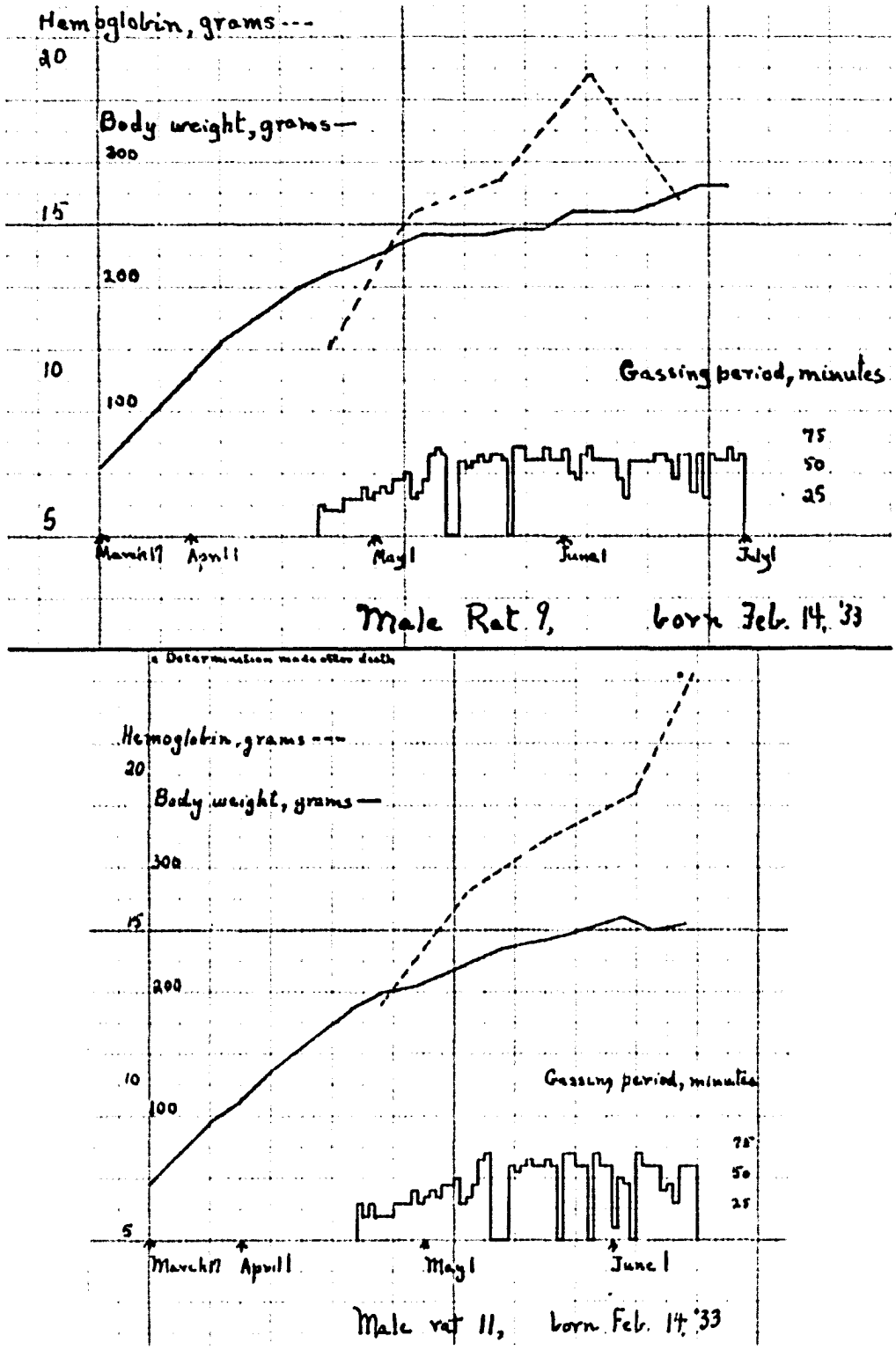

Hemoglobin, grams---
20
Body weight, grams—
300
15
200
10
100
Gassing period, minutes
75
50
25
5
March 17
April 1
May 1
June 1
July 1
Male Rat 9, born Feb. 14, '33
a Determination made after death
Hemoglobin, grams---
20
Body weight, grams—
300
15
200
10
100
Gassing period, minutes
75
50
25
5
March 17
April 1
May 1
June 1
Male rat 11, born Feb. 14 '33

Figure 20.

Male rat 13 produced no offspring. It was placed with normal females November 23-28, and December 18-23.

Male rat 15 did not reproduce, It was placed with normal females November 23-28, and December 18-23.

When it was killed, no motile spermatozoa were observed. Testis weight was found to be 1.7 gm. as compared to the expected normal of 2.5 gm. for this rat.

Figure 20.

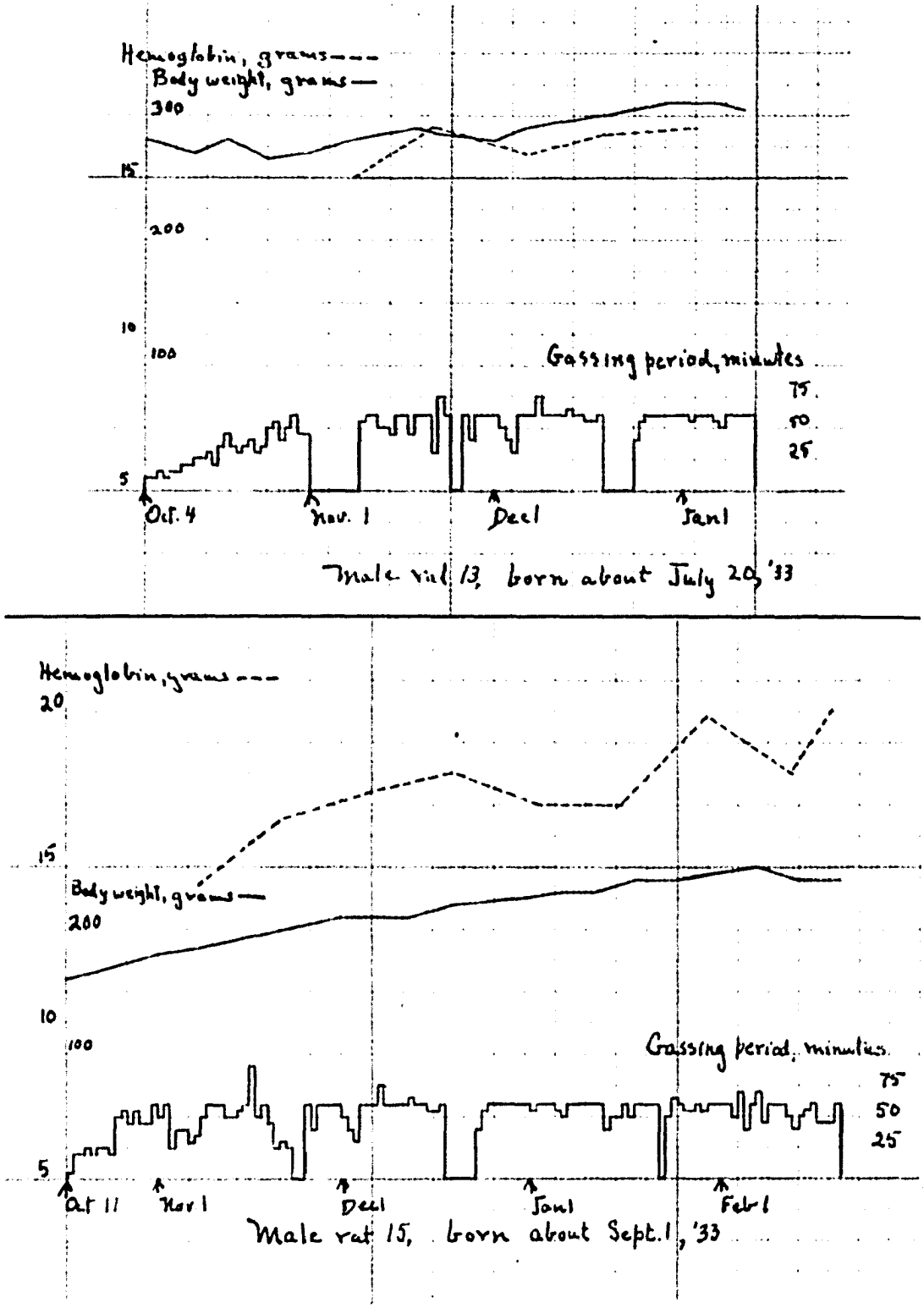

Hemoglobin, grams---
Body weight, grams—
300
15
200
10
100
5
Gassing period, minutes
75
50
25
Oct. 4
Nov. 1
Dec 1
Jan 1
Male rat 13, born about July 20, '33
Hemoglobin, grams---
20
15
Body weight, grams—
200
10
100
5
Gassing period, minutes
75
50
25
Oct 11
Nov 1
Dec 1
Jan 1
Feb 1
Male rat 15, born about Sept. 1, '33

Figure 21.

Male rat 17 gave rise to no progeny. It was placed with normal females November 23-28 and December 18-23.

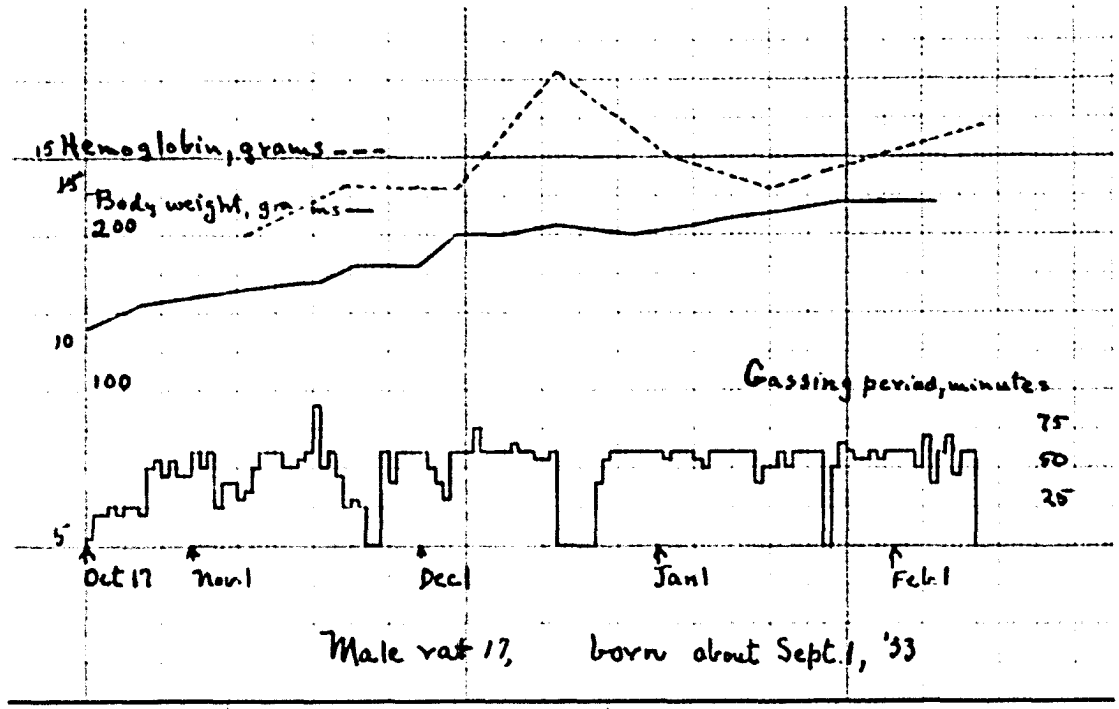
At death, no motile spermatozoa were found. Testis weight was 0.8 gm., as compared to 2.3 gm., the expected normal.

Male rat 19 was placed with two normal females from May 27 to May 30. On June 20, 22, and 23 normal females in proestrus were placed in the cage with this male. These females were removed June 25. None bore litters.

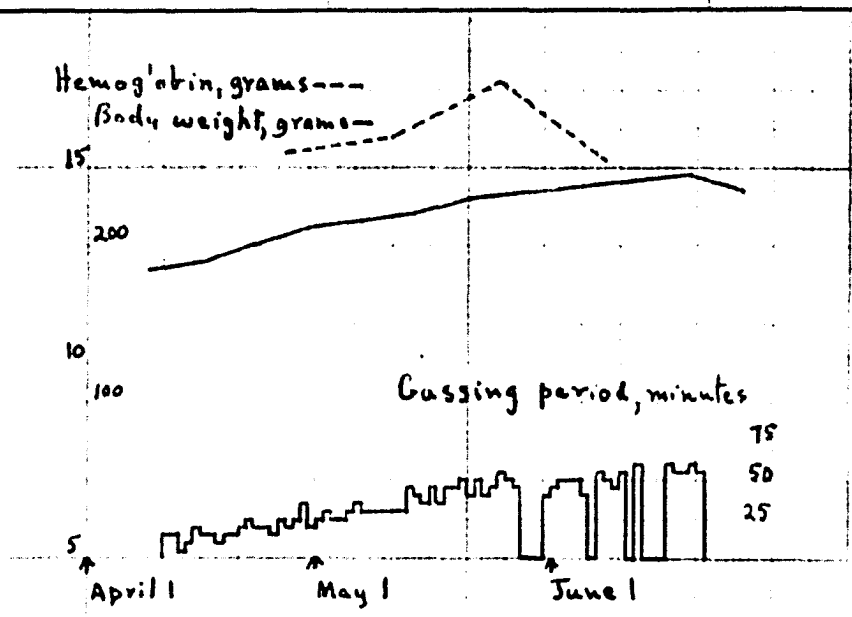
The weight of the females for a three week period following association with the male are given:

Female placed with male	June 20	June 22	June 23
Date	Weight grams	Weight grams	Weight grams
6-20-34	180	210	188
6-25-34	186	214	190
6-30-34	184	206	190
7- 4-34	188	208	196
7- 8-34	190		
7- 9-34		212	198

Figure 21.



Male rat 17, born about Sept. 1, '33



Male rat 19, born Jan. 12, '34

Figure 22.

Male rat 21 was placed with two normal females May 27-30, and with one normal female determined to be in pro-oestrus, June 20-25. No offspring resulted.

The weight record of the female placed with the male June 20 is as follows:

Date	Weight grams
6-20-34	202
6-25-34	200
6-30-34	208
7- 4-34	210
7- 9-34	208

When the rat was killed June 26, no motile spermatozoa were observed. The testis weighed 0.895 gm. Normal weight for the testis of this rat would be 2.7 gm.

Male rat 23 was placed with two normal females May 27-30. On June 21, one of these females was seen to be about to deliver a litter. She was isolated, and not observed for a few hours. None of the litter was ever seen, but the weight of the female dropped 20 grams over-night.

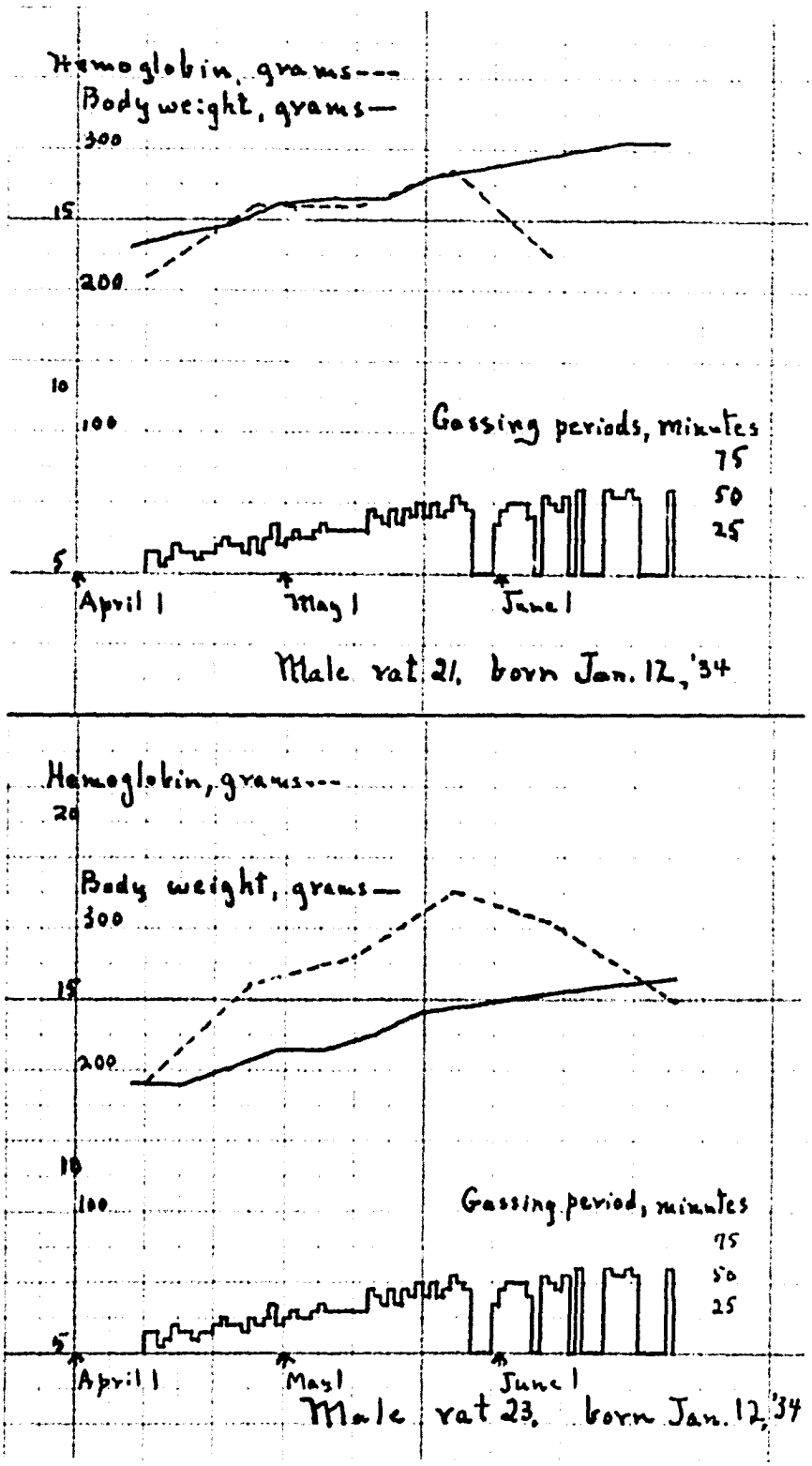
On June 20-25, the male rat was with a normal female known to be in pro-oestrus. No litter resulted.

The weight record of the female is given:

Date	Weight grams
6-20-34	220
6-25-34	218
6-30-34	220
7- 4-34	216
7- 9-34	222

When the male rat was killed June 26, a few (two) very slightly motile spermatozoa were observed. Testis weight was found to be 0.708 gm. Normal expected weight for this rat is 2.5 gm.

Figure 22.


Hemoglobin, grams---
Body weight, grams—
300
15
200
10
100
5
Gassing periods, minutes
75
50
25
April 1
May 1
June 1
Male rat 21, born Jan. 12, '34
Hemoglobin, grams---
20
Body weight, grams—
300
15
200
10
100
5
Gassing period, minutes
75
50
25
April 1
May 1
June 1
Male rat 23, born Jan. 12, '34

Formation of Carbon Monoxide Hemoglobin

Between March 16 and June 2, 1933, and again in June 1934, a number of determinations were made of the percentage of carbon monoxide hemoglobin in the rats kept in the laboratory, and in the experimental animals under different conditions. The Sayers and Yant Pyrotannic Acid method used is accurate within ten per cent. The results obtained are given in the following table:

Table 1. Carbon monoxide hemoglobin determinations.

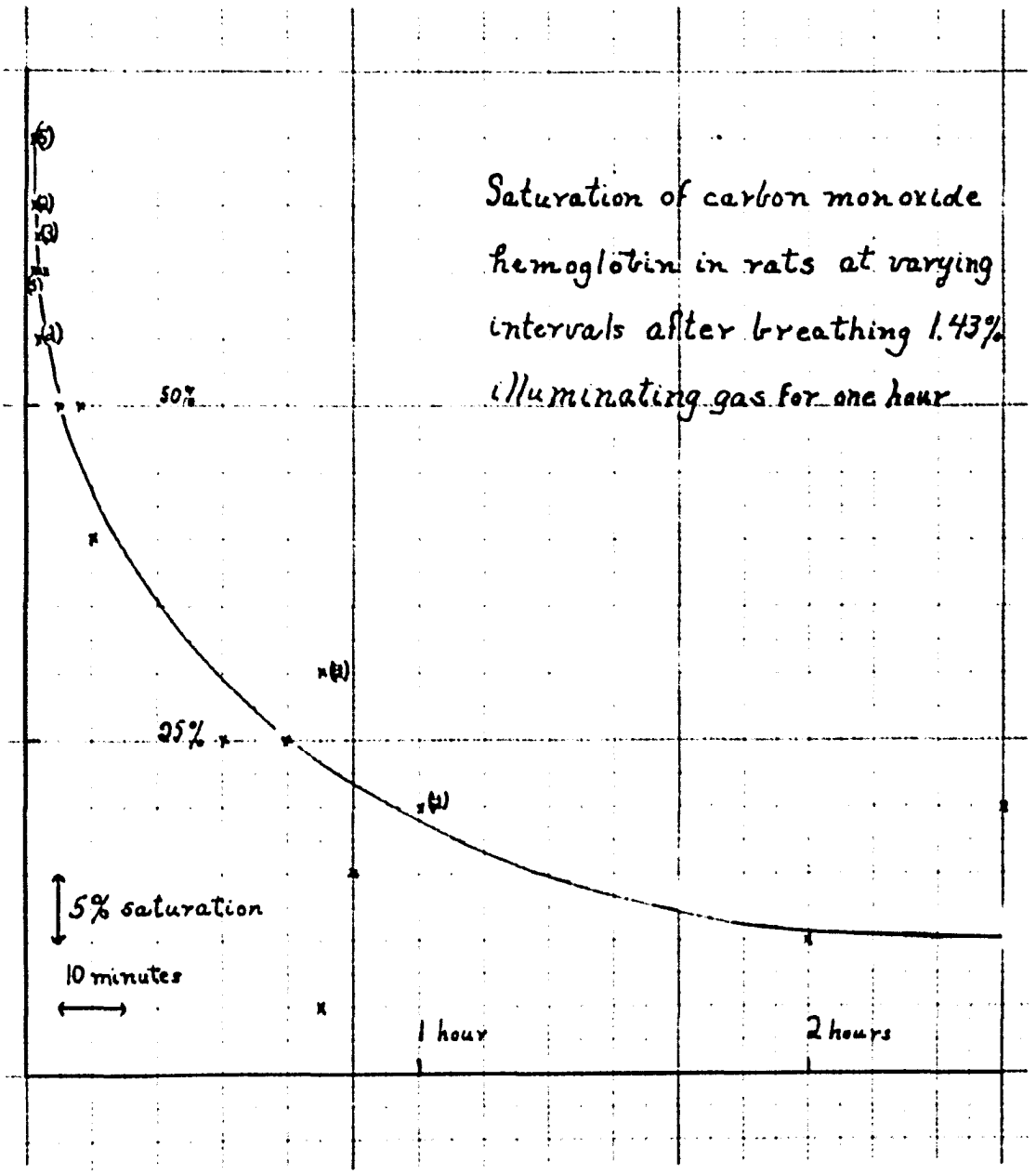
Condition of rat	No. of rats	Per cent carbon monoxide hemoglobin
Never gassed: stock and control	11	0-5
24 hours after gassing	11	0-5
	3	5-10
21 hours after gassing	3	20-30
2 hours after gassing	2	10-20
1 hour after gassing	3	10-20
45 minutes after gassing	1	0-10
	3	20-30
30 minutes after gassing	1	20-30
8-10 minutes after gassing	2	40-50
3-5 minutes after gassing	2	50-60
1 minute after gassing	15	60-70
Killed by illuminating gas	7	70-80
	2	80-90

It may be noted here that considerable difficulty was experienced in obtaining blood from the tail of the rat immediately following a gassing period. This impairment in the circulation caused by illuminating gas poisoning is in accordance with an observation of Mott (1907).

Figure 23.

The dissociation curve of carbon monoxide hemoglobin in rats given is estimated from the points located.

Figure 23.


Saturation of carbon monoxide hemoglobin in rats at varying intervals after breathing 1.43% illuminating gas for one hour
50%
25%
5% saturation
10 minutes
1 hour
2 hours

Hemoglobin Determinations

The hemoglobins of the animals subjected to the illuminating gas-air mixture are definitely higher than those of their litter-mate controls. Among the control males, the highest value recorded is a hemoglobin of 17.8 grams. Six of the gassed males (50 per cent) had at one time in their life, a hemoglobin exceeding 18 grams, and the peak reached among these animals was a value of 21 grams (excepting the value of 23.2 grams excluded because of possible change in the animal body after death).

The highest hemoglobin found in a control female animal was 15.8 grams. Four of the nine females reported had a hemoglobin that was greater than 18 grams at one time in their life. The highest hemoglobin recorded in this series of rats--22.8 grams--was found in female rat 17. As may be observed from the charts of female rats 15 and 17, an increase in the gassing periods to two hours daily is followed by an increased hemoglobin value.

As another means of comparing the hemoglobins of the gassed rats with those of the control rats, and with the accepted normal (Williamson and Ets, 1926), the charts of all of the rats upon which hemoglobin determinations were made after the fifth month of life were examined; and from them was interpolated the hemoglobin value of each of these rats on the one hundred and fiftieth day of life. Those rats whose exact age was not known

were included in the group examined, because the error in the assignment of the birth date to these rats is probably less than twenty days, and the value of 15.51 ± 0.25 grams of hemoglobin is given by Williamson and Ets as the normal for rats from 140 to 159 days old. Only rats which had been gassed an appreciable length of time were included in the group of gassed rats.

Table 2. Hemoglobins of rats aged 150 days.

	: Rat :	Hemoglobin, grams
Control	♀ 4	13.0
	♀ 6	12.0
	♂ 4	15.2
	♂ 6	14.0
	♂ 8	14.2
	♂ 10	14.8
	♂ 12	14.6
	♂ 14	15.2
	♂ 16	13.8
	♂ 18	12.8
Average		13.96 ± 0.07
Experimental	♀ 5	13.8
	♀ 7	15.2
	♀ 13	18.8
	♂ 7	18.8
	♂ 13	16.2
	♂ 15	18.4
	♂ 17	14.8
	♂ 23	17.0
Average		16.625 ± 0.43

The mean difference, 2.665 ± 0.484 is highly significant.

The records of female rats 15 and 17, which were gassed from October 2, 1933 till the end of July 1934 do not indicate any marked tendency toward a decline in hemoglobin value as the length of the experimental period increases.

Cell-plasma Ratio

In this tabulation are included six determinations made upon five female rats used in an earlier experiment to determine the influence of the gassing procedure upon the rate of regeneration of hemoglobin. One of these rats had the highest hemoglobin of any rat in any group studied. Possibly the stimulus for the regeneration of hemoglobin caused by the period of nutritional anemia, coupled with that caused by the daily formation of carbon monoxide hemoglobin resulted in the extraordinarily high hemoglobin values obtained on this rat.

Table 3. Hemoglobin and cell plasma ratios.

Rat	: Hemoglobin, grams	: Cell/Plasma
♀ 2	10.36	54
♂ 2	12.55	59
♀ a	13.30	55
♀ 6	13.33	59
♀ Not gassed.*	13.47	67
♀ 2	14.03	56
♀ b	14.70	52
♂ 2	15.44	66
♀ c	16.23	58
♂ 2	17.14	59
Rats not gassed average	14.065	58.5
♂ 3	10.04	54
♀ 3	10.53	55
♀ 3	12.17	48
♂ 1	13.47	54
♀ 1	13.47	63
♀ 15	14.03	63
♀ 1	15.08	63
♀ 17	15.43	65
Rats gassed average	13.028	58

Table 3. Continued.

Rat	Hemoglobin, grams	Cell/Plasma
♂ 3	16.01	61
♂ 1	18.04	63
♀ Gassed*	18.04	81
♀ Gassed*	18.64	85
♀ d	19.30	75
♀ e	24.10	84
♀ f	25.25	76
Rats gassed average	19.91	75

*Litter mates to females 6, 15, and 17.

♀ a Not gassed; milk diet October 8 to November 5
♀ b Not gassed; milk diet October 8-25
♀ c Not gassed; adequate diet always
♀ d Gassed; milk diet October 8-25
♀ e Gassed; milk diet October 8-25

Determination of Fragility of the Erythrocytes

Fragility of the red blood cells was determined on a few animals which had been kept on an experiment for a very long period of time (from September 1933 to August 1934). Certain of these animals had been splenectomized July 1933, but as the splenectomy *per se* does not seem to have affected the cell fragility, the records of these rats are included with those of their litter-mate controls, some of which were gassed, and some of which were not gassed.

Table 4. Fragility test.

Condition of animal	Concentration of NaCl solutions causing hemolysis: Marked	Distinct
Not gassed	0.0028	0.0040
Not gassed; splenectomized	0.0028	0.0036
Not gassed	0.0028	0.0032
Average	0.0028	0.0036
Gassed	0.0028	0.0040
Gassed	0.0028	0.0040
Gassed	0.0028	0.0040
Gassed; splenectomized	0.0028	0.0040
Gassed; splenectomized	0.0028	0.0040
Gassed	0.0028	0.0036
Average	0.0028	0.00393

In this small group of animals there seems to be a tendency for the gassed animals to show an increased fragility of the red blood cells.

Weights of the Experimental and Control Rats

A comparison was made between the weights of the gassed and the control animals of the same sex on the fiftieth, one hundredth, and one hundred and fiftieth days of life. In this way the animals were divided according to experimental treatment, sex, and age into twelve groups. The data were too meager to admit of reliable statistical analysis in every case, but in certain groups significant differences were found. Comparison is also made with rats of the same age and sex of the Wistar experimental colony strain, as tabulated by Greenman and Duhring (1931).

On the fiftieth day, the average weight of the gassed females was 90 grams. The average weight of two control females at this age was 85 grams, but the number of cases is so small that no valid comparison can be made. The average weight of females of this age of the Wistar experimental colony is 110.6 grams.

At the age of 100 days the average weight of the gassed females was 155.7 grams. Again the average weight of the two control females was less, being 141.3 grams. No explanation, except that of the lack of sufficient data, can be offered to explain this situation, as the control females here being compared against their litter mates were chosen by chance, and were not the smallest females in their respective litters. Wistar experimental colony rats weight 181.4 grams at this age.

When 150 days old, the gassed female rats weighed an average of 178.9 grams, 9.85 grams less than the three females which were not gassed. This difference is highly significant, and indicates one of the results of the gassing procedure on the rats. The Wistar rats average 193 grams in weight at this age.

That the subjection to the air-illuminating gas mixture affects the male rat more seriously than it does the female rat is indicated by the fact that in every age group analyzed the control rats weigh more than the gassed rats, the differences in weight being in every case, highly significant.

In the rats 50 days old, the control male rats weighed

125 grams, and the gassed male rats 109 grams. The weight given by Greenman and Duhring for normal males of this age is 123.8 grams.

The control male rats 100 days old weighed 271.67 grams, 56.67 grams more than the gassed rats. The difference between control and experimental rats was greater at this age than at either of the other ages studied. The number of individuals from which the statistics for this group were compiled was larger than those of any of the other groups studied. The Wistar experimental colony male rats of this age are reported as weighing 235.7 grams.

At 150 days of age, the control male rats weighed 275 grams, and the gassed rats, 251 grams. The rats reported by Greenman and Duhring weighed at this age an average of 275 grams.

The averages obtained are tabulated:

Table 5. Weights of rats.

	Female			Male		
Days	Control	Gassed	Mean difference	Control	Gassed	Mean difference
50	85 ±1.5	90 ±0.6	5.0 ±2.4	125.0±1.8	109±1.6	16±2.5
100	141.3±2.2	155.7±0.8	14.4 ±2.3	271.7±2.4	215±1.3	57±2.7
150	188.8±1.8	178.9±0.8	9.8 ±2.0	275.0±1.8	251±1.7	24±2.2

Litter Histories

The data on reproduction may be summarized as follows:

Case in which both parent rats were subjected to illuminating gas: A litter of seven was born to litter-mate parents, both of which had been gassed only over a period of one month, a total gassing period of only about three hours for each parent at the time of coition. The young survived two days.

Cases in which the male rat only was gassed: The gassed male which impregnated the gassed female as recorded above, at about the same time impregnated a non-gassed, control female rat. A litter of three was born, the young surviving eight days.

A male which had been gassed for two months (a total of 23 gassing hours) impregnated a normal female. The female was observed to be about to deliver a litter, but no young were ever seen.

Six of the gassed males were kept constantly with normal females, which had raised young. Excepting the litters of seven and three noted above, which were born early in the experimental period, these rats gave rise to no young. The other six gassed males were placed for several days with normal females, at two different times. From these matings, only one pregnancy, that described above, was known to result. In no case did a gassed male rat give rise to progeny which survived.

Matings of the control, non-gassed, male rats: During this time the nine control male rats gave rise to twenty litters. Of these litters, the young of nine litters did not survive to weaning age, but in six of these cases, the female was a gassed rat. Five stock males in the laboratory, mated at times with the gassed females, were also mated with normal females, and during this time gave rise to 18 litters, only two of which did not survive.

Cases in which the female rat only was gassed: Two gassed female rats placed with normal males for four days (July 9-13, 1934) did not bear litters. (The control rat bore a litter August 5.)

Seven of the gassed female rats were kept with normal male rats all the time that they were neither pregnant nor lactating. As the litters, with one exception, did not survive over three days, it is conceivable that during a period of three months, each rat might have produced three litters, twice as many litters as were actually produced.

The history of these litters is tabulated:

Table 6. History of litters borne by gassed females.

Mother	Litter size	Number alive at end of 2 hours	24 hours	48 hours	Age (days) of last survivor
♀ 1	6	0	0	0	0
♀ 1	4	3	3	1	3
♀ 1	4	3	1	1	2
♀ 1	?	No data recorded; litter did not survive.			
♀ 3	3	3	0	0	1
♀ 3	5	5	2	0	2
♀ 5	7	4	1	0	2
♀ 5	2?	0	0	0	0
♀ 7	10	10	8	2	24*
♀ 9	8	8	1	0	2
♀ 11	6	6	2	2	3
♀ 13	1	1	0	0	1
♀ 13	Pregnant when killed; five foeti found.				

*Lone surviving rat killed at weaning age; its last surviving litter mate died at 17 days.

Cases in which neither the male or the female rat was gassed: Very few of the normal control males in the experiment were mated with the normal control females. Therefore, records of litters produced by the stock rats from April till July 1933 were kept for purposes of comparison. For the same reason, the number of young in each litter surviving to be weaned was recorded. (No attempt was made to identify the mother of one litter as having raised previous litters.)

Table 7. History of litters borne by control and stock rats.

Mother	Litter size	Number surviving
♀2	8	0
♀2	12	0
♀4	6	0
♀4	7	7
♀6	7	0
Stock ♀	4?	4
	6	6
	10	10
	13	12
	9	9
	10	10
	13	12
	13	12
	6?	0
	6?	3
	9	8
	14	13
	10	10
	9	9
	6	6
	9	9
	6	6
	14	13
	10	0

Motility of Sperm

In no case investigated were normally motile spermatozoa found in a drop of semen obtained from the epididymis of a gassed rat. Only once was even slight motility observed. This was in male 23 which had recently impregnated a female. This rat was gassed twenty-three hours over a period of two and one-half months, evidently an insufficient amount to render it impotent.

Exceedingly great motility was exhibited by the spermatozoa

of the control rats in every individual examined.

Testis Weight

Testis weight was markedly below normal in the gassed males. Below is tabulated a comparison between the testis weight actually found, and that expected, for both experimental and control animals:

Table 8. Testis weight.

	Observed grams	Expected grams
Control		
♂ 12	2.6	2.66
♂ 14	2.2	2.6
♂ 16	2.1	2.96
Experimental		
♂ 15	1.7	2.5
♂ 17	0.8	2.3
♂ 21	0.895	2.7
♂ 23	0.708	2.5

Determinations of the Oestrus Cycle

In the first groups of rats in whom the reproductive cycles was studied were six albino white rats, three gray rats, and three pied rats (rats A to L inclusive). These rats were born about October 18, 1932, and determinations of their oestrus cycles were made from January 21, 1933 till April 3. The length of the cycle of all of the rats under the usual laboratory conditions, was determined from January 21 till February 14. One

white rat, one gray rat, and one pied rat were set aside to serve as controls, and the other nine rats were gassed, beginning February 14. The length of the cycles of these rats during the period of subjection to the air-illuminating gas mixture from February 14 till April 3 was determined.

Following the conclusion of the work on these rats, a second group of rats (rats M to Z, inclusive) was chosen, and a similar experiment begun. Fourteen rats of the Ames-Wistar strain were chosen for this, and their normal cycle lengths determined. Unfortunately most of these rats were killed by accident so early in the experimental period that but few subsequent cycle lengths were determined.

(It has been observed throughout the course of all the experiments conducted on the effects of sublethal percentages of illuminating gas upon rats that during the hot months of the summer it is extremely difficult not to kill the rats by accident during the experimental procedure.)

One case of pseudopregnancy was accidentally induced (the eleventh cycle in rat D). This cycle was omitted from the calculations. Oestrus cycles which included the day the gassing period was initiated were classed with the cycles of the control period. The cycles are graphically represented in Figures 24 to 26, inclusive.

The control period of the first group studied showed the rats to have a mean cycle length of 4.74 ± 0.144 days. During

the experimental period, the cycle length increased to 5.56 * 0.2 days. The value of the mean difference, 0.82 * 0.24 days indicates that the increase is significant, but not highly so, since the mean difference is less than four times its probable error.

The control period of the rats of the second group studied indicated that the average cycle of this group was of the same length as that of the first group: 4.78 * 0.1 days. During the experimental period the mean cycle length of these rats was 5.0 * 0.34 days. The mean difference in this case was 1.12 * 0.34 days. Again, the results are significant, but not highly so.

The total duration of the experimental period to which these rats were subjected was not long, and it is possible that a more extensive experimental treatment would have increased the significance of the determinations. The rats in the first group were gassed for seven weeks. With the exception of rat N, which was gassed for eight weeks, no rat in the second group was gassed for more than four weeks.

Microscopic Study of the Ovary

Five of the rats whose reproductive cycles had been determined were killed on the day of oestrus. Serial sections were made of the ovaries of each of these rats, and were examined in an attempt to determine if any appreciable anatomical change

Figures 24, 25, 26.

Each point located indicates the length of one oestrus cycle. All cycles found on and to the right of the broken line were cycles determined after the initiation of the experimental period.

The eleventh cycle in rat D was prolonged due to a condition of pseudopregnancy induced by the experimental procedure. None of the other prolonged cycles may be attributed to that condition.

Figure 24.

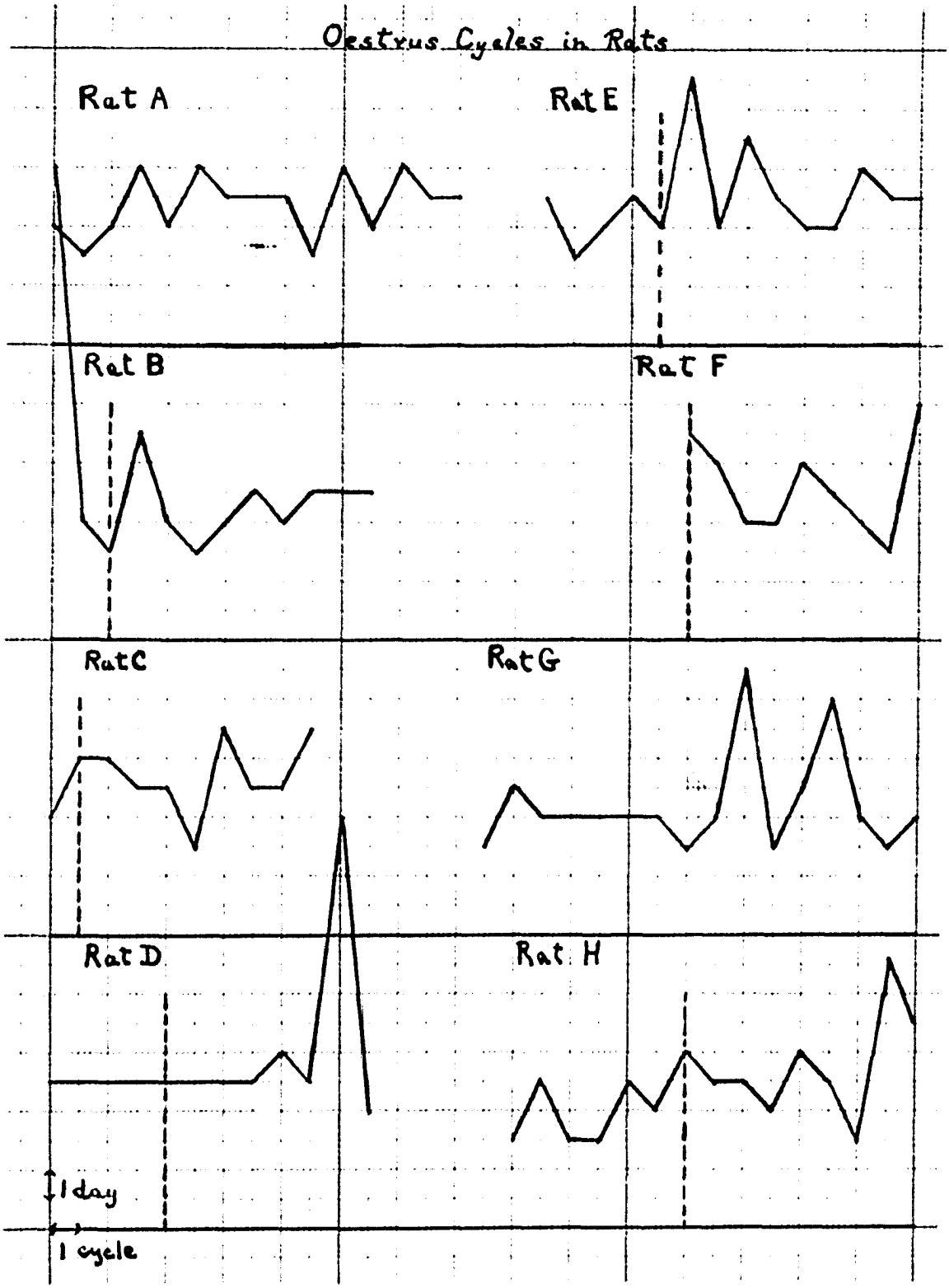

Oestrus Cycles in Rats
Rat A
Rat E
Rat B
Rat F
Rat C
Rat G
Rat D
Rat H
1 day
1 cycle

Figure 25.

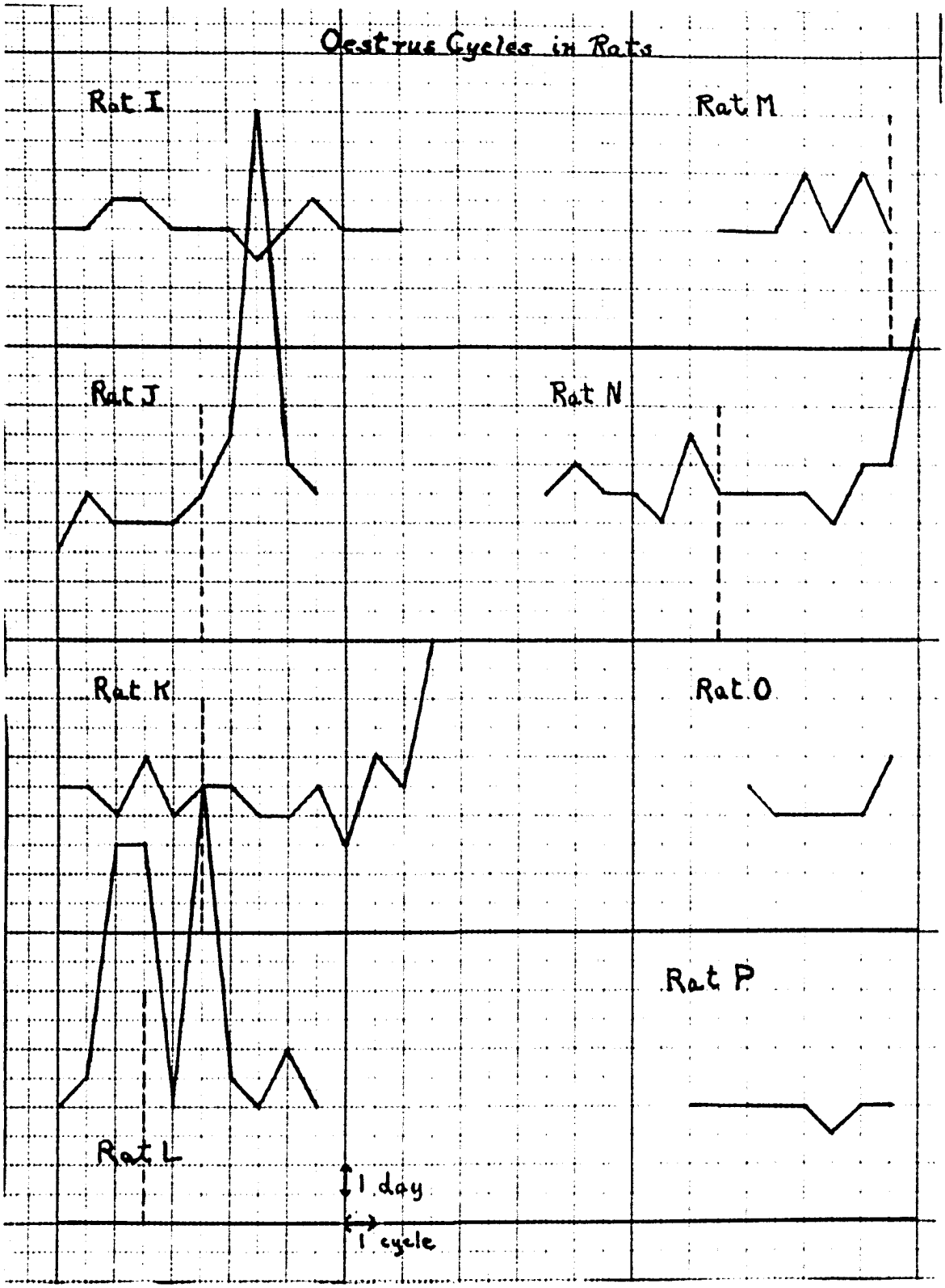
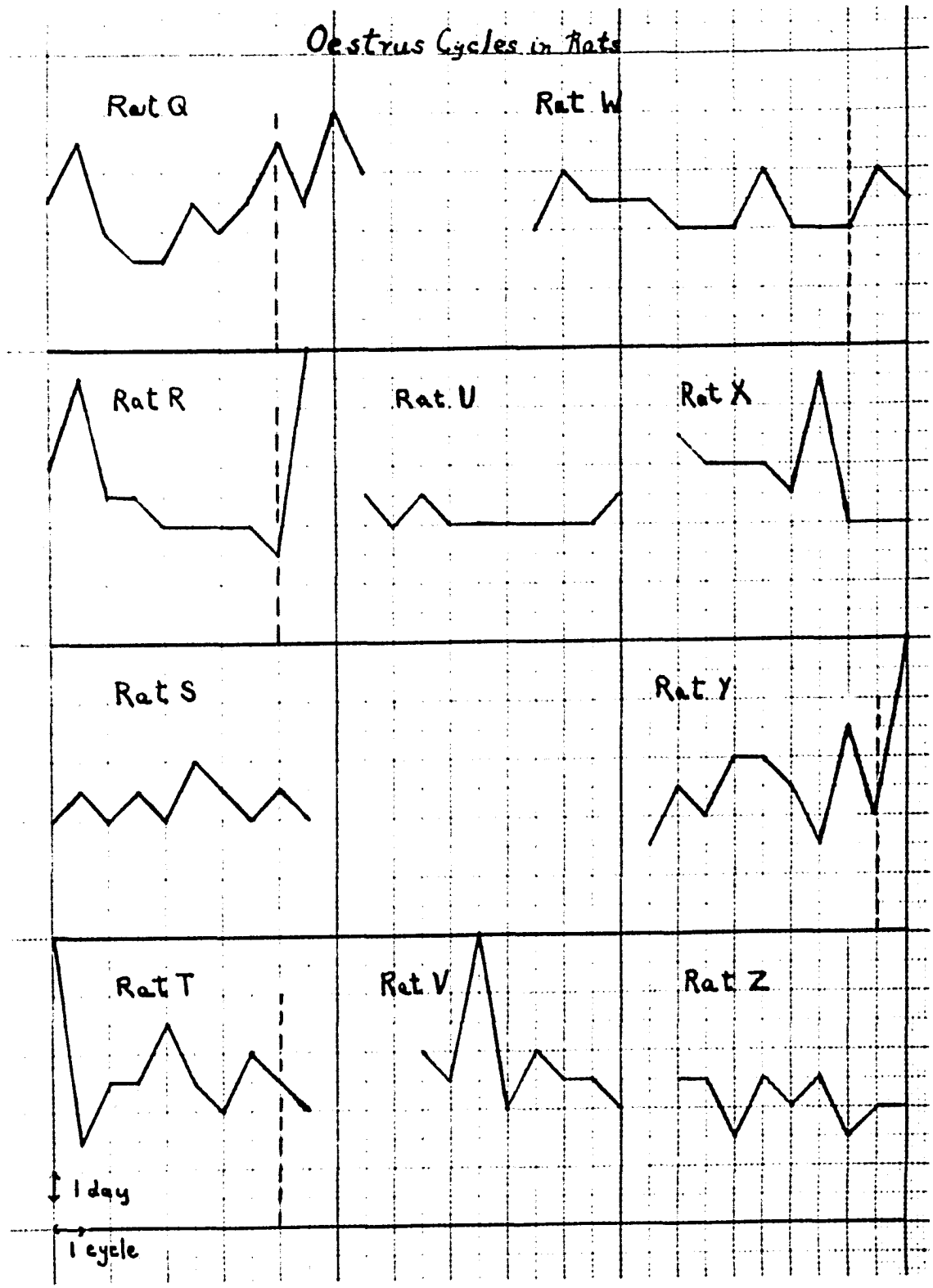

Oestrus Cycles in Rats
Rat I
Rat M
Rat J
Rat N
Rat K
Rat O
Rat P
Rat L
1 day
1 cycle

Figure 26.


Oestrus Cycles in Rats
Rat Q
Rat W
Rat R
Rat U
Rat X
Rat S
Rat Y
Rat T
Rat V
Rat Z
1 day
1 cycle

had taken place in the ovaries of the rats with the longer cycles induced by the experimental procedure. Serial sections were made of the ovaries of eight other experimental and control rats, but as these rats were in varying periods of the reproductive cycle when killed, comparison of amount or nature of the follecular tissues present in these ovaries cannot be made.

All of the Graafian follicles in the central six slides of each of the five series of ovary slides were measured, to discover if any difference in size existed which might be attributed to the effects of the experimental procedure. The following data were obtained:

Table 9. Measurements of Graafian follicles containing ova with nuclei found in central six slides of the rats killed in oestrus.

Rat	Age (days)	9.6-54μ	55-154μ	155-254μ	255-354μ	355-680μ
G	ca.236	8	40	20	15	6
P	96	10	69	35	16	14
E	ca.236	12	62	17	4	6
C	ca.236	10	46	20	8	6
H	ca.236	15	37	15	9	6

Rats G and P were not gassed.

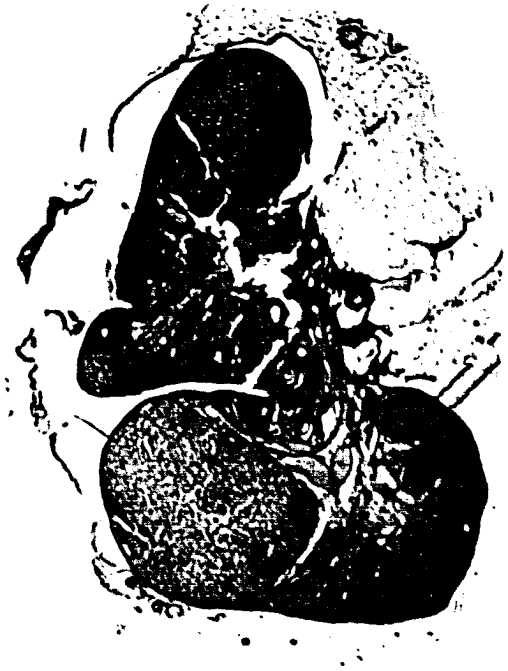
The sections photomicrographed, Figure 27, show the ovaries of the gassed rats to contain more blood than the ovaries of the control rats. This seemed to be true throughout the series of thirteen ovaries examined. A difference in the size of the ovary also may be noted.

Figure 27.

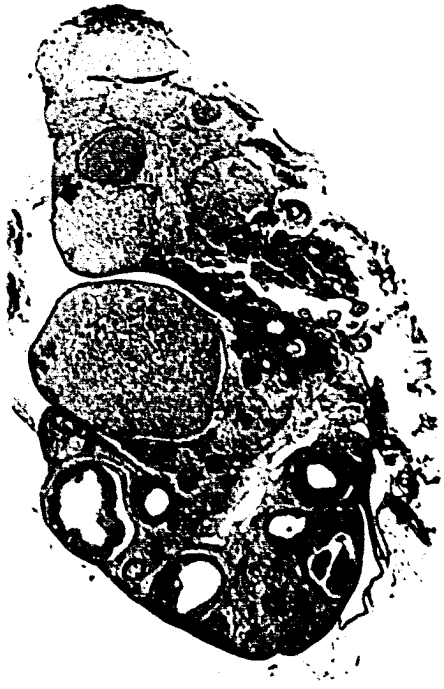
Sections through the center of ovaries of control rats G and P and experimental rats E and H.

Magnification X25.

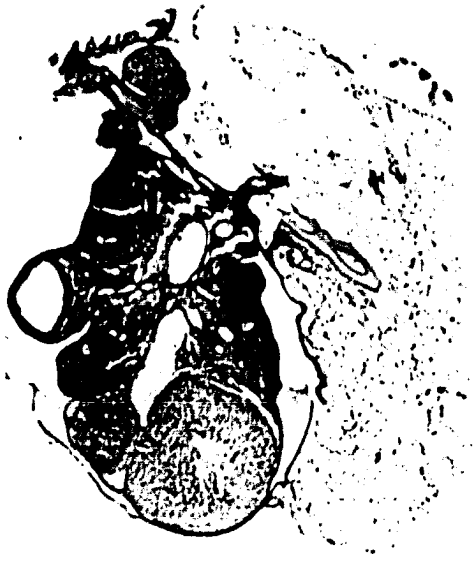
Figure 27.



Rat G



Rat P



Rat E

Rat H

DISCUSSION

Concerning Blood Determinations

The Pyrotannic Acid method of Sayers and Yant for determination of carbon monoxide in blood and air was used. By this method the saturation of carbon monoxide in the blood of the rats killed with illuminating gas was about 80 per cent in every case. This indicates that death occurred with a greater degree of saturation of the blood with carbon monoxide than Henderson and Haggard (1922) found in dogs killed by illuminating gas. These authors base their conclusion that illuminating gas is more toxic than pure carbon monoxide on two facts: first, that pure carbon monoxide in air caused death when the animal had a percentage saturation of carbon monoxide hemoglobin of 84; and second, that illuminating gas in air caused death when the percentage saturation of carbon monoxide in the blood was only 72. The results obtained on the Ames-Wistar rats indicate that either the illuminating gas used in the experiments reported contains a lesser percentage of toxic constituents in proportion to the carbon monoxide content than did that used by Henderson and Haggard, or that these other toxic constituents are less toxic for rats than for dogs. The data does not indicate that the tissues and organs of the rat can survive under a greater degree

of anoxia than can the tissues of the dog, because of the results obtained by Henderson and Haggard using pure carbon monoxide as the asphyxiating agent.

The rate of dissociation of the carbon monoxide hemoglobin following a gassing period may be seen from the graph (Figure 23) to be very rapid during the first ten minutes, and quite slow after eighty minutes. The curve obtained from the determinations on rats is not as straight as that given by Stadie and Martin (1925), based on data obtained from dogs allowed to recover in air from a severe degree of carbon monoxide poisoning. In the rats reported the recovery period is more rapid than that of the dogs. This is to be expected,because of the differences in the affinity of the hemoglobins of the two animals for carbon monoxide. Nicloux (1920) states that the ratio between the affinity of the hemoglobin of dog blood for carbon monoxide and for oxygen is 320 to one. Anson et al.(1924) find that the hemoglobin of the rat has an affinity for carbon monoxide only 280 times its affinity for oxygen, at 15°C. This affinity is less at body temperature. In man, the rate of dissociation of carbon monoxide hemoglobin is found to be slower than that of the rats here reported. Henderson and Haggard (1922a) find a five to fifteen per cent reduction of the carbon monoxide hemoglobin percentage saturation in one hour. The affinity of human hemoglobin for carbon monoxide is considered to be about 300 to one.

The experimental animals showed an increased hemoglobin. This was accompanied by an increased number of red blood cells, as the increased cell volume indicates. McMillin (1932), working in this laboratory, and with rats under the same conditions, made total red blood cell counts, and found that the gassed rats had a greater number of red blood cells than did the control animals. This increase in hemoglobin and in numbers of erythrocytes undoubtedly is a response to the anoxic condition repeatedly induced by the experimental procedure, and is analogous to that following reduced oxygen pressure. If the experimental period is terminated, the rats lose the compensatory extra number of red blood cells, together with the hemoglobin they contain, a response which occurs very shortly following the last gassing procedure. Equally fast is the rise in hemoglobin following an increase in the length of the daily gassing periods.

Color volume index may be calculated from the data given in Table 3. The data are divided into three groups: the control rats, and the gassed rats with hemoglobin values below normal, and the gassed rats with hemoglobin values above normal. Color volume index in man has a value of from 0.9 to 1.0, under normal conditions (Lamb, 1930). The color volume index of the ten control rats upon which there are data is 0.776 $\pm$ 0.0227. That of the gassed rats with hemoglobins below normal is 0.72 $\pm$ 0.0153. That of the gassed rats with hemoglobins above normal is 0.86 $\pm$ 0.0512. An increased color volume ratio would indicate a larger

amount of hemoglobin in each individual cell. Error in the determination of the cell-plasma ratio such that too large a ratio of red cells was obtained would lower the value of the index, and the opposite error, which might occur with slight loss of cells in the hematocrit tubes, would raise the index value. Either of these errors may be present, as the method used for determining cell-plasma volume is not absolutely reliable. The results obtained with these groups of rats indicate, in so far as the method can be relied upon, that there is no significant difference in the color volume indeces among the groups, and that those rats possessing the higher hemoglobins have no more hemoglobin per unit volume of erythrocytes than do the control and gassed rats with lower hemoglobins.

Boedikor (1932) reported a slight increase in fragility of the erythrocytes of dogs which he subjected to repeated doses of pure carbon monoxide in air. Landsorp (1928) found that in her experiments five out of six rats showed a definite increase in cell fragility. McMillin (1932) observed no change. The rats here reported in which increased fragility of the red cells was found were animals which were subjected for an extremely long interval to the gassing procedure--from September 1933 till July 1934. Their controls were litter mates, so age is not a factor. Takenouchi (1919) reports that age increases red cell fragility.

Concerning the Effect of Gassing on the Males as Compared with that on the Females

There is ample support for the belief that the daily subjection to sublethal doses of illuminating gas is more serious for the male rats than for the females. Herbits (1917) had statistical evidence that this was true for human beings, and Webster (1930) reports that in law the female is judged to have survived longer than the male, other conditions being equal, when both have been killed by carbon monoxide. Among the rats, in all the experiments that have been performed in this laboratory, it has always been found to be very difficult to keep from killing the male rats during the experimental procedure. Females kept in the animal chamber for three hours have survived. Males gassed so strenuously would be found dead in their cages later the same day, if they survived the gassing period.

As was shown, the weights of the male animals were more markedly decreased than those of the females.

The highest hemoglobin ever recorded in this laboratory (25.25 grams) was found in a female rat. The highest value recorded for a male is 21 grams. This difference, of course, may be correlated with the fact that the female rats can survive, and consequently are subjected to, more prolonged gassing periods.

The effect on the reproductive organs is evidently more serious in the male than in the female. The female rats maintain a reproductive cycle. They bear young as long as four

months after the inception of gassing, though not as readily as the normal animal. With greater duration of the experimental period, the females no longer can b e impregnated. They lose their young (one exception) within a few days after birth. But the males which have been subjected to the air-illuminating gas mixture for a much shorter length of time than four months lose their ability to procreate. One male impregnated a female after six weeks of being gassed daily. Every other male was affected so severely that after a few hours (more than three) total gassing time, no male successfully impregnated a female.

A study of the weight charts of the normal females kept with gassed male rats does not indicate that impregnation, implantation, development of foeti, and subsequent resorption tookplace. The females gained weight while with the males, but with the exception of one of the females with male rat 11, did not gain weight steadily for about fifteen days, and then lose it, the weight subsequently remaining at the lower level. The fact that the females were kept constantly with the males perhaps makes the evaluation of their weight changes less certain.

Concerning Testis Weight

Mason (1926) studied testicular degeneration in albino rats induced by inadequate diets. He made a histological study of the degenerating testes of his experimental rats, and found that degeneration of cells occurred in reverse order to that of genesis.

He found the spermatozoa to disappear first, next the spermatids, and later the spermatocytes and spermatogonia. He states that this progression of degeneration is that described by all the workers in this field, except those who have caused testicular degeneration by means of x-rays.

Testis weight is appreciably below normal (about 80 per cent of normal) at the time when the spermatids are degenerating, and according to Mason, at this time the testis feels "soft". Mason found the testis in the final stage of degeneration to be essentially the same as the cryptorchid testis: "All the germ cells of the germinal epithelium of the tubules have disappeared, and all that remains is a syncytium of Sertoli-cell nuclei in a greatly reduced layer of fibrous cytoplasm." He says further that the interstitial tissue is more prominent in this stage, but that this is due to the great decrease in size of the tubules, rather than to any hypertrophy of the tissue itself, or any hyperplasia of the interstitial cells. In the final stage of degeneration Mason found the testis to weigh from 43 to 58 per cent of the normal. Three of the testis weights reported in this study showed even greater atrophy: weighing only 28, 33, and 35 per cent of the normal, respectively. An extreme degree of degeneration would appear to be indicated.

In Mason's animals, the process of degeneration, once initiated, progressed to the final stage in from thirty-five to fifty days. Histological study of testes twenty-five to seventy-five days after return to a normal diet showed the second testis

to be more degenerate than the first (which had been removed for study before placing the animals on the adequate diet). Lettuce added to the diet had no effect, if degeneration had begun. However it was found that lettuce added to the deficiency diet used by Mason was effective in preserving a normal condition of the tubules.

Alcoholization over long periods produces testicular degeneration in the rat (Bouin and Garnier, 1900; Allen, 1919). This degeneration is considered to be more probably the indirect result of lowered vitality and other body disturbances, than the result of a specific action of the alcohol on the germinal epithelium. It seems reasonable to believe that in the rats subjected to illuminating gas, it is the lowered vitality attendant on the experimental treatment which brings about the loss of ability to reproduce.

It is interesting to note that temporary loss of sexual power in men poisoned by illuminating gas is reported by McCombs (1918), and that loss of libido in individual suffering from chronic carbon monoxide poisoning is reported by Rossiter (1928).

Concerning the Effect on the Oestrus Cycle and on Ovarian Tissue

The daily subjection to the air-illuminating gas mixture upset the normal body metabolism of the female rats to such an extent that body weights were below normal, after a certain

length of experimental interval had elapsed, and the animals felt soft when picked up, indicating loss of muscle tone. The hair of the experimental rats became rough and lacking in luster, and the animals increasingly unkept, as the experiment proceeded.

A further indication of lack of well-being on the part of the female rats was the significant increase in the length of the oestrus cycles. Under optimum conditions the cycle length may be as short as four days, and Long and Evans (1922) suggest that possibly this is the true normal length. Longer cycles in the control rats may be caused by individual variations among the rats, and by the impossibility of maintaining the rats under optimum conditions. But the significant increase in cycle length of the experimental animals over their own cycle lengths during the control periods, and over the cycle length of the control animals must be attributed to the gassing procedure. There is no reason to believe that the effect is caused by any specific toxic action of carbon monoxide poisoning on the reproductive organs, but that it is rather a response to the impaired vitality induced by the experimental procedure.

Pseudopregnancy is a complicating factor when an attempt is made to establish the mean of the cycles of a rat over a period of time. The condition can be accidentally induced by the investigator while obtaining a smear from the vagina. If the condition is induced, and not recognized, it will alter the findings appreciably. Slonaker (1929) found in the rats

he studied the average length of pseudopregnancy to be 14.58 days, and the range to be from seven to nineteen days.

The fact that a cycle is long is no proof that the cause of this increased length is due to the presence of the condition of pseudopregnancy. Long cycles in which the interval between succeeding oestrus periods shows smears containing leucocytes and epithelial cells only were considered to be conditions of pseudopregnancy. When all parts of the cycle--the cornified cell stage, the epithelial cell stage, the diœstrus interval--were more prolonged than normally, but followed in the usual sequence, the cycle was considered to be a prolonged cycle, and the cause was not considered to be pseudopregnancy. Edgar Allen (1923) reported that mice with a prolonged cycle containing a longer than normal period of oestrus were more prolific than mice with shorter cycles. When a few cornified cells were found in the middle of a long cycle, these were considered to indicate an abortive attempt at oestrus. Such long cycles are not considered to be caused by pseudopregnancy. Edgar Allen found both of these types of cycles in his mice. Another variation which may be found is a very prolonged period of constant oestrus.

The histological study of the ovaries of the experimental and gassed rats showed no change that could be ascribed to a greater cycle length. The number of Graafian follicles containing ova cannot be used as an indication of the condition of the rat, since the number of ova found in an ovary at any given

time depends upon age (Arai, 1920). The rats studied were of two age groups. The Graafian follicles in comparable parts of the ovary were measured, but no difference in the proportion of follicles falling in each size group was apparent.

If in each case the size of the ovum be taken to be slightly smaller than the size of the Graafian follicle measured, a comparison may be made between the data collected on the control and experimental animals, with that reported by Arai (1920). If this is done a considerable discrepancy will be found. Arai found by far the largest number of ova to fall in his smallest size group, and he found very few ova as large as most of the ova in the Graafian follicles which were measured. The ovaries studied, and reported in this investigation were from rats killed in oestrus, and consequently the ovary tissue was fixed while in the stage just preceding ovulation, a time when it would contain very large follicles. Arai does not state in what condition of the reproductive cycle the rats whose ovaries he examined were. In making a complete count of the ova found in an ovary, Arai examined a very large amount of peripheral tissue in which the very smallest ova are found. An examination of the six central slides of a series of ovarian tissues would result in a very small proportion of the tissue examined being peripheral.

As no obvious significant change could be detected in the ovaries examined, no further investigation into this problem was made.

SUMMARY

1. Rats killed by illuminating gas were found to have 80 per cent carbon monoxide hemoglobin.

2. Rats allowed to recover in air after breathing for one hour a 1.43 per cent illuminating gas-air mixture lost 30 per cent of the carbon monoxide hemoglobin in the first half hour, 10 per cent more carbon monoxide hemoglobin in the second half hour, and five per cent more in the third half hour.

3. The hemoglobin and the red cell volume rose in response to the daily breathing of 1.43 per cent illuminating gas.

4. Rats subjected to a long duration of daily periods of breathing illuminating gas showed a slightly increased fragility of the erythrocytes.

5. The gassed male rats at 50, 100, and 150 days weighed significantly less than the control rats. The females weighed significantly less at 150 days.

6. One litter was born, of which both the parents had been gassed for a very short time. It did not survive.

7. Two litters were born, of which the male parent was gassed for a short time. Neither survived.

8. Twelve litters were born, of which the female parent only was gassed, for varying periods, up to four months. With the exception of one offspring, no young survived.

9. Female rats gassed nine months did not bear young.

10. Inability to procreate in the male correlated with lack of motility of the spermatozoa and marked decrease from the normal in testis weight.

11. The length of the oestrus cycle in the gassed female rats was increased, but the cycle was maintained throughout the experimental period.

12. Microscopic examination of serial sections of the ovary tissue revealed increased blood supply. No interpretation of the histology of the ovary is made.

CONCLUSIONS

Subjection of rats to daily period of sublethal percentages of illuminating gas impairs reproductive ability.

This effect is more marked in male rats, which, after a certain experimental period, are unable to procreate. At the same time male rats exhibit evidences of degeneration of germinal tissue.

To induce serious effects in female rats a more prolonged experimental period is necessary. Early in the experimental period, the females bear young, but these do not survive. Females gassed over a longer period do not bear young. The oestrus cycle is maintained.

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