THE ACUTE RADIATION SYNDROME IN LARGE DOMESTIC ANIMALS WITH SPECIAL REFERENCE TO X-IRRADIATION IN GOATS

BY

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PREFACE

The author has interested himself in nuclear science especially as it affects veterinary medical matters for over fifteen years. He attended many Service courses on the subject and has written a number of theoretical articles. (Wilkins 1949, 1955, 1956, 1957, 1959).

In 1960 he was posted to the Radiobiological Research Unit of the Medical Research Council at Harwell to help in a project to discover the effects of neutrons using large animals as the test subjects. His specific task was to acquire and maintain in health a large colony of goats. He took the opportunity to study some of the effects of X-irradiation on these animals and, in a very small measure, to put theory into practice.

The study was divided into two sections :

SECTION I was a general review of the acute radiation syndrome in domestic animals.

SECTION II consisted of general observations on a colony of goats together with an investigation into certain aspects of the effects of X-irradiation on adult goats.

The main significance of the work is in the fact that this is the first recording of data of the effects of whole body irradiation on a larger ruminant domestic animal in the United Kingdom. The general pattern of the findings is similar in most respects to that manifested by other animal species.

The programme of work could not have been completed without the encouragement given to the author by Dr. J.F. Loutit, Director of the Radiobiological Research Unit, Harwell and by Dr. J.R. Greening, Director of the Medical Physics Unit, Edinburgh. Grateful acknowledgement is made for assistance obtained from various members of the staff of the Radiobiological Research Unit Harwell especially Mr. M.J. Corp who arranged the irradiations. The thesis is dedicated to the memory of my two sons, Pip and Peter.

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SECTION I

A REVIEW OF THE RADIATION SYNDROME IN DOMESTIC ANIMALS

A REVIEW OF THE RADIATION SYNDROME IN DOMESTIC ANIMALS

Lieut. Colonel J. H. WILKINS, B.Sc., M.R.C.V.S., RAVC.

PART 1—LETHAL STUDIES

Introduction.

In his quest for knowledge on the biological effects of irradiation man has employed most of the domestic animals in his experiments. Perhaps the first were dogs used by Regaud et al. (1912) in France. However, most of the experimental irradiation of larger domestic animals in recent years has been carried out in the U.S.A.

Justification for using animals in this work can be made mainly on the grounds that the results will benefit the understanding of the biological effects of ionizing radiation on man. Present knowledge of the effects of radiation on human beings has been obtained either directly from studies of the survivors of Hiroshima and Nagasaki (Howland and Warren, 1947 ; Liebow et al. 1949), or from the response of patients to radiotherapy, or is based upon studies of persons injured by accidental exposure to critical nuclear assemblies (Hempelmann et al. 1952 ; Hasterlik and Marinelli, 1955 ; Gouskvoa and Baissogolor, 1955 ; Brucer, 1958 ; Jammet et al. 1959 ; Wald and Thoma, 1961). Much knowledge has also been accumulated *indirectly* from controlled experiments with animals.

These sources of information have their own inherent drawbacks which include for example :

(*a*) studies of the Japanese victims have been necessarily incomplete from the lack of precise information,

(*b*) radiotherapeutic measures are employed only on abnormal conditions where a small surface of the body is exposed to a large dose of radiation,

(*c*) the seven accidents involving critical nuclear assemblies which have occurred in the world since 1945 could not obviously be controlled although perhaps they are the source of the most reliable information,

(*d*) extrapolation of data from experiments with animals to man depends on the variations of radiosensitivity of different tissues in different species.

The concept then of biological injury caused by ionizing radiations is old. However, injury as a result of total whole-body irradiation is a comparatively new idea having been accentuated by the effects on the victims of the atomic explosions in 1945. Total body irradiation means the exposure of the whole body to ionizing radiation so that as uniform a dose as possible is delivered to all tissues. The injury caused differs from that caused by radiotherapy and also from repeated small doses of radiation. The biological disturbances which follow irradiation depends on dosage, duration and manner of irradiation but are essentially similar in character no matter whether the ionizing radiations are X-rays, gamma rays, neutrons or any other kind of radiation, (Bloom, 1947). These effects are brought about by damaging the tissue cells and thus altering their functions or killing them. There is a complex chain of events some of which are a direct result of the initial injury e.g. the classic alteration in the blood picture, while others are secondary to it, e.g. alteration in the body fluid balance. Nevertheless, all the changes are distinguished by their lack of specificity (Patt and Brues, 1954). A less

penetrating radiation is on the whole less effective than one which has a more even distribution. Similarly, a bilateral or multilateral exposure is more effective than a unilateral one. This has been shown to be true in pigs (Tullis et al. 1952) and in dogs (Bond et al. 1957). Much of the understanding of the effects of irradiating on animals has come about by observations directly on the whole animal rather than from work on the individual cell, important as this may be. It is the total body irradiation of the whole animal which reveals the biological properties and idiosyncrasies of the individual animal. The mutual interaction of the various tissues, which determines the outcome of the irradiation, is manifested to the clinician in the symptoms and clinical signs to which collectively is given the name of ' Radiation Syndrome.' There is no single clinical reaction specific for irradiation damage to tissues. However, all species of animals are affected in much the same way and the median lethal dose for all domestic animals is somewhere in the region of 500r. An important factor in understanding the response of large animals to ionizing radiations is the proper measuring and definition of the dose used. Various expressions are used and need careful differentiation if confusion is to be avoided. The recommendations of the International Commission on Radiological Units is that the dose should be expressed either in roentgens (r) or in rads. **The Air-Dose (Free-In-Air Dose, or Exposure Dose)** is expressed in roentgens and refers to the dose measured essentially free in air.

The Tissue Dose is also expressed in roentgens and refers to the dose measured in the animal's body and includes all the scatter and build-up thus caused.

The Absorbed Dose is expressed in rads and is an expression of the relative absorption of radiation energy in tissue as compared with air and is found by means of tables or graphs.

The Mid-Line Dose is an estimated dose in rads at the mid-line of the animal's body.

Even when the tissue dose and absorbed dose are known, the inhomogeneity of the tissues of a large animal (bone, muscle etc.) make it extremely difficult to decide on the actual total dose received. The air dose, which can be measured accurately, may be the most accurate to use (Bond and Robertson, 1957). Where there is a mixture of radiations a selective determination of the dose of each type is necessary, e.g. the measurement of neutron dose is invariably complicated by the fact that neutrons are almost always accompanied by electromagnetic radiations (X-rays or Gamma rays). The dose is additive in these cases (Vogel et al., 1955) and is cumulative in so far as the effects of previously received irradiations have an effect upon subsequent exposures, since the biological disturbances also depend on the relationship between repairable and irrepairable injuries caused. Although the biological effects of ionizing radiations are proportional to the dose of radiations of moderate intensity no demonstrable clinical effects are visible at very low intensities. However, the rate of delivery or dose-rate is important in lethal studies.

LETHALITY STUDIES IN DOMESTIC ANIMALS WITH SPECIAL REFERENCE TO DOSE RATE

The lethal dose of ionizing radiations for whole body exposure varies among the different species of animals and also among individual animals of the same species. The dose of whole body radiation which will kill half the number of animals used within 30 days is known as the $LD_{50(30)}$ and has proved to be a useful radiobiological dosimetric measurement. However, in the large domestic animals it is tentatively suggested that a period of 60 or 90

days i.e. a $LD_{50(60)}$ or $LD_{50(90)}$ would be more suitable because of the distinctive temporal relationship between injury and recovery of particular cellular elements and their rate of utilization by the animal.

It is difficult to explain the differences of $LD_{50(30)}$ between different animals, e.g. the guinea pig at 175r to 200r and the rabbit at 800r.

Death following total body irradiation may occur in a variety of modes depending on the species, dose, type of radiation and survival time after irradiation. The causes of death depend in part upon the degree of exposure (Quastler, 1956) but waves of mortality have been reported in all experimental animals making assessment of individual cases more difficult. Gerstner (1958) described a dose-survival time curve containing three " plateaus" which were dose-independent regions. These regions are ascribed to three different pathogenic mechanisms :

(*a*) in the low dose plateau (300r to 700r) death is caused by haematopoietic depression.

(*b*) in the mid-dose region (800r to 2000r) death is caused by gastro-intestinal denudation.

(*c*) in the high-dose plateau (4000r and upwards) by failure of the CNS.

This curve has been estimated for man from clinical data and experiments on animals (Allen et al., 1960).

It is convenient to tabulate these modes of death as follows :—

(*a*) Hyperacute (CNS Death)
(*b*) Acute (Gastro Intestinal Death)
(*c*) Subacute (Bone Marrow Death)
(*d*) Chronic (Latent : " Survivors ")

The $LD_{50(30)}$ of the adult large domestic animal is in the region of 500r (between 300r and 600r) if the total exposure is done in less than 24 hours. The acute median lethal doses for a number of domestic animals is given in the accompanying table. Variations in mortality response are found in families, or among individuals, of the same species (Kohn et al., 1956). The absolute roentgen values will differ depending upon conditions of exposure and it is sometimes difficult to compare results from different countries because of inherent differences in physical measurements used and biological criteria employed. Certain physiological factors also determine the individual response. Metabolic rate and body size are two factors which affect the susceptibility of individual animals especially on survival although they have little effect on the actual response to radiations. Although age may be a factor in the mouse it has not as yet been demonstrated in the large animal (Kohn et al., 1956) and sex differences in radiosensitivity have not been consistently demonstrated. (Abrams, 1951 ; Hurst and Casarett, 1955). The nutritional state of the animal does have some effect on radiosensitivity. (Bond and Robertson, 1957). A low protein diet before irradiation increases susceptibility as does a severe protein deficiency after exposure. (Smith et. al., 1952). A fat deficient diet also increases the susceptibility to protracted, but not single, X-ray exposure. (Cheng et al., 1952). Trum (1956) found that pigs could survive several times as long as burros while receiving identical doses of gamma radiation and it was assumed that the fat of the pigs protected them. This was a refutation of the finding of Rust et al. (1954) who showed that in acute radiation studies pigs were more sensitive than the burro. Lineolate depletion of the tissues as a factor of increased susceptibility has been put forward by Cheng et al. (1954). Biochemical factors affecting the radiosensitivity of the large animals include changes in enzymes and enzyme systems.

The blood-glucose increases after whole body irradiation indicating alterations in carbohydrate metabolism and liver-function (Lourau and Lartigue, 1955) : breakdown of proteins increases the aminoacid level of both blood and urine (Katz and Hasterlik, 1955) and the carbohydrates thus made available by deamination serve as precursors of glycogen. Lane et al. (1955) have measured a significant increase in blood pyruvate in irradiated large animals and this may be associated with the above process.

Radiation death is obviously a very complex process and may perhaps reflect a particular susceptibility of the individual animal to the various causes which lead to sickness, such as shock, anorexia, haemorrhage, septicaemia, leucopaenia and so on. The recovery from radiation injury is also different in different animals of the same species and may account for the differences in survival time. Many physiological and histological effects, however, are quite independeant of species (Bloom 1947) and may depend not only on the dose but with local measures of restraint and sedation of the animal concerned. Thus individuals in an apparently uniform population do not respond equally and variations in mortality may be considerable.

EQUIDAE

Many lethal dose investigations have been carried out in the **Burro** and the $LD_{50(30)}$ using total body gamma radiation has been determined to be from 532r to 784r.

The gamma radiation, measured free in air, from zirconium 95/niobium 95 was more effective with an $LD_{50(30)}$ of 585r than cobalt 60 with an $LD_{50(30)}$ of 784r or tantalum 182 with an $LD_{50(30)}$ of 651r (Rust et al., 1953, 1954).

The rates of exposure in these three studies were all different. The rate for the cobalt 60 was 49.8 to 51.2r/hr for the tantalum 182 it was 17.9 to 22.7 and for zirconium 95/niobium 95 it was 19-20r/hr.

It is generally agreed that a slower rate of exposure for a given amount of radiation decreases its effectiveness. This appears to be the reverse of the above observations. This may mean that the rate of exposure did not account for the differences of the MLD's and some other factor or factors is concerned.

Survival time too varied and was influenced by the rate of the exposures. At rates of 18r/hr to 23r/hr there was a unimodal death pattern with the peak at 25 days post-irradiation. At 50r/hr there was a bimodal death pattern with peaks at 3 days and 26 days. (Trum et al., 1952, 1953 ; Rust et al., 1952, 1953, 1954 ; Lane et al., 1956 ; Trum et al., 1959). Kuhn et al. (1958) observed that the $LD_{50(30)}$ for a nuclear explosion (neutron-gamma radiation) was 402 reps but a similar pattern of death as previously was noted. However, Thomas and Brown (1961) studied the response of burros to neutron-gamma radiation and found that out of seven animals which received 180 rads (145 rads of neutrons, and 35 rads gamma) at a dose rate of 6 rads/min, two animals died. One died in 71 hours and the other 119 hours after irradiation with clinical signs resembling CNS damage, confirmed on histological examination. The five survivors also exhibited neurological derangement and an interesting feature was the delayed epilation which occurred and the indication of a weight-response relationship. Previous studies of the acute response of burros exposed to Co_{60} gamma radiation failed to demonstrate a weight-response relationship (Rust et al., 1954) but Kuhn et al. (1958) had observed the highest mortality in the smaller burros exposed to the neutron-gamma radiation of an atomic explosion.

Generally speaking, death in burros has been found by all workers to be often unpredictable. Some animals have died without any clinical signs being shewn. In early deaths, a shock-like collapse with neurological disturbances such as coma has been commonly reported. Those dying later, have shown a general debility, ulcers, spontaneous rupture of organs or vessels causing massive internal haemorrhages. All deaths have occurred within 45 days (Trum, 1953).

Pigs exposed to the same cobalt 60 source of gamma radiation as the burro (*vide supra*) under identically similar conditions had an $LD_{50(30)}$ value of 618r (Rust et al., 1954). Pigs exposed to 1000kv X-rays and 2000kv X-rays both unilaterally and bilaterally had an $LD_{50(30)}$ value of between 388r (2000kv) to 510r (1000kv) depending on the method of exposure. (Tullis et al., 1954). Trum et al. (1959) found that pigs lived from 83 to 385 days if they were exposed to an initial dose of ionizing radiation, then allowed 90 days to recuperate and finally subjected to 50r of gamma rays daily until death. The mean survival times were directly related to the size of the initial radiation dose. Lethal dose studies in **Cattle** have been carried out by Rosenfeld (1958), Schultze et al. (1959) and Brown et al. (1961). An $LD_{50(30)}$ value of 543r with an estimated MLD at the mid-line of 160 rads and a mean survival time of 20.3 days was arrived at by Brown et al. (1961) when adult cattle were exposed to total body cobalt 60 gamma radiation. The dose rate in air was between 53r/hr and 57r/hr and the animals were allowed freedom of movement throughout the exposure field. Rosenfeld (1958) found that a single lethal dose of 600r total body irradiation from cobalt 60 in a 4π exposure field where the dose rate was between 44.3r/min and 51.2r/min represented a $LD_{40(9)}$ or the $LD_{100(14)}$ for male calves from 3 to 5 months old. If the rectal temperature rose before the fourth day after irradiation this was prognostic of a fatal outcome. Dyspnoea and apathy with marked salivation were apparent for 24 hours before death.

It is apparent that **cattle** with an MLD of 543r (Brown et al., 1961) are more sensitive to gamma radiation than either burros or pigs, the $LD_{50(30)}$ values of which are 784r and 618r respectively (Rust et al., 1954). The difference in radiosensitivity cannot be associated with body size for the pigs were smaller than either the burros or the cattle. The same source of irradiation and comparable dose rates were used for burros, pigs and cattle which reinforces the argument of a special species sensitivity as far as cattle are concerned.

Sheep exposed to a single total body gamma radiation from zirconium 95 and niobium 95 at a rate of 17 to 19r/hour had an $LD_{50(30)}$ value of 524r with limits between 450r and 658r (Trum, 1955). Some animals remained lying down from 12 to 72 hours before death (1955 ; ORO-145). However, the 30-day LD_{50} for total body 200 kv X-irradiation of **Goats** at a dose rate of 3.3r/min was calculated to be about 350r based on an experimental series of only 11 animals. Survival time in the lethal range varied from 7 to 20 days. (Swift et al., 1946). Recent personal observations using 250 kv X-irradiation at a dose rate between 11 to 13r/min puts the MLD of goats somewhere between 500 and 650r (Wilkins, 1962) which is similar to that of sheep (Trum, 1955). Survival time was also longer than that found by Swift et al. (1946).

Dogs. Casarett (1950) found that the $LD_{50(30)}$ for dogs varied considerably depending on dose rate :—

Dose rate (*r/hr.*)	*Dose* $LD_{50(30)}$ (*r*)
456.6	335
160.0	430
21 to 25	530

Cronkite et al. (1950) reported that 600r of X-irradiation was 100% lethal to dogs which had shown to have an $LD_{50(30)}$ of 315r. However, the same dose 600r of gamma radiation was not so (Trum and Rust, 1952).

Other workers have found the MLD for dogs using X-radiation to vary from 236r to 450r (Prosser et al., 1946 ; Benner et al., 1951 ; Allen et al., 1951 ; Gleiser, 1953 ; Cronkite et al., 1955 ; Shively et al., 1956 ; Bond et al., 1956 ; Bond et al., 1957 ; Alpen et al., 1958, 1960).

LETHALITY STUDIES USING FRACTIONAL DOSES

The methods of exposure probably cause more response differences in individual animals than do specific idiosyncrasies. The biological effects of a given radiation dose is decreased when it is administered in a series of fractions instead of in a single exposure. The size of the fraction seems to be more important than the time of exposure. When the exposure time is short it does not appear to matter how rapidly the body is subjected to radiations and the survival value may be a fair indication of total body dose received. As living tissue has the power of repair, the rate of exposure becomes important if the total dose is extended over a very long period of time. Fractionation has been studied by exposing animals at different daily doses over a specific interval of time and then comparing the effect produced by the same total dose given in a single exposure. Alternatively the daily exposures are continued until death. Such studies have provided much valuable data on the mechanisms of radiation death, recovery and tolerance.

The phenomenon of the precision of the acute lethal response of mammals to single large doses of total body irradiation has been stressed by Mole (1959). A fractional change in dose makes a large difference in the proportion of animals dying, even when the dose is spread out over days or weeks.

Recently, Brown et al. (1960) have shown that the dose of X-radiation to kill mice acutely depends almost wholly on the overall exposure time, i.e. for continuous radiation the actual exposure time, or for fractionated doses the time between the start of the first dose and the end of the last one. The longer the duration of exposure, the larger the dose required but changes in dose rate and the number of fractions appeared to be of no consequence.

Burros, sheep and **cattle** lived in a constant flux of cobalt 60 gamma radiation at 40r to 50r per hour for 90 to 120 hours before total physical collapse, i.e. a mean lethal dose of 3600r to 6000r. (Trum and Rust, 1952 ; Wasserman and Rust, 1955). Studies undertaken to determine the lethal dose of fractionated total body irradiation in burros using cobalt 60 gamma radiation revealed the following :—

Dose per day	*Survival Time (Days)*	*Mean Lethal Dose (r)*
400 r	8.3 ± 1.4	3320
200 r	14.1 ± 3.3	2820
100 r	23.3 ± 1.0	2330
50 r	30.2 ± 3.3	1510
25 r	63.0 ± 13.2	1575

There was a relationship between the average survival time of the burro and dose rate. The log of survival in days plotted against the log of the dose rate gave a straight line. This occurrence is of interest as a similar finding was reported by Thomson et al. (1953) in survival observations in small laboratory animals. However, comparisons are not strictly valid as their study used a continuous exposure to cobalt 60 gamma radiation whereas in the burro studies there was a daily exposure.

Dose Rate	*Mean Survival Times (Days)*			
	Burros	*Guinea Pigs*	*Rats*	*Mice*
90—100 r/day ...	23.3	20.2	48.4	51.6
20— 30 r/day ...	63.0	62.8	332.6	—

The burros and the guinea pig had very similar mean survival times although the $LD_{50(30)}$ for the burro is 784r and that for the guinea pig is 200r. On the other hand the mean survival times for rats and mice were significantly longer (Trum et al., 1953 ; Rust et al., 1955 ; Haley et al., 1955).

As it is the opinion of many eminent radiobiologists that the reparable recovery rate of large mammals is slower than that of the small laboratory animal, the above data is rather mystifying and warrants further research. PIGS have been given fractionated doses of 50r/day until death (Trum, 1956) and accumulated a mean lethal dose several times greater than the burro. This is an interesting anomaly viz, that although the $LD_{50(30)}$ for the burro (784r) is greater than that of the pig (618r) yet as far as fractionated doses are concerned the burro is more radiosusceptible than the pig.

The $LD_{50(30)}$ for the dog was found to be 450r by Rugh (1953) but if the radiation was spread out over 24 hours it rose to 530r.

LETHALITY STUDIES AND QUALITY OF RADIATION

" Quality " means the type and energy of the radiation used which is in turn directly related to its penetrating power in tissue. Biological effects are supposedly caused by absorption of dose or transference of energy to the tissue concerned. Only where the animal is practically homogeneous in structure and the dose is uniform throughout can a true response depend on the quality of the radiation. Although these conditions may obtain in the small laboratory animals it does not do so in the large animal. This is because the biological effects depends on absorption of dose and energy transference to the tissues which itself depends on tissue characteristics (i.e. bone, muscle,

fat, connective tissue etc.) The significance of dose distribution in the large animal is very important and is now appreciated. Lethality depends on total ion production within the animal system and this accounts for the fact that equal energies of radiation absorbed may give rise to different biological reactions and responses. This in effect is what is implied by the term " relative biological effectiveness (RBE) " of the different radiations.

It is many years since X-rays were found to be 50% more effective than gamma radiations and since neutrons to be twice or more times effective than X-rays. (Suiguira (1939) ; Aebersold and Lawrence, 1942). More gamma radiation is required to produce the same biological effect than X-rays : similarly, more X-radiation is required to produce the same effects in tissues than neutrons. The inverse ratio of the doses required of different radiations to produce a standard amount of given biological effect is in fact the RBE of those radiations. It is influenced by the endpoint observed : death is perhaps the commonest end-point employed in the form of the $LD_{(50)30}$ but other end-points used include organ weights, cataract formation and cancer production. Most studies indicate that as the energy of the radiation is increased so the RBE is decreased. The $LD_{50(30)}$ for dogs exposed to 1000 kv X-rays was 335r whereas when exposed to 250 kv X-rays it was 300r (Boche and Bishop, 1946). From this the RBE of 1000 kv X-rays to 250 kv X-rays was calculated to be 0.8. However, Bond et al. (1956) found no significant difference in the lethal response of dogs to X-irradiation when mid-line doses were compared :—

Radiation	*Mid-Line* $LD_{50(30)}$ *(r)*
250 kv X-rays	252r
1000 ,, ,,	255r
2000 ,, ,,	268r

Burros exposed to gamma radiation of different mean energies showed a variation in MLD when the measured air dose was considered.

Source	*Mean Energy (MeV)*	$LD_{50(30)}$		
		Measured Air Dose	*Estimated Mid-Line Dose*	*Rate r/hr*
Co^{60} ...	1.25	784 r	280 rads	50
Ta^{182} ...	1.2 to 0.18	651 r	290 rads	18 to 23
Zr^{95}/Nb^{95} ...	0.74	585 r	250 rads	19 to 20

However, the estimated mid-line doses again showed no significant differences. Early discrepancies in the RBE of neutrons have recently tended to disappear. The pattern of death studies can now be divided into two general classes *viz* the small laboratory animals have values which range from 1.7 to 2.0. Whereas for large animals it varies from 0.8 to 0.9 when compared to 250 kv X-radiation (Bond et al., 1956 ; Alpen et al., 1960). It is due to the unequal ion distribution along the paths of the radiations concerned, *i.e.* the depth dose patterns, which explains the different biological effects produced by them in the tissues of the living animal. The energy of the radiation falls off rapidly in tissue

and therefore the type of exposure will be important. This fall off is very considerable in large animals as was shown by Tullis et al. (1952), who demonstrated that unilateral radiation in pigs is less effective than bilateral. Generally speaking, the lower the energy and the larger the species the bigger the $LD_{50(30)}$ becomes (Grahn et al., 1956 ; Jones et al., 1956).

However, as far as neutrons are concerned the large contribution to the total dose made by the associated gamma radiation generated by the neutrons in tissues especially at very low (thermal) energies tend to lower their overall RBE in large animals (Bond et al., 1956).

EFFECTS OF IONIZING RADIATIONS
$LD_{50(30)}$ VALUES FOR LARGE ANIMALS

Species	*Measured Air Dose (r)*	*Estimated Mid-Line Absorbed Dose (rads)*	*Radiation used*	*Dose Rate (r/hr)*	*Reference*
BURRO	784	280	Co^{60} Gamma	50	Rust et al, 1954
	651	290	Ta^{182} Gamma	18—23	Rust et al, 1954
	585	350	Zr^{95}/Nb^{95} Gamma	19—23	Lane et al, 1956
					Oro—145, 1955
	—	180	Neutron/Gamma	360	Thomas & Brown, 1961
CATTLE	543	160	Co^{60} Gamma	53—57	Brown et al, 1961
SHEEP	524	205	Zr^{95}/Nb^{95} Gamma	17—19	Trum, 1955 Oro—145, 1955
GOATS	350	237	200 kV X-rays	200	Swift et al, 1946
PIGS	618	242	Co^{60} Gamma	50	Rust et al, 1954
	510	247	1000 kv X-rays	1800—2000	Bond et al, 1957
	388	237	2000 kv X-rays	800—900	Tullis et al, 1952
	225	187	Atomic Explosion		Tullis et al, 1954
DOGS	664	316	100 kv X-rays		Alpen et al, 1958
	465	335	Co^{60} Gamma		Shively et al, 1956
	312	265	2000 kv X-rays	900	Gleiser, 1953 Cronkite et al, 1955
	304	255	1000 kv X-rays	1620	Bond et al, 1957
	285	239	Neutron/Gamma		Alpen et al, 1960
	281	252	250 kv X-rays	900	Bond et al, 1956
	281	228	250 kv X-rays	900	Alpen et al, 1958
	271	250	Atomic Explosion		WT-18, 1952 TIS, Oak Ridge
	236	212	250 kv X-rays	900	Alpen et al, 1960

PART II—CLINICAL SIGNS AND SYMPTOMS

Introduction

The term " radiation syndrome " is used to cover the complex of clinical signs shown by animals after exposure to total whole body radiation. Although the basic effects of ionizing radiations on living cells are the same whichever type of radiation is used (Bloom 1947) the injury to the whole body depends on many other intricate and variable factors : these include the dose, dose rate, length of time under exposure, quality of radiation and the capacity for repair. Radiations are always injurious to the tissue cells which absorb them and the injuries produced are either transitory (reversible effects) or permanent (irreversible effects) with an intermediate class of effect where the radiation changes disappear completely but leave the tissue in a state of lowered resistance to further radiation (conditioned reversible effect) (Spear 1946). There is a variable latent period between the irradiation and clinical recognition of the biological effects. Experimental evidence suggests that the greatest injury is caused to the chromosomes of the cells although decomposition of the cellular protein molecules is another factor. If enough cells are fatally injured, the damage is reflected in the various clinical signs and symptoms known to occur in animals after severe exposure. If the damage is less severe the cells will continue to function temporarily without apparent immediate ill-effects : the full effect will become visible only after the latent period.

Any ionizing radiation, it is repeated, is lethal to the living cell if a sufficiently high dose is absorbed. When applied to the whole body in these high doses the amount necessary to cause death varies with the different species of animals (*Vide* Part I (Lethal Studies) RAVC Journal, Autumn 1962). If the exposure is in the lethal range, death is preceded generally speaking by the appearance of petechial and ecchymotic haemorrhages, cardio-vascular dysfunction and damage, terminal pyrexia, intermittent anorexia and asthenia. For exposures not lethal within a short time the syndrome may manifest itself as subacute or delayed in its effects.

It is often difficult to correlate between the clinical signs shown by a larger animal and the dose of radiation received. This will depend very much upon factors such as the techniques used, the abnormalities looked for and above all on the experimental biologist concerned. The trained veterinary officer is the best to assess the clinical manifestations shown by large animals exposed to radiation in the same way as the doctor is considered the best assessor where human beings are affected. The analysis of the radiation syndrome is made infinitely more complicated by the fact that it is almost impossible to segregate the clinical phenomena which are the direct result of the radiation from those that may be the result of bacterial invasion or of the normal physiological reparative processes within the body.

That bacteraemia and toxaemia are important factors involved in the clinical manifestations of the radiation syndrome goes without saying. Many experimental biologists go so far as to believe that they are major causes of the death of irradiated animals. Certainly, the author has found that the clinical signs and post-mortem appearances of goats dying acutely from lethal doses of X-irradiation are similar in some respects to Clostridium welchii Type D intoxication found in Pulpy Kidney Disease and Enterotoxaemia (Wilkins, 1962).

Warren and Whipple (1923) found a correlation between septicaemia and the destruction of the gut epithelium in irradiated dogs. Organisms can more readily traverse the damaged intestinal wall of irradiated than of normal animals, due possibly *inter alia* to the fact that bacteria adhere to the wall more readily in irradiated than in non-irradiated animals (Chrom, 1938). Even avirulent bacteria have been shown to invade the tissues of an irradiated animal (Miller et al. 1950 ; 1951, Tullis, 1949 ; Mayhew et al. 1955). Resistance to infection is lowered due partly to the reduction of blood leucocytes which always occurs after irradiation and partly to the fact that the immune response of the animal is affected (Taliaferro et al. 1951).

The survival time of an irradiated animal often appears to be a function of the relative degree of gut injury. Mayhew et al., 1955 observed that the bacterial invasion of irradiated burros did not occur before death at 9 days when irradiated at the rate of 400r/day whereas at rates of 100r/day organisms were found in the blood and internal organs between 12 to 48 hrs afterwards. The animals exposed at the rate of 100r/day survived 22 days. Cronkite et al., (1952) and Trum (1953) had already observed that when the burro received a dose of irradiation sufficiently high to cause an early death the haemorrhagic phase does not manifest itself in the radiation syndrome. It appears therefore that bacterial invasion and the haemorrhagic phase are concurrent physiological changes associated with actual survival time but are not physiologically correlated.

Haemorrhage also plays a major part in the radiation syndrome. It is very rare that a haemorrhage by its site or extensiveness has caused death. Trum et al., (1952) observed that a female burro died from internal haemorrhage following a rupture of an ovarian cyst. More usually there is a tendency to petechial or ecchymotic bleeding throughout the organs and tissues. This tendency is paralleled by the lowering of the circulating platelets but the correlation appears not to be a strong one for it is impossible to tell which individual animal is going to survive from its platelet count. It is interesting to note that with small laboratory animals and dogs the correlation is exact as in man. The original observation that the cause of the haemorrhage was due to a hyper-heparinaemia (Allen et al., 1948) has been adequately refuted by Jackson et al., (1952).

The pathogenesis of haemorrhagic lesions following whole body irradiation is obscure. Andersen et al., (1958) postulates that the degree of haemorrhage correlates with the size and amount of a special intravascular substance which is formed as a result of radiation. These intravascular poly-saccharide globules may act as emboli inducing endothelial damage in the presence of such factors as a low platelet level.

By and large, however, the principal cause of haemorrhage is still considered to be thrombocytopaenia. As soon as bacterial invasion and haemorrhage occur the direct effects of radiation become obscured. The " subacute " and " delayed " clinical effects of radiation must then be put into their proper context and a reasonable assessment made keeping in mind also the tissue reparative processes which have been going on throughout the period.

THE ACUTE RADIATION SYNDROME

The clinical signs reported below were usually noted in animals which had received lethal or near lethal doses and lived long enough after irradiation to show symptoms.

EQUIDAE (BURRO)

Collapse and coma usually preceded early deaths but often burros died without evidence of illness (Trum et al., 1952). Some animals died showing extreme debility with necrotic ulcers in the mouth and anus. Others after showing a temporary recovery gradually weakened with an increasingly severe haemorrhagic diathesis. This showed itself by bleeding externally from wounds, tumours or parasitic lesions. Death in these cases was due to internal bleeding from rupture of some organ or vessel.

It is almost an axiom that if anything atypical of mammals in general can be shown the burro will show it, according to U.S. experience.

General Observations

Immediately after irradiation there was a period of apparent well being lasting 24 to 48 hours. Apathy and malaise then set in and lasted 2 to 5 days during which early deaths occurred. Survivors then seemed to improve showing initially a general hyperaesthesia and later quietening down. This period of improvement lasted approximately a week after which another period of malaise began. Those destined to recover improved gradually from this time, the rest died within a few days. Certain specific signs shown included (Trum, 1953) :—

a. Anorexia : food and water intake varied with the well-being of the individual animal and was reflected in the weight and condition.

b. Locomotion : from the second day onwards animals did not like bearing weight upon their legs and showed lameness when trotted up.

c. Skin : there was no shedding of hair but the skin of the head became oedematous producing a "bighead" appearance. Wounds even grossly contaminated did not suppurate.

d. Mucous membranes : In 2-7 days necrotic ulcers were evident on mouth and lips leading to facial and gutteral oedema. After the second week petechial haemorrhages and purpura appeared on lip, prepuce and vagina.

e. Eyes : lachrimation, a severe conjunctivitis and oedema were noticed between 2-7 days in some and between 14-18 days in others. Spontaneous recovery occurred and the signs were not related to dose.

f. Pulse, Respiration and Temperature : no specific or constant change was noted. Dyspnoea was common due to partial occlusion of the upper respiratory passage caused by facial and gutteral oedema. At such times there was a bloody froth from the nostrils. Temperatures were normal except during a short interval before death when they became abnormally high (up to 107°F) or subnormal (95-98°F).

g. Alimentary tract : diarrhoea was uncommon but haemorrhages into the intestinal tract occurred. At post mortem ulcers were found in all parts from lips to anus.

h. Genito-Urinary tract : all females showed polycystic or monocystic ovaries which in many cases were blood-filled. Survivors exhibited a normal cycle one year after exposure.

In males, the testes were retracted a few days after irradiation and became diminished in size. Normal libido was displayed except during periods of apathy.

i. Neuro-Muscular signs : signs characteristic of encephalitis occurred. Animals stood for hours pressing their heads against the wall. Others circled continuously or walked backwards. Other signs seen included

twitching of the facial muscles, spasmodic retraction of the lips, spasmodic flexion of the hocks (stringhalt), head-jerking and tail-switching. Occasionally paralysis of ears or eyelids occurred. The neuro-motor signs lasted for one or two days after which animals either died or recovered.

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS OF ACUTE RADIATION SYNDROME IN BURROS

Time after Irradiation	Clinical Signs
0—36 hours	Normal or euphoric.
24—48 hours	Apathy, malaise, anorexia, occasional deaths.
2—7 days	Malaise and anorexia continue. Hyperaesthesia, neuromotor and locomotion disturbances. Testes retract. Foetid odour from mouth. Necrosis and ulceration of mucous membranes. Sudden deaths. Conjunctivitis and lachrimation.
7—14 days	Appetite improves. Serous exudate followed by bleeding from skin. Symptoms of first week continue. Clotting defect appears in blood.
14—21 days	Anorexia may return. Haemorrhagic syndrome becomes apparent. Deaths casually follow prolonged debility. Bloody froth from nostrils. Bleeding from body orifices.
21—30 days	Occasional typical deaths with previous symptoms. Improvement noted in survivors.
Over 30 days	Deaths from acute radiation rare. Ageing process accelerated.

THE CLINICAL RESPONSE OF BURROS TO NEUTRON IRRADIATION

Thomas and Brown (1961) irradiated seven burros with 180 rads (145 rads neutron radiation plus 35 rads gamma radiation) at a rate of 6 rads per minute. Two burros died unexpectedly with clinical signs resembling CNS damage, heretofore seen with much larger doses and much higher dose rates using gamma radiation alone. The five survivors exhibited CNS symptoms as well but to a much lesser degree. Although epilation has not been observed in burros exposed to lethal or sublethal doses of gamma radiation this did occur with neutron-exposure. There was also evidence of a weight-response relationship *i.e.* the clinical response in the smaller animals was more severe than the larger ones. In previous studies of the acute response of three species of large animals (cattle, pigs and burros) exposed to Cobalt 60 gamma radiation alone, a weight-response relationship was not evident. It is interesting to observe also that the highest mortality occurred in the smaller burros when exposed to an atomic explosion (Kuhn et al., 1958).

From this study it appears that neutrons are more effective than gamma radiation on the burro at relatively low dose rates. Also that burros are more sensitive to vascular changes contributing to the CNS effects and death at relatively low radiation dose levels (Brown et al., 1962).

RUMINANTS

General observations in Adult Cattle

Brown et al., (1961) exposed adult grade Hereford cattle to whole body Cobalt-60 gamma radiation at doses of 450r to 700r.

During the first 3 days after irradiation the animals were more easily excited when handled.

Except for this observation the cattle were normal and had a good appetite for 10 to 12 days after irradiation.

The first clinical signs of irradiation sickness were mild diarrhoea which was often blood-tinged. This was followed chronologically by knuckling of the fetlock joints of the hind legs, generalized weakness, depressed appetite, dyspnoea, anorexia, and severe diarrhoea.

Dyspnoea, caused by oedema of the larynx and lungs, usually progressed from a mild to a pronounced state over a period of 3 to 4 days. Near the animals' death severe haemorrhage in the large intestines occurred shown by large amounts of bloody faeces being passed.

Weight loss over the 30-day period was less than 10%. At the end of the irradiation period the rectal temperature was 1 to 3 degrees above normal.

Within 24 hours after irradiation all temperatures had dropped within the normal range (101° to 102.5°F) and remained so until approximately the 14th day. Temperatures of 108°F to 110°F were recorded in several animals which died.

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS IN ADULT CATTLE

Time after Irradiation	Clinical Signs
0—24 hours	Temperature up 1 to 3 degrees. More easily excited.
1—3 days	More easily excited otherwise normal.
4—14 days	Normal.
14—30 days	Mild diarrhoea which progressively became more severe and terminally contained large amounts of blood. Pyrexia, ataxia, generalized weakness, anorexia and dyspnoea preceded death.
30 days and over	Continuance of signs with debility until death or gradual improvement in survivors.

Observation in calves

The clinical course of calves exposed to a single lethal dose of 600r of total whole body gamma radiation was characterized by progressive weight loss, recovery from an early diarrhoea and anorexia, an essentially asymptomatic period of 4 to 5 days, followed by the reappearance of watery diarrhoea and anorexia. By the 8th to 9th day the exposed calves generally manifested inflammation of the eye-lids, lachrimation, a viscid nasal discharge and signs of a locomotor disorder characterized by stiffness of the hind legs and an unsteady wobbly gait. The mean survival time was 10 days and for 24 hours before death there was pronounced malaise, apathy and respiratory distress. If the rectal temperature rose by the 4th day after irradiation this was usually prognostic of an early or delayed fatal outcome. Once the temperature of an animal rose to around 105°F, which is approximately 3 degrees above normal, death usually supervened within 3 or 4 days.

Neurological signs, epilation, and bloody exudates from mouth or anus were *not* observed (Rosenfeld, 1958).

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS IN CALVES

Time after Irradiation	Clinical Sjgns
0—12 hours	Normal.
12—24 hours	Listless, off food, not ruminating, watery diarrhoea.
2—5 days	Normal.
5—8 days	Pyrexia, anorexia, watery diarrhoea, lying down.
8—10 days	Conjunctivitis, lachrymation, viscid nasal discharge, unsteady gait, malaise, apathy, dyspnoea with marked salivation. Death may occur.
10—30 days	Continuance of signs with severe loss of condition 5 to 7 days before death. Terminal dyspnoea and pyrexia.
30 days and over	Gradual improvement in survivors. Others show increasing debility until death.

SHEEP

During the first 2-3 days after irradiation sheep appeared essentially normal although a certain number showed muscular tremors mostly of the hind quarters.

About 48 hours following exposure anorexia appeared and a progressive loss in weight occurred in most animals after this.

Occasionally sheep showed ataxia and a disinclination to bear weight on their legs. Knuckling over and bent knees as frequently observed in burros and goats were seen. Some animals remained down from 12-72 hours before death. A blood-tinged frothy discharge from the nostrils and blood-tinged faeces occurred in some sheep (Trum, B. F. and Rust, J. H. 1958).

GOATS

Cronkite (1945, 1950) described the signs of the radiation syndrome in the goats exposed to the atomic explosion at Bikini. Death usually occurred between 3-14 days after exposure with signs of apathy, anorexia, rhinitis and bloody diarrhoea. Epilation was seen after 7 days in some goats but rarely in survivors. Goats refused to drink and sudden unexpected deaths sometimes were observed. Swift et al (1946) irradiated 11 goats with 300r to 600r of X radiation. Some became lethargic and refused food and water for 2 to 3 hours after irradiation, after which time they appeared normal. No other signs of illness were observed until 2 or 3 days before death. At this time mucous discharge and bleeding from the nose and mouth, extreme lethargy and prostration occurred. Petechiae were seen in the mouth and blood occasionally in the faeces. Little food was eaten during the terminal period and skin-bleeding was observed in some goats. Wilkins (1962) irradiated 20 goats with 400r to 2000r of X-irradiation and observed that they varied in their clinical responses considerably. Initially animals appeared normal for varying periods of time during which muscular tremors were apparent. An early sign was a lessening, or complete loss, of water consumption which continued intermittently thereafter. Terminally animals often put their mouths into the water but appeared not to be able to swallow allowing the

water to drool out. A reddening of the mucous membrane of the fauces with oedema and inflammation of the lingual lymphatic follicle was noted in some cases and could have been the cause of this.

Rectal temperatures remained essentially normal (100° to 102.5°F) for most of the post-irradiation period although a higher normal plateau (103° to 104°F) often occurred intermittently. Two to four days before death temperatures rose precipitously to between 106°F and 110°F. On the day of death it usually dropped below normal (98°F to 99°F).

Anorexia and some apathy usually occurred soon after exposure but there was a quick return to normal.

Intermittent bouts of inappetence and non-rumination were observed for the following 14 to 21 days and continued in non-survivors until death. These goats spent a lot of time lying down and grinding their teeth. An oedematous swelling of the subcutaneous tissues of the head especially around the buccal region gave the animal a characteristic appearance. Epilation was observed in some goats about the tenth day after irradiation and continued thereafter for varying periods. A " stripe effect " due to shielding was noted.

Intermittent cardiac arrhythmia, heart block and fibrillation occurred in many goats. Some goats showed a transient irritability but a general depression combined with an apathetic fatigue was more common. Weakness of the hindquarters was often shown in the later stages but other animals showed jerky head movements, flicking at imaginary flies with the fore feet and other neuro-motor signs.

The passing of normal pelletted faeces was the rule and intermittent diarrhoea with some melaena the exception even at supra-lethal doses. During the terminal asthenic period some 2 to 3 days before death urination and defaecation usually ceased although a soiled anal region and tenesmus were often seen. Frank diarrhoea was uncommon. The temperature suddenly began going up in step-like rises to as much as 111°F, dyspnoea occurred and a viscid discharge was observed from nose, mouth and eyes. The animal pushed its head into a corner and stood or lay in a forlornly apathetic fashion for hours on end. Death was sudden with little or no struggling.

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS IN SHEEP AND GOATS

Time after Irradiation	Clinical Signs
0—36 hours	Normal. Muscular tremors and cardiac signs in some.
1—2 days	Anorexia. Malaise.
2—7 days	Intermittent anorexia. Reduction or suppression of water consumption. Otherwise normal.
7—14 days	Progressive weight loss. Weakness and apathy : knuckling over in front. Ataxia. Death may occur.
14—21 days	Signs continue. Occasional diarrhoea and discharge from eyes. Deaths may occur.
21—30 days	Terminal pyrexia. Discharge from eyes, nose and mouth. Diarrhoea in some. Intense apathy. Sudden death.
30 days and over	Survivors normal. Non-survivors remain normal until 2 to 3 days before death.

PIGS

The clinical signs shown by pigs exposed to ionizing radiation have been described by many workers including :— Tullis and Warren (1947), Cronkite (1950), Tullis (1951), Rust et al., (1954) and Trum et al., (1959). Perhaps the most noteworthy fact was the remarkable survival times of pigs irradiated at 50r/day until death (Trum er al., 1959). Whereas burros irradiated under the same conditions had survival times between 25 to 35 days (Rust et al., 1955), pigs survived from 83 to 385 days. The chronological sequence of signs are presented in the accompanying table :—

CLINICAL RESPONSE OF PIGS EXPOSED TO 50r PER DAY GAMMA RADIATION UNTIL DEATH

Days after Irradiation	Dose (r)	Signs
0—60	0—3000	Normal. Transitory hyperaesthesia.
60—100	3000—5000	Lethargic, disinclination to move. Few sudden deaths.
100—220	5000—11000	Lethargic, polyarthritis, dyspnoea, string-halt, ulcerations on coronary band.
220—260	11000—13000	Survivors appeared normal and were asymptomatic.
260—400	13000—20000	Lethargy and lameness reappear, final deaths.

The typical clinical picture after receiving the overwhelming dose of ionizing radiation at the Bikini bomb explosion showed pigs at first looking and acting normally. After the first 2-3 days of apparent well-being, dyspnoea and a diarrhoea developed which in 7 days became bloody, tarry or both. They became hyper-irritable but stayed on their feet, and refused food but drank water. As the diarrhoea and anaemia progressed weakness developed and the animals spent considerable time lying down. They usually responded when approached, struggled to their feet and even walked until suddenly they died. Death was often unexpected from clinical observations alone. Four of ten pigs were alive five days after receiving 20,000r. Those pigs receiving a lethal but lower dose or after exposure to high doses of X-rays or gamma radiation were normal but hyper-irritable until 6 days after irradiation when diarrhoea, apathy and anorexia then set in. The diarrhoea often persisted until death. Between the 9th and 10th day petechiae appeared and purpura after the 10th day. Blood trickled from the nose, vagina, urethra and rectum of all animals.

Other signs shown included occasional lameness, lying down and huddling together a great deal, oedema of the face and scrotum, and elevation of rectal temperature before death. Some pigs showed an involuntary muscular spasm similar to the skin fly response of horses and cattle. Occasional exaggerated spasmodic flexion of the hocks (" stringhalt ") was displayed by some animals. Anorexia and diarrhoea sometimes were not seen. In fact, contrary to the response by most acutely irradiated animals, pigs were sometimes constipated producing hard faecal balls (Trum et al., 1959).

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS IN PIGS

Time after Irradiation	Clinical Signs
0—5 days	Occasional lameness, irritable otherwise normal.
6—10 days	Diarrhoea commences and may continue until death. Apathy, anorexia. Petechial haemorrhages in eyes and mouth. Blood in faeces and urine.
10—21 days	Purpura of skin. Oedema of face and scrotum. Conjunctivitis.
21 days and over	Lameness, huddling, terminal pyrexia, bloody exudate from natural orifices. Death.

DOGS

The clinical signs of the radiation syndrome in dogs resemble more closely those seen in human beings than other domestic animals. Prosser et al., (1946) described them as one of toxaemia in the acute stage and of severe anaemia in the subacute. Many other workers have described the sequence of symptoms observed in dogs exposed to medium lethal doses of whole body irradiation. (Cronkite et al., 1950 ; Brecher et al., 1951 ; Jackson et al., 1952 ; Gleiser, 1953 ; Bond et al., 1956 ; Conrad et al., 1956 ; Alpen et al., 1958 ; Alpen et al., 1960).

During the first 24 hours after irradiation they exhibited lassitude, anorexia and vomiting. Some vomited within one hour after irradiation. Rectal temperatures remained normal.

From the 2nd day until the 8th or 9th day after irradiation dogs regained their appetite and appeared to be well. Rectal temperatures remained normal during this stage. During the later part of this intermediate period the skin of some dogs became ulcerated and others showed a footsore condition.

The terminal phase was frequently ushered in by the onset of stomatitis, which was followed by a refusal to eat, fever, dyspnoea, emaciation, bleeding gums, bloody diarrhoea, subepidermal petechiae and ecchymoses, conjunctivitis and bleeding of small wounds.

Large haematomas and cellulitis of one or more limbs frequently appeared before death.

Effects of MLD-plus Doses of Radiation in Dogs

The dog is relatively resistant to involvement of the gastrointestinal tract after ionizing radiation (Conard, 1956). The X-ray dose required for injury is several times as great as the $LD_{50(30)}$ dose in which death results almost entirely from haematopoietic failure. Characteristics of the gastrointestinal syndrome in dogs as observed by Conard were retching and vomiting shortly after irradiation, anorexia, and death on the third or fourth day after irradiation.

CHRONOLOGICAL SEQUENCE OF CLINICAL SIGNS IN DOGS

Time after Irradiation	Clinical Signs
0—24 hours	Apathy, vomiting, anorexia.
2—9 days	Normal : some show lameness or footsoreness.
9—14 days	Stomatitis, anorexia, pyrexia, dyspnoea, bleeding gums, bloody diarrhoea, sub-epidermal petechiae/ecchymoses.
14—21 days	Above signs continue. Extreme apathy. Emaciation. Weight loss.
21—30 days	Necrotic gingivitis and laryngopharyngitis. Large sub-epidermal haematomas. Cellulitis. Fever. Bloody diarrhoea. Death.
30 days and over	Non-survivors continue as above and die eventually with signs of a severe anaemia. Survivors recover slowly after a long convalescence.

PART III—PATHOLOGICAL ASPECTS

The biological effects of ionizing radiation from all sources on normal mammalian tissues are qualitatively similar (Warren & Gates, 1940). The effect on a given tissue is proportionate to the amount absorbed and not to the amount of radiation delivered. A large part of these effects are due to scattering of the radiation within the tissue; consequently the field of exposure is a most important factor, as is the rate of irradiation, for with low rates regeneration will compensate for effects on somatic cells and no visible result may be apparent. The first report on the acute general constitutional effects of radiation was made by Walsh in 1897.

One of the most important and striking effects of radiation are those induced in the blood vessels. The endothelium is the most susceptible of all the vascular tissues and the major changes are seen in the smallest vessels where the endothelium makes up a proportionately larger part of the wall ; injury to large vessels is rare. The vascular changes are of importance because of the resulting thrombosis and the lowering of resistance and reparative powers of the surrounding tissues. There is tissue starvation which explains in part the intractable character of radiation lesions.

From work on the respiratory quotients of irradiated animals (Hervey *et al*, 1951 ; Lane *et al*, 1955) it can be assumed that there is a profound disturbance of metabolism probably related to the citric acid cycle for there is usually an aminoaciduria with an increase of blood pyruvates. Alterations in hormone production have also been reported *viz* decrease of gonadotrophic hormone (Lane *et al*, 1954), increase in ACTH (Patt *et al*, 1947), increase in thyroid stimulation and decrease in alkaline phosphatase (Schooler *et al*, 1954).

Effects on Cells.

Some of the morphological changes seen in cells of irradiated tissues include :

Cellular changes :

(i) Necrosis and necrobiosis
(ii) Metaplasia

Cytoplasmic changes :

(i) Vacuolization
(ii) Disintegration
(iii) Atrophy and hypertrophy

(iv) Changed staining characteristics

Nuclear changes :

(i) Pyknosis
(ii) Karyorrhexis
(iii) Micronucleation
(iv) Giantism
(v) Vacuolization
(vi) Mitosis abnormalities

Tissue and Organ changes

Generally speaking, length of survival has more effect on the pathological alterations seen in tissues than dosage.

(i) Animals dying within a week usually show changes in the gastro-intestinal epithelium and the lymph nodes, possibly also in the central nervous system.

(ii) Those surviving from 2 to 5 weeks would show a partially or wholly recovered gastro-intestinal epithelium, haemorrhagic manifestations in the form of massive subendothelial haemorrhages in the heart and other organs, seriously damaged lymph nodes and blood forming tissues, plus a generalised pulmonary oedema. Colonies of bacteria with little or no tissue response would be seen.

(iii) Animals surviving after 6 weeks would show recovering lymph nodes and blood forming tissues, a recovered gastro-intestinal epithelium and fibrotic proliferation of the septal walls of the lungs. Colonies of bacteria may be seen showing neutrophilic response.

EFFECTS OF IRRADIATION ON THE SYSTEMS OF THE BODY

Heavy irradiation sufficient to cause the acute radiation syndrome is also capable of adversely affecting the functions of the testes and ovaries.

TESTES.

The spermatogonia of the testicle are amongst the most radiosensitive of all mammalian cells. It was first shown by Albers-Schonberg in 1903 that the injury resulting from exposure to X-rays consisted of a decrease in spermatogenesis and destruction of the epithelium lining the seminiferous tubules. The spermatogonia undergo degeneration in a similar fashion to other radiosensitive cells; the spermatocytes and spermatozoa are relatively much more resistant to radiation. The net result as far as the testicle is concerned is that it rapidly atrophies. The duration of the hypoplastic atrophy and the degree of recovery are dose dependent. After doses greater than 800 to 1000r permanent atrophy and sterility result.

OVARIES.

The ovary was studied extensively in the early years of the century. Atrophic changes have been described by Halberstadter (1905) and Lacassagne (1913) and (Warren) 1943. The developing ovarian follicles are very radiosensitive whilst the corpora lutea are relatively radioresistant. After doses of about 800r ova disintegrate and follicles become atrophic. The destructive effect of the radiation is progressive being maximal at between 20 and 30 days. However, primitive ova survive in the dense stroma and these can develop into fertile viable ova. The dose to sterilize the ovaries is not known at the moment.

EFFECTS ON HAEMATOPOIETIC TISSUE

Blood and Bone Marrow.

Most of the blood cells are formed in the red bone marrow. Radiation injury is influenced by:

(*a*) differences in the life-span of the cells and
(*b*) differences in the radiosensitivity of the cells.

Source and Life-Span of Blood Cells

Cells	Source	Life Span
Lymphocytes	Lymphatic Tissue	8—24 hrs.
Granulocytes	Bone Marrow	2—3 days
Platelets	Bone Marrow	3—6 days
RBC's	Bone Marrow	15—17 weeks

The general effects are reduced supplies of blood cells, which account for reduction in resistance to infection, loss of clotting, haemorrhages, late anaemia and asthenia.

Lymphatic System.

The spleen is the largest mass of lymphatic tissue in the body but it is primarily involved in blood, rather than in lymph circulation. The function of the spleen can be taken over by other lymphatic tissues. Since the lymph nodes filter foreign substances out of the lymph, they show the first stages of haemorrhage and infection after acute irradiation.

Haematology.

The general character of the blood changes in all species of animals after irradiation is very similar (Taylor 1919). Since the pioneer work of Heineke in 1903 and 1904 on the effect of X-radiation on the circulating blood much work has been done in this field. The destructive effects of total whole-body irradiation on the blood and blood-forming tissues has been studied in most of the domestic animals (burro, Rust *et al* 1954 ; cattle, Rosenfeld 1958 ; Brown *et al*, 1961 ; sheep and goats, Swift *et al*, 1946 ; Cronkite, 1950 ; Trum *et al*, 1958 ; Wilkins, 1962 ; pigs, Cronkite *et al*, 1949 ; Cronkite, 1950 ; monkey, Eldred and Eldred, 1953 ; dog, Cronkite and Brecher, 1955). Sufficient irradiation of the body causes a reduction in the number of cells of all series in the circulating blood. A great source of confusion in investigating the blood picture of animals is the progressive changes which occur after irradiation. Although the primary injury occurs during the actual period of exposure, some of the subsequent changes which appear only after a significant latent period, may be progressive for weeks or even months.

Probably the most important factor in determining the picture of the circulating blood is the almost consistent finding of widespread cell destruction in the blood-forming organs after irradiation. Some observers, however, consider that the mature circulating leucocytes are affected directly by ionizing radiations and destroyed by lysis. "Toxic substances" are thereby released and these are supposed subsequently to have a secondary injurious effect on the blood-forming tissues (Storer *et al*, 1954). Probably both effects *i.e.* direct effect on the haematopoietic tissues and the indirect effects on the circulating blood, are ultimately responsible. Be this as it may, there is no doubt that the whole haematopoietic system is a most sensitive indicator of the effects of whole body irradiation.

The changes in the circulating blood after irradiation may be summarized, not in strict chronological order, as follows :—

1. During the earliest phase there is a transient leucocytosis due almost entirely to a neutrophilia. Trowell (1952) has suggested that this is due to the mobilisation of existing mature cells in the bone marrow: however the release of marginated leucocytes in the capillary bed, the probability of the quicker maturation of granulocytic precursor cells in the bone marrow and their subsequent release into the circulation cannot be ignored. This phase lasts for a variable period of time and has a variable pattern; however, 24 hours is rarely exceeded and it is followed by

2. A period of extreme leucopenia which in fatal cases consists of a progressive neutropenia leading to agranulocytosis and death. In animals which recover there is a gradual return to normal; this recovery in numbers is dose and species dependent and may vary from the 20th to the 30th day after irradiation (Cronkite and Brecher, 1955).

3. There is an immediate profound drop in the number of lymphocytes in the circulating blood (lymphopenia), and they almost disappear within 48 hours. Heineke (1903) suggests that this was due to a selective destruction of lymphogenic and lymphatic tissue by the radiation. Lymphocytes show a remarkable degree of radiosensitivity but also show strong powers of recovery (Trowell, 1952). In the sublethal range the degree of depression is a fair index of the amount of radiation exposure. The count may remain essentially at about the same level for many months after the initial lymphopenia.

4. In many animals a profound thrombocytopenia is preceded by a transient increase in the number of circulating platelets. The depression is a function of dose in the sublethal ranges. In the lethal range the depression is maximal with platelets almost disappearing from the circulating blood. They remain at the minimum levels until death or spontaneous regeneration.

5. A late anaemia occurs at MLD doses followed by a slow recovery in survivors. Erythrocytes possess a greater radioresistance than other blood cells; this factor coupled with the ability of erythropoietic foci in the bone marrow to regenerate plus the longer life of the red blood cell make the actual number of erythrocytes remaining in the circulation at any given time after irradiation to be relatively greater. After massive doses of radiation *e.g.* over MLD doses, there is a progressive increase in the haematocrit (packed cell volume) due to extreme fluid loss. As a result of the failure of haematopoiesis the red blood cell count falls progressively in proportion to their natural life span ; consequently, as the life span of the erythrocytes of animals is comparatively long *i.e.* 100 days or longer, the decrease in the haematocrit and the onset of anaemia is relatively slow. Acute anaemia usually precedes death after lethal doses of irradiation but after sublethal exposures anaemia develops very slowly and is followed by a slow, prolonged recovery (Clarkson *et al*, 1938).

6. Eosinophils, Basophils and Monocytes. Significant changes are occasionally seen in the counts and characteristics of these cells but they are not regular nor predictable.

The circulating blood does not accurately reflect the state of the activity of the haematopoietic tissues after irradiation: lymphatic tissue and bone marrow may show varying degrees of hypoplasia or hyperplasia long after the blood picture has returned to apparent normal.

The Cardio-Vascular System

The effect on the heart are secondary to those on the connective tissue of the interstitial tissue and the blood vessels. Perivascular bleeding is followed by hyalinization of the connective tissue elements, possible atrophy of muscle cells and changes in the ganglion cells. The contractility of the heart may thus be affected, producing clinical evidence of cardiac dysfunction and disturbances (Dogs, Hartman *et al*, 1927. Burro, Rust 1956. Goats, Wilkins, 1962). Effects on the right heart may possibly be correlated to the lesions in the lungs. Secondary effects are due to circulatory disturbances.The effect on the blood vessels have already been referred to (*q.v.*).

EFFECTS ON THE RESPIRATORY TRACT

The lungs are moderately radiosensitive organs. Inflammation of the respiratory tract frequently follows irradiation and this is clinically evident by a cough, dyspnoea, fever, pleural effusion and thoracic pain. The chief danger is a lowering of tissue resistance to infection. In the mild case there is congestion, oedema, lymphectasia with slight bronchiolar and alveolar changes. In the more serious case there is in addition obvious pneumonic injury with active regeneration of bronchilar and alveolar cells with the presence of a well-defined "hyaline membrane". The late effects include patchy atalectasis and fibrosis as a sequel to infection.

EFFECTS ON THE ALIMENTARY CANAL

The first effect of irradiation is impaired secretion and discontinued cell production. Cell breakdown follows and large numbers are released so that the folds of the intestines become cluttered with debris. The extensive exposure of tissue under the epithelium gives rise to ulcers. Diarrhoea and blood in faeces is a sequel.

The primary effects of radiation on the gastro-intestinal mucosa are probably direct. However, secondary factors play a part almost from the beginning. Mechanical trauma, damage to vascular connective tissue (especially the endothelium) secondary infection, dysfunction, adhesions or immobilisation of the wall due to parasites, all have their ultimate effect.

Liver and Gall Bladder.

Radiation damage impairs digestion. By altering production of fatty acids in the liver, metabolism is affected. Large doses produce focal areas of necrosis and haemorrhaging in liver and gall bladder.

The primary changes in the liver occur soon after exposure to radiation. These include hyperaemia, haemorrhages, cloudy swelling, disappearance of glycogen and granular degeneration. Necrosis may be seen 72 hours later. Repair is coincident with injury but secondary degeneration or retarded repair confuses the picture.

In the gall bladder there is congestion, oedema, haemorrhage and necrosis of the epithelium with an inflammatory reaction most noticeable about 14 days after exposure. Calcification near the gall bladder was found in dogs after heavy doses of X-rays by Bolliger *et al.*

EFFECTS ON THE URINARY TRACT

The kidneys are moderately responsive to radiation. Doub *et al* (1927) regarded the kidneys as the most susceptible organ as far as loss of function and anatomical changes were concerned but many other observers regard them as relatively radio-resistant. Early renal changes include hyperaemia, swelling and desquamation of the tubular epithelium. Later changes include fibrosis producing shrinkage and capsular thickening. The ureter may show stenosis as a result of fibrosis and the bladder shows oedema and signs of vascular injury. Tenesmus may be the resulting clinical manifestation.

SKELETAL AND MUSCULAR SYSTEMS

Radiation has little effect on bone or muscle cells but is a serious threat to the radiosensitive marrow contained within bones.

THE SKIN AND HAIR FOLLICLES

The skin is easily injured but its capacity for local repair is remarkable. Irradiation of the hair follicle cells can stop growth and cause temporary baldness (epilation).

THE ADRENALS

Damage by irradiation causes the interval of restoration to the normal after stimulation to be longer. The animal is thus more susceptible to heat, cold, injury and infection; the heart beat becomes irregular, blood pressure drops and the balance of salts in the plasma becomes upset.

THE THYROID

The thyroid is not considered very sensitive to external radiation : however it concentrates internally absorbed radioactive iodine which is used in the production of thyroxine. The subsequent *beta* and *gamma* bombardment causes a decrease in thyroxine thus reducing the basal metabolism rate and impairing health.

SOME POSSIBLE SEQUELAE OF IRRADIATION

Caractogenesis

Direct irradiation of the eyes with X-rays and *gamma*-rays causes cataracts. Only 1 or 2 damaged lens cells are necessary to initiate cataract formation. Subsequent surveys revealed a higher incidence of cataracts among Japanese atom bomb survivors than among non-exposed Japanese. Susceptibility to radiation induced cataracts depends on age: cataracts from natural causes are commoner in older animals. Because of the continuing growth of the lens in the young, cataracts are more likely to be caused in the young from radiation.

Carcinogenesis

There are three methods by which radiation can produce cancer:

Direct: Where cancer appears in a part that has been exposed to a large dose of radiation.

Boost: Where cancer appears in a part exposed to a small amount of radiation it can be assumed that the part contains cancer-producing agents and that the cancer was triggered off by the radiation.

Abscopal Effect: Is due to production of special cells or chemicals which travel via the blood stream throughout the body and effect remote tissues.

Cancer is most likely to occur in the skin, lungs, the bones and the bone marrow.

Radiation disturbs the interrelationships of tissues so that benign tumours may become highly malignant, metastatasing and invasive.

"Premature Ageing"

There seems to exist in the irradiated animal a diffuse state of deterioration. Radiation injury is partly reparable and partly irreparable. The reparable part subsides exponentially and the irreparable part accumulates in proportion to the dose. The sum of the reparable and irreparable parts accounts for the life shortening process at any given time. This process appears to some observers to be akin to premature ageing.

GENERAL CONCLUSIONS

(I) LETHAL ACTIONS

(a) LD_{50} values

The measured air dose LD_{50} values of the adult large domestic animal varies between 250r and 700r whereas the estimated mid-

line absorbed dose is approximately 250r. This constancy of the absorbed dose as compared with the air dose is probably due to the fact that large animals provide within the large mass of their bodies a constant maximum radiation scatter.

(b) **Dose, Time and Fractionation**

(i) Uniform doses of radiation produce greater acute effects if delivered quickly over minutes or hours than if delivered slowly over days or weeks.

(ii) When administered in a series of fractions instead of as a single exposure, a given dose of radiation produces less injurious biological effects.

(iii) The overall exposure time determines acute deaths. Animals which survive the acute effects, or have been exposed to radiation without showing acute effects, may nevertheless develop any of a large number of late effects.

(c) **Quality and RBE**

Quality of radiation is closely correlated with dose distribution within the animal body. This effects lethality and other biological responses which give rise to differing values of effectiveness (RBE) between different kinds of radiation of varying beam energies.

(d) **Mechanism of Death**

The mechanism of death after whole body irradiation is still obscure. The changes in the blood are probably a secondary consequence to a prime exhaustion of somatic stem cells and individual radiosensitivity probably ultimately depends on this fact. Bacterial invasion of the tissues from an injured gut combined with a leucopenia and cessation of antibody production contributes to, but is itself not the prime cause of death.

(II) CLINICAL SYNDROME AND PATHOLOGICAL ASPECTS

(a) Knowledge about reactions of ionizing radiations within cells is still uncertain. By studying the effects of total body irradiation on the whole animal the clinical picture can be more accurately assessed.

(b) The most radiosensitive cells in the adult mammal are the lymphocyte and the spermatogonia. A drop in the lymphocyte count of the circulating blood is the earliest, most marked, and most consistent observation. On the contrary, the lymphocyte and the lymphopoietic tissue show a remarkable ability of recovery as a reaction to radiation injury. The polymorphonuclear leucocyte is less sensitive and recovers less adequately than the lymphocyte. The thrombocytopenia is generally considered to be the prime cause of the haemorrhagic phase. The occurrence of haemorrhage and the prolonged life span of the animal erythrocyte combine to produce a late anaemia.

(c) There are three general types of acute radiation syndrome in animals, *viz* :

(i) The Cerebral Form, which is produced by very high doses of radiation and is always fatal. Transient cerebral signs often occur at lower doses. Two main groups of clinical signs may be manifested.

Apathy, which is probably due to non-inflammatory reaction in the CNS.

Tremors and ataxia, which is probably due to inflammatory processes in the CNS with degeneration of cerebellar cells.

(ii) The Gastro-Intestinal Form occurs when the doses are smaller and there is denudation of the intestinal epithelium leading to massive fluid loss and loss of electrolytes. Death occurs quickly unless anti-shock therapy is substituted. Most of the histopathological changes seen in the bowel are non-specific combined with normal non-specific attempts at repair. There are few, if any, effects specifically due to radiation damage.

(iii) The Haematopoietic Form, occurs at MLD Doses. The prodromal sign of apathy and anorexia is followed by a clinical phase showing no symptoms during which however there occurs a rapid degeneration of radiosensitive tissues. This may arise from circulating toxins akin to histamine. The characteristic pancytopenia and haemorrhagic diathesis give rise to intestinal ulcerations and dys function producing clinical signs 2 to 4 weeks after exposure very similar to the gastro-intestinal form (ii).

(d) Although the general pattern of the acute radiation syndrome, in large domestic animals is similar individual species differences are detectable. For example, the dog has clinical signs not unlike the human whereas the burro is inconsistent, unpredictable and often anomalous in its reactions. The ruminants are, by and large, similar in character to one another; the large colon in the ruminant retains its capacity as a water absorber and thus prevents or delays fluid and electrolyte loss. This fact probably accounts for the inconsistent and often prolonged survival times noted.

(e) Recovery from early clinical signs does not signify complete recovery. In this sense the acute radiation syndrome differs from conventional injury of the tissues for there is, after radiation injury, a latent period during which undetectable histochemical and histological changes occur within the tissue until the signs become clinically detectable. These latter changes are irreparable and are carried by the animal until death.

(f) No specific clinical sign is pathognomonic of the radiation syndrome in animals. A combination of a characteristic symptom (*e.g.* apathy) with a characteristic laboratory finding (*e.g.* clotting defect in the blood) coupled with characteristic morbid anatomical and histopathological findings (*e.g.* widespread haemorrhages/nuclear pleomorphism) is suspicious.

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SECTION II

X IRRADIATION OF GOATS

Introduction

A review of the radiation syndrome in large domestic animals was undertaken as a preliminary study (SECTION I) and as a result of this it was decided to pursue a generalised research programme on the effects of X-irradiation on goats.

The objectives of the research study were to discern and describe the clinical course in goats of the biological effects of median lethal doses of X-irradiation. More specifically the intention was to discover the physiological and pathological response of the British domestic goat, maintained under laboratory conditions, to total body irradiation with 250 kV X-rays.

It was hoped to establish with the very limited number of animals used an approximate LD50 dose and to estimate generally the effectiveness of X-irradiation on an intact large mammal comparable in body mass to man. As a final objective the data might aid military, medical and veterinary authorities in formulating future research and civil defence policies.

I EXPERIMENTAL ANIMALS

a) Choice of Animal

The goat was chosen because when adult it is approximately the same bulk as the average grown man. In addition, other points in its favour were i) it is a hardy, tractable and trainable animal and ii) its body fluids and tissues could be obtained easily. The disadvantages of its being a ruminant herbivore with a peculiar characteristic anatomy and physiology were considered on balance not to be as important as the above mentioned advantages.

b) Breeds

(Photos 1 to 5)

There are many breeds of goats in the world but only four are of importance in Great Britain. All four breeds were represented in the colony built up at Harwell. These were :-

i) British Saanen, which is white in colour

ii) British Toggenburg, with a coat varying in colour from dark chocolate to pale fawn with white markings on the face and hindquarters.

iii) British Alpine, a short-coated goat black in colour with white markings

iv) Anglo-Nubian, a brownish short-coated goat with pendulous ears and a Roman nose

There were horned and hornless individuals in each breed. A fifth category comprising hybrids was placed under the general term "British".

Throughout the text the following abbreviations have been used to indicate breed, viz :

BS . . . . . British Saanen

BT . . . . . British Toggenburg

BA . . . . . British Alpine

AN . . . . . Anglo-Nubian

BTS) . . . . British of Toggenburg type

BSA) . . . . British of Alpine type

The largest total number of goats at any one time in the colony was 119, made up as follows:

	Adults	Goatlings
Female	48	1
Male	6	58
Castrated Males	6	

Table I Distribution of Goats by Breeds and Sex.

Sex	BS	BA	BT	AN	BRITISH	Total
Male	43	10	7	2	2	64
Female	29	3	9	1	7	49
Cast. M.	4	2	-	-	-	6
	76	15	16	3	9	119

c) Age

The age of a goat can be told fairly accurately by an examination of its incisor teeth. Table II shows the distribution of the goats of the colony by sex and age.

Table II Distribution of Goats by Age and Sex.

Sex	Total	Under 2 yrs	2 - 3 yrs	3 - 4 yrs	4 - 5 yrs	5 - 6 yrs	6 or over yrs
Male	64	58	1	1	2	1	1
Female	49	1	2	3	18	16	9
Cast. M.	6	-	-	1	5	-	-
	119	59	3	5	25	17	10

d) Horns

Some goats grow horns naturally, and others, equally naturally, have none. There is evidence that hermaphrodism and hornlessness are closely associated. Table III shows the distribution of the Goats to hornlessness.

Table III Distribution of Goats by Breed Sex and Hornlessness.

Sex	BS	BA	BT	AN	BRITISH	Total	Percent.
Male	26	4	2	1	1	34	53
Female	14	3	6	1	4	28	57
Cast.M.	3	1	-	-	-	4	66
	43	8	8	2	5	66	55½

THE GOAT COLONY

Photo 1. A Section of the Goat Colony

Photo 2. British Alpine and British Saanen (Females)

Photo 3. British Toggenburg (Male)

Photo 4. Anglo-Nubian (Male)

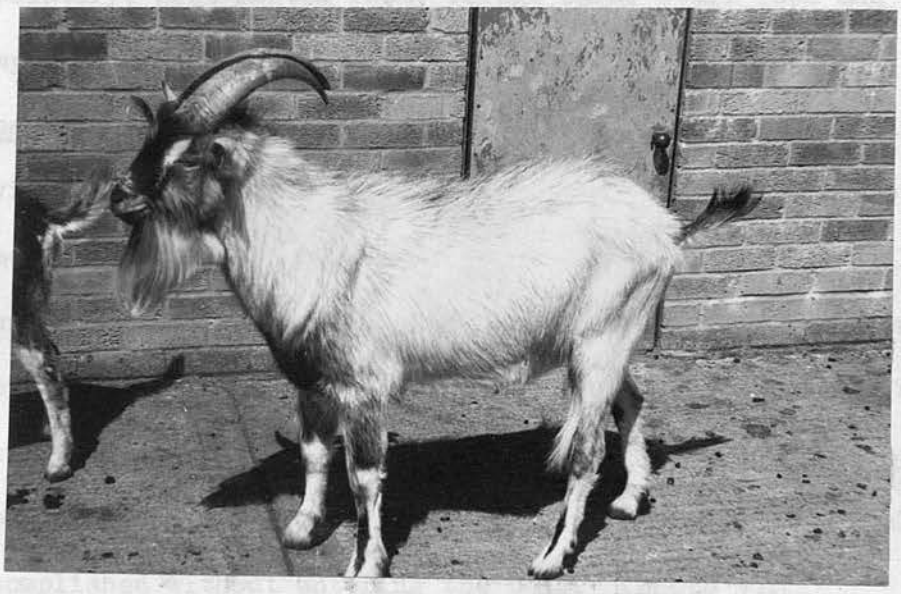


Photo 5. British (Male) Toggenburg Type

Photo 6. Goat Farm : Exercise Yard

e) Care and Management of Goats

i) The Goat Farm

The goats were housed in a specially constructed building with concrete floors and yards (Photos 6 to 8).

The building consisted essentially of two wings, one of which held the male goats while the other housed the females. Each wing consisted of ten bays and each bay contained three pens. The total number of pens was sixty and each pen could accommodate two adult goats. A small quarantine wing, of two bays and six pens, was also provided. A laboratory, post-mortem room, forage barn, staff room and office completed the quadrangle (Fig.1).

Pens Each pen was approximately 8 feet long and 5 feet wide; it was divided from its neighbour by a tubular metal framework to which removeable metal-sheet partitions could be attached at will. A constant supply of water was available to each goat from water bowls provided with automatic ball cocks. Feeding troughs were affixed to portholes in the gate to each pen and hay-racks of tubular metal were provided conveniently at goat head level. All feeding could thus be accomplished without entering the pens. (Photo 9).

Yards. Access doors at a low level led from each bay to a concrete inner exercise yard. An outer exercise yard joined the manure tip. Lysol foot baths were located at all entrances to the goat farm as a precaution against needless introduction of infection.

ii) Management

Generally speaking, the system of management adopted was to ensure a warm, dry, well-ventilated and adequately lighted goat house coupled with a simple routine of feeding good-quality food with sufficient free exercise.

The Daily Routine varied from time to time according to the season and prevailing weather conditions but consisted essentially of turning out the goats into the yards (males into one and females into the other of course) in the mornings whilst the pens were "mucked out" and "hayed-up".. In the afternoons the goats were returned to their pens and fed their ration of concentrate food whilst the yards were brushed down and hosed thoroughly.

Feeding. The daily ration for adult goats was as follows :

i) ½ to 1 lb. concentrates, which consisted of

bruised oats	..... 1 part
flaked maize	..... 1 part
bran	..... 1 part
linseed cake	..... 1 part

ii) 2 to 5 lbs. green food, such as cabbage, kale, carrots swedes or mangles

iii) 5 lbs. hay, of the best quality usually a good "seeds" mixture including some clover or a rough meadow hay.

When the kids were very young they were fed a special pelleted supplementary concentrate food which was fully mineralised with added vitamins A and D3 and with the following composition:

Oil	3½%
Protein	18%
Fibre	6%
Starch Equivalent	67
Protein Equivalent	14.5

In each pen there was a mineralised salt lick fixed to the wall; and in the late autumn and mid-winter all goats received injections of vitamin D concentrate.

Watering. Each pen was provided with a water bowl in which the water was kept at a constant level by an automatic ball-cock. Thus all goats normally had access ad libitum to water.

Bedding. Only best wheat straw, usually long and not the "combine" type, was used as bedding.

iii) Isolation and Quarantine.

All goats on arrival were isolated in the quarantine wing for fourteen days. During this period they were numbered and a full description entered into a clinical history sheet which was kept for each goat. Faeces was examined for helminths eggs; urine was usually clinically analysed; blood was taken for haematological and immunological examinations and the intradermal tuberculin test and the johnin test for tuberculosis and johnes disease respectively were carried out. All goats were inoculated with enterotoxaemia vaccine. After the quarantine period the goats passed into the male or female wing of the main goat house depending on sex.

Routine steaming and disinfection of all removeable metal troughs and equipment was carried out weekly if possible. All entrances and exits of the goat farm were barred by "lysol" foot baths to help to reduce the introduction of disease. (Photo 10).

Office
staff
store
FOOD
barn
LAB.
P.M.
Entrance
females
YARD
EXERCISE
males
YARD
Quarantine

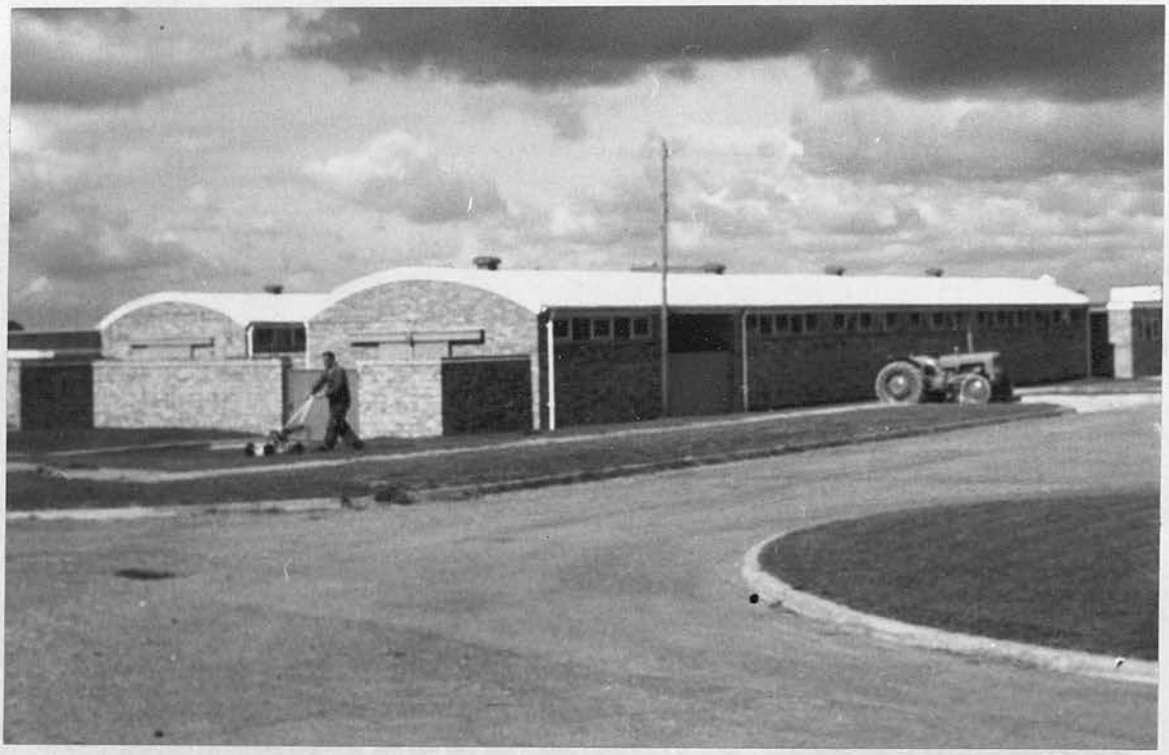


Photo 7. General View of Goat Farm, MRC, Harwell

Photo 8. Female Goat Wing

Photo 9. Goat Pens

Photo 10. Quarantine and Isolation Wing.

II OBSERVATIONS AND PROCEDURES

Observations were made on the whole colony of goats, which numbered 119 at its height, to obtain certain normal control values but it was often found necessary to express experimental results both on colony control and individual control goats.

Twenty goats were used in the irradiation experiment. They were mature animals of various breeds, sex and age as detailed in the following table :

Table IV

Goat No.	Markings	Breed	Age (yrs)	Body Wt. (lbs)	Horns (h)
Females (11)					
F 14	White	BS	3½	96	h
F 20	Wh. with Bl. spots	BSA	5	136	-
F 24	Ditto	BSA	3½	94	-
F 27	White	BS	6	111	h
F 30	White	BS	5½	165	h
F 31	Brn. & Bl.	AN	6½	134	-
F 40	White	BS	4½	108	-
C175	Bl. & Wh.	BA	4	119	-
C200	Fawn	BT	4½	120	-
W302	White	BS	4½	153	-
W316	Bl. & Wh.	BA	4½	141	-
Males (4)					
M 10	White	BS	4	190	-
M 9	Bl. & Wh.	BA	3	174	-
M 3	White	BS	6½	190	-
M 1	Fawn & Wh.	BT	4	174	-
Castrated Males (5)					
C118	White	BS	4	235	h
C229	White	BS	4	210	-
C234	Bl. & Wh.	BA	4	164	h
C240	Bl. & Wh.	BA	4	165	-
C315	White	BS	4	142	h

A. Clinical Examinations were carried out at variable regular periods

i) rectal temperatures initially were taken three times daily at 9 am, mid-day and 5 pm. A ½-minute clinical thermometer was used for recording temperatures which were read after 1 minute. Rototherm thermometers were also used but were found not to be so satisfactory.

ii) pulse and heart rates were examined at variable periods The pulse was taken by manual palpation of the femoral artery or coccygeal artery and the heart rate was recorded by stethoscope.

iii) respiratory frequency was recorded by visual examination of the flanks and nostril movements, occasionally by auscultation.

iv) water consumption was measured when required by using specially marked buckets.

v) food consumption was measured roughly by deducting weight remaining in the trough from the normal ration scale.

B. Body Weight

An ordinary pig-weighing machine was used to weigh the goats. A specially adapted indicator scale was added to enable the weighing of animals up to 250 lbs. (Photo 11).

Weights of all goats were normally determined initially at weekly intervals as a routine measure. Animals became accustomed very quickly to the weighing routine and the procedure became simple. Most goats would enter the weigh-cage as soon as the gate was opened and would leave quite voluntarily after weighing.

C. Restraint

Goats were effectively restrained for examination by the use of leather neck straps or head collars. A special tubular metal cradle was employed quite frequently to immobilise animals for clinical examinations and injections. The apparatus was very heavy and rigidly immoveable when being used as the tubular metal had been filled with lead.

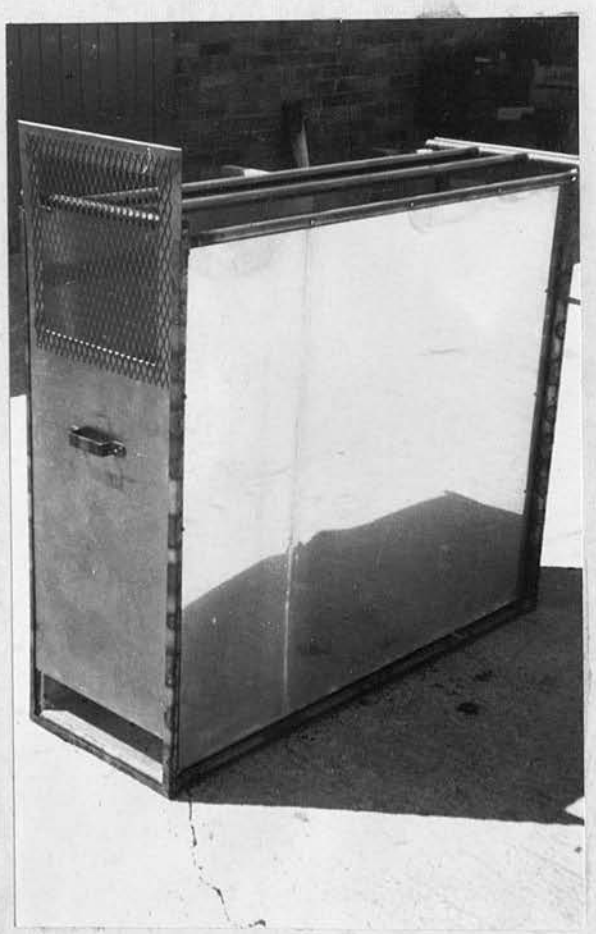


Photo 13. Aluminium Goat Cage

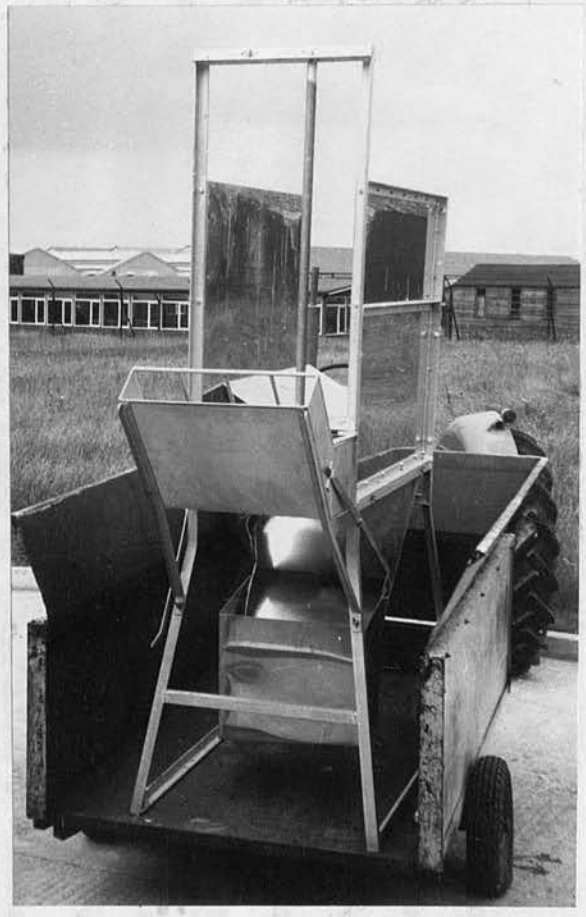


Photo 12. Metabolism Cage

Photo 11. Goat Weighing Machine

A "metabolism cage" (photo 12) modelled on one loaned from the National Institute for Research in Dairying at Reading was used occasionally to calculate urine and faeces output; it consisted essentially of a metal compartment to fit the largest goat and was 3 to 4 feet off the ground. Faeces pellets fell through a wire floor on to a metal platform suitably inclined and were collected in polythene buckets. Urine flowed down the same metal panel to a separate channel leading to a polythene collecting vessel. In front there was a removeable manger and water bucket with a hayrack, all suitably and conveniently placed for the confined goat to reach easily. Restraint was achieved by a neck cradle consisting of two moveable tubular metal rods.

Restraint by Sedation

The requirement was for immobilisation of the goat for a period of about two hours. All volatile anaesthetic agents were therefore ruled out. Studies were carried out on certain non-volatile agents the principal ones being sodium pentobarbitone, thiopentone sodium and promazine hydrochloride. Other more extensive studies were carried out with a new psychotropic drug known as phencyclidine. This product especially when combined with the previously mentioned agents proved most satisfactory. All these drugs were administered either by intravenous or intramuscular injection. As phencyclidine was eventually used as the basic induction agent its effects on goats and on the radiosensitivity of mice was given some consideration (see Appendix Wilkins 1961, 1962, 1964).
The procedures adopted as a routine measure kept the goat quietly recumbent, often asleep, with minimal salivation for periods up to 3 hours.

D. Parasites

a) Enumeration of Helminth Eggs.

A modification of the techniques described by Stoll (1923), Lane (1928) and Gordon & Whitlock (1939) was employed. The objective was to determine per gramme of faeces the number of helminth eggs

and protozoan cysts of the more common gastro-intestinal parasites of goats.

Method

i) 3 grammes faeces weighed out.

ii) Placed in glass bottle containing glass balls.

iii) 42 ml. saturated salt solution added.

iv) Bottle shaken untill all faeces homogeneously broken down.

v) Poured into sieve, collecting fluid in a glass bowl.

vi) Both chambers of the egg-counting slide were filled with well-mixed fluid by means of a Pasteur pipette.

vii) Slide examined systematically under 2/3 and 1/6 objectives. All eggs and oocysts were counted in two chambers. The average was then obtained.

viii) Average number of eggs or oocysts multipled by 100 gave the total number of eggs oocysts per gramme.

Method of Calculation

The original volume was 45 ml i.e. 3 gms faeces plus 42 ml. saturated salt solution.

The volume of the lined centimetre square of the Gordon-Whitlock slide was 0.15 ml.

Vol. on slide is 0.15/45 which is 1/300.

Each egg/oocyst counted represented 300 per 3 grammes which is equivalent to 100 per gramme.

By this simple routine method a general idea of the helminth and/or protozoan burden carried by each goat could be determined and its possible effects on the clinical syndrome produced by irradiation assessed.

b) Examination for Presence of Larvae

i) A piece of rubber tubing carrying an adjustable clip was fixed to the stem of a large glass filter funnel.

ii) A piece of wire gauze was placed in the mouth of the funnel.

iii) A small quantity of macerated faeces was placed on the wire gauze.

iv) Sufficient water was added to cover the faeces. The temperature of the water was about 20°C (68°F).

v) The apparatus was allowed to stand for three hours in a warm atmosphere. Larvae passed through the wire sieve to the bottom of the stem of the funnel.

vi) Examination and identification of the larvae was done by examining a drop of fluid under a microscope.

c) Recognition of helminth eggs, larvae and protozoan cocysts in faeces of goats.

Eggs : i) "Strongyloid" spp: were all ovoid in shape, small in size (80 x 40μ) with a segmenting embryo. The specific identity of many of the strongyloid eggs was not attempted as this would have required time-consuming specialized techniques as those of Kates and Shorb (1943) or Cunliffe and Crofton (1953).

ii) Nematodirus filicollis: very large in size, ovoid in shape with an early segmenting embryo. (200 x 70μ).

iii) Trichuris ovis: were very easily distinguishable. They were barrel-shaped, small in size (75 x 35μ) with a plug at each end and an unsegmented embryo.

iv) Moniezia expansa: (Tapeworm): a small (56μ)

triangular shaped coarse-looking egg containing a hexacanth embryo.

v) Fasciola hepatica: (Liver Fluke): were large (140 x 80 μ) yellow brown ovoid eggs with a faint plug at one end.

Oocysts: vi) Coccidia: were very small (10 to 30 μ), refractile eggs either ovoid globular or ellipsoidal in shape. There is very little information to be found on coccidial infections of goats. A number of species has been described by Christensen (1938) and Honess (1942).

Larvae: vii) Lungworms:

a) Dictyocaulus filaria were larger (560 μ) with a small button at the head end.

b) Muellerius Capillaris were smaller (250 μ) with a spine at the tail end.

Care was always needed to distinguish eggs from air bubbles, pollen grains and fungi spores. Larvae had to be distinguished from hairs, which lacked an intestine and were coarse in appearance.

d) Interpretation of Egg Counts

A count of 500 and over eggs per gramme was considered to be significant and to warrant treatment.

Treatment: initially phenothiazine was employed at a dosage of 5 gms. for kids and goatlings; adults received 10 gms. The dose was administered in the form of a draught per os.

The method of treatment was later replaced by the subcutaneous injection of 2-(B-methoxyethyl) pyridine, better known as methyridine or "promintic". The dose used was 1 ml. per 10 lb. body weight. This treatment was very effective but often caused local abscesses at the sites of injection. The intraperitoneal route for the injections was later adopted in isolated cases with completely satisfactory results. Two doses at a months

interval cleared the goats of mature as well as immature helminths. Reinfection took place mainly through the faeces (in which the larvae developed, reached the coat of other goats and were then licked off) and the unavoidable feeding of previously contaminated hay.

Coccidiosis was treated with sulphamezathine in a suspended powder given as a draught per os. A first dose of 0.2 gm/kg body weight was followed by half the initial dose given daily for five days.

E. Urine Analysis

A sufficiently comprehensive and accurate system of urine analysis was adopted for routine purposes without being unduly laborious.

Collection of Urine

Female Goats : It was a simple matter to collect urine of female goats. Usually it was sufficient to rustle the bedding or throw some straw on the floor nearby for the goat to squat down to urinate when it could easily be collected in a clean glass vessel! Difficult cases were catheterized: others were placed in the metabolism cage.

Male Goats : owing to the possession of a delicate processus urethrae at the end of the glans penis it is impossible to pass the catheter in a male goat. It was difficult collecting urine from a male goat other than by using a metabolism cage.

Urine Analysis

a) Physical Characteristics

(i) Inspection: The presence and colour of any deposit was noted and the urine classified as turbid or limpid.

(ii) Reaction was conveniently determined by dipping a piece of litmus paper into the urine. Acid turns litmus red, alkali turns litmus blue.

(iii) Specific Gravity at 15°C was estimated by floating a hydrometer (urinometer) with a range from 1.000 to 1.060 in the urine in a cylinder. When only a small quantity of urine was available correction was made for any dilution necessary by using the formula

$$S.G. = \frac{y(a+b) - b}{a}$$ where

y = specific gravity observed in dilute urine

a = mls of urine used

b = mls of distilled water added

A correction was made for temperature as follows:-

ADD 0.001 for every 3°C above 15°C

SUBTRACT 0.001 for every 3°C below 15°C

b) Chemical Tests for Pathological Constituents

General : Each urine was subjected to routine chemical tests normally used in clinical pathology laboratories and also to tablet and paper tests.

(i) Estimation of Reducing Substances was always carried out on fresh urine or boiled fresh urine.

(a) Benedicts Test:

To 2.5 ml Benedicts qualitative reagent four drops of urine were added and boiled for two minutes. A positive test for reducing substances was considered to have been given when a red, yellow or green colour developed with a colour precipitate on standing. A green colour without a precipitate was not a positive reaction. The degree and speed of reduction was a rough guide to the amount of reducing substances present.

b) "Clinistix" Reagent Strips for the Detection of Glucose

A strip of stiff paper with one end impregnanted with a mixture containing glucose oxidase, a vegetable peroxidase and orthotolidine was dipped into a sample of urine and then exposed to the air. If glucose was present a BLUE colour developed. The test is highly specific for glucose and is extremely sensitive, capable of detecting as little as 0.1% of glucose (Hunt et al 1956).

c) "Clinistix" Tablets for Detection and Estimation of Reducing Substances

A tablet containing a mixture of copper sulphate, sodium hydroxide, citric acid and sodium bicarbonate was dropped into a small test tube containing 5 drops of goats urine diluted with 10 drops of water. A marked generation of heat and effervescence occurred and if reducing substances were present there was a change in colour from green to orange, depending on the concentration of these substances. Assessment of the concentration was effected by comparison with a standard colour chart.

(ii) Detection and Estimation of Protein

a) Heat Plus Acidulation Coagulation Test

Tests for protein can only be performed satisfactorily if the urine is clear : turbid specimens were therefore filtered and rendered just acid to litmus with 3% acetic acid.

A one inch column of urine in a test tube was inclined over a small bunsen flame. The top layer was boiled. A turbidity indicated the presence of either protein or phosphates.

Two drops of 3% acetic acid were then added.

Any remaining turbidity indicated protein.

b) Salicyl-Sulphonic Acid Test.

1 ml of a 20% aqueous solution of salicyl-sulphonic acid was placed in a test-tube and an equal quantity of urine was layered on top. If protein was present in amounts greater than 5 mg per 100 ml urine a white ring developed at the junction of the two fluids. Gentle mixing produced a white opalescence which persisted.

c) "Albustix" Strips.

A paper stick with one end impregnated with tetra-bromphenol-blue plus a citrate buffer was dipped into a sample of urine. After removal should the end develop a greenish-blue colour almost immediately it indicated the presence of protein. The intensity of colour produced was compared with a colour scale which indicated the amount of protein present.

This test is considered specific for the common urinary proteins and Bence-Jones protein although highly buffered alkaline urines may give weak positive reactions. The test is capable of detecting a concentration of 5 mg of protein per 100 ml urine and is thus comparable in sensitivity to the salicyl-sulphonic acid test but is not quite as sensitive as the heat test.

(Baron and Oakley, 1957).

iii) Detection and Estimation of Blood Pigment.

All routine chemical tests for blood fail to differentiate between haemoglobinuria and myoglobinuria. The Spectroscopic Test was carried out as a routine measure to differentiate myoglobin from oxyhaemoglobin.

a) Benzidine Test.

2 ml of glacial acetic acid was saturated with 4 mgms of benzidine base in a test tube; to this was added 3 drops of hydrogen peroxide (20 vols) and 2 ml of urine and mixed. The appearance of a blue colour indicated the presence of blood pigment. The test was then repeated and the urine was added drop by drop; an approximate indication of the degree of concentration of blood pigment in the urine was given in this roughly quantitative method.

b) "Occultest" Tablet Test.

This diagnostic test is based upon the oxidation of O-tolidine by the peroxidase activity of haemoglobin which results in the development of a blue colour. A drop of well-mixed uncentrifuged urine is placed on to the centre of a small square of filter paper. The "Occultest" Tablet, which contains O-tolidine and strontium peroxide as the main active ingredients, was placed in the centre of this moistened area and 2 drops of water placed on the tablet. A positive result was shown within 2 minutes by the appearance of diffuse area of blue colour surrounding the tablet. Absence of a blue colour within 2 minutes indicated a negative result.

This test will detect one part of blood in 100,000 parts of urine equivalent to approximately 50 red blood cells per cubic mm.

c) "Haematest" Tablet Test

Faeces was routinely examined for blood pigment using the benzidine test and the "Haematest" Tablet.

A small amount of faeces was smeared on to a square of filter paper and a tablet was placed in the centre of this. Two drops of water were added on top of the tablet. A positive result was indicated by a blue colour developing around the tablet within 2 minutes. This test will detect 1 part of blood in 20,000 parts of faeces.

General.

All blood tests are very sensitive and great care was taken to use only clean receptacles for collection and manipulation. Only distilled water was used in all cases. A blank control was used in every case.

Microscopic examination of the urinary deposits showed red blood cells if a haematuria was present.

(iv) Ketones

a) Rothera's Test (Modified).

Rothera's Test and the "Acetest" Tablet Test were carried out as routine measures.

5 ml of urine were carefully run into a test tube containing approximately half an inch of powdered Rotheras Modified Reagent. Without mixing, the tube was set aside for five minutes. The development of a purple colour at the junction was taken as indicating the presence of ketone bodies (Acetone or diacetic acid). Rotheras Reagent (Modified) consisted of

Ammonium Sulphate	-	100 grammes	All finely powdered and mixed.
Sodium Carbonate (Anhydrous)	-	50 grammes	
Sodium Nitroprusside	-	3 grammes	

(v) Bile and Bile Pigments.

a) Bile Salts

Hay's Test for bile salts was carried out by sprinkling a

few grains of flowers of sulphur on to urine in a test tube. Bile salts, if present, lowered the surface tension and the sulphur would sink.

b) Bile Pigments.

There is no satisfactory test for the bile pigment in goats since their urine gives confusing colour reactions. The Ictotest Tablet was used in every case.

"Ictotest" Tablet Test.

A tablet containing a diazo dye, salicyl sulphonic acid, sodium bicarbonate and boric acid was placed in the centre of an area of a cellulose asbestos mat moistened with 5 drops of urine. Two drops of water were placed on to the tablet. If bilirubin was present, a bluish-purple colour developed on the mat surrounding the tablet within 30 seconds. (Hoe and Wilkinson, 1958).

c) Microscopical Examination

Deposits

The urine remaining was centrifuged and a wet smear on a slide was made from a drop of the deposit. The preparation was examined with the aperture of the sub-stage condenser nearly closed. Examination was carried out using 2/3 inch and 1/6 inch objective. The deposits were classified as follows :-

a) Cells:- RBCs, WBCs, or Epithelial

b) Casts:- Hyaline, Granular or Epithelial

c) Crystals

d) Amorphous Material

F. Intradermal Tuberculin and Johnin Tests.

The single intradermal comparative test was applied to all goats with protein-precipitated-derivative (P.P.D) tuberculin and johnin manufactured by the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory at Weybridge in Surrey.

Composition of the Tuberculin and Johnin

Two kinds of tuberculin were used viz: mammalian and avian, and one of johnin.

i) P.P.D. Tuberculin (Mammalian) . . . . . 2.0 mg per ml
Phenol 0.5%
Glycerol 10%
100,000 international units per ml.

ii) P.P.D. Tuberculin (Avian) . . . . . 0.5 mg per ml
Phenol 0.5%
Glycerol 10%
25,000 international units per ml.

iii) P.P.D. Johnin . . . . . 0.5 mg per ml
Phenol 0.5%
Glycerol 10%

The tuberculin and johnin were stored in the refrigerator and were used immediately after the vial was opened. Syringes and needles were freshly steam sterilised before use. All three tests were performed simultaneously in the same animal and there was only one post-injection observation at the 72nd hour.

Site:

Mammalain Test : middle third of RIGHT side of neck

Avian Test : " " " LEFT " " "

Johnin Test : lower third of CENTRE of neck

Preparation of Site :

At the selected sites small areas of hair about the size of a half-crown were clipped close and cleaned with a pledget of cotton wool soaked in surgical spirit.

Measurement of Skin :

A fold of skin at the site was taken between the forefinger and thumb and the double thickness measured in millimetres with calipers.

Intradermal Injections :

A small dental needle, with bevelled edge outwards, was inserted obliquely into the fold of skin and 0.1 ml of the product injected. Considerable pressure on the plunger was always necessary. A small pea-like swelling was the result in a correctly performed injection.

Interpretation of Results :

Tests were read at the 72nd hour post-injection. Skin measurements were again recorded, and the nature of the swelling, if any, noted. Any swelling with surrounding oedema was regarded as positive. In the absence of oedema, swellings up to 4 mm. were regarded as negative. swellings up to 5 to 6 mm were regarded as doubtful
swellings over 6 mm were regarded as positive.
For the purpose of describing reactions, the following abbreviations were used: S.O. - slight oedema. D.O. - diffuse oedema.
E.O. - extensive oedema. C. - circumscribed.

G. The Complement Fixation Test for Johnes Disease was carried out only once in all adult goats on entry to the colony.

Method

The method was based on that used at the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory at Weybridge. Goat sera were tested in one dilution only.

The serum was taken off the clot, freed from red blood cells by centrifugation and diluted to 1 in 5 with 0.5% phenol saline. Immediately prior to testing, 0.4 ml was taken and inactivated in a water bath at 56 degrees centigrade for half-hour.

The antigen. After cooling, the inactivated serum was mixed with 0.4 ml antigen, which was a suspension of Mycobacterium johnei in 0.5% phenol saline. The antigen was supplied by the Central Veterinary Laboratory at Weybridge.

The mixture was allowed to stand at room temperature for one hour and it was then centrifuged for a few minutes. The supernatant fluid was discarded. The tubes were then stored overnight in the refrigerator.

The Complement. The organisms in the deposit were then re-suspended in 0.8 ml of 0.5% phenol saline and 0.4 ml of guinea pig complement added. The mixture was allowed to stand for one hour at room temperature for fixation.

The indicator system consisted of equal parts of a 2% suspension of sheep erythrocytes and equine antisheep haemolysin. 0.8 ml of the indicator system was added to the mixture in the tubes. The tubes were then placed in a water bath at 37 degrees centigrade for half-hour.

Reading. The tubes were centrifuged to throw down the organisms and residual erythrocytes to facilitate reading of results. Reading was done against an illuminated screen and the degree of haemolysis in each tube was recorded. Readings were recorded as follows:

1. Negative . . . . . over 50% to 100% haemolysis.
2. Doubtful . . . . . over 12½% to 50% haemolysis.
3. Weak Positive . . Trace to 12½% haemolysis.
4. Positive . . . . . no haemolysis.

H. Haematological Methods.

1. Blood Collection. Blood samples were taken from the jugular vein with a 5 ml or a 10 ml siliconised hypodermic syringe fitted with a sharp needle. Platelet dilutions and blood smears were made as quickly as possible from the freshly-drawn blood. The remainder of the blood in the syringe was squirted gently into a "Camlab" polythene bottle containing dilithium sequestrene as anti-coagulant. This was shaken by hand for a few minutes and then placed in a "Matburn" Blood Cell Suspension Mixer until required for examination.

2. Blood Smears for Differential Counts. Three smears were made from each blood sample in the conventional way. One was stained with Leishmans stain, another with Jenner-Giemsa and the third with May-Grunwald-Giemsa respectively.

Staining : Blood films were dried in air and then covered with absolute methanol to fix. Subsequently, the procedure followed was :

Jenner-Giemsa and May-Grunwald-Giemsa.

i) films were covered with 15 drops of stain and were
ii) diluted with 30 drops of buffered distilled water. (pH 6.8)
iii) This was allowed to act for 5 minutes, after which
iv) it was washed off with distilled water.
v) A freshly-prepared dilute Giemsa made by adding 1 drop of Giemsa stain to 1 ml of distilled water was used as a counterstain. This was allowed to act for 12 minutes.

Leishmans Stain

The dried films were covered with 15 drops of stain and were diluted with 30 drops of buffered distilled water (pH 6.8) and allowed to stain for 10 minutes.

After drying out on filter paper the stained smears were examined under the oil immersionl/12th lens objective and 200 leucocytes were classified in every case whenever possible.

3. Counting of Red Blood Cells. 20 c.mm (0.02 ml) of well-mixed blood was added to 12 ml of the diluting fluid in a screw-top McCartney bottle. The diluting fluid was a solution of 1% formalin in 3% sodium citrate. The McCartney bottle was placed in a Matburn Blood Cell Suspension Mixer and allowed to spin for at least 30 minutes. This mixing gave a dilution of 1 in 600.

The number of red blood cells in five squares (i.e. 80 small squares) were counted in an improved Neubauer Haematocytometer and the result was multiplied by 30,000 to give the number of red blood cells per cubic millimetre.

Not less than 500 cells were counted on each side of the haematocytometer and the average taken as the reading.

Counting of White Blood Cells. 0.1 ml of well-mixed blood were added to 1.9 ml of a 1% glacial acetic acid solution tinged with gentian violet. This gave a dilution of 1 in 20. The mixture was spun in a Matburn Mixer for at least 10 minutes before charging the haematocytometer.

The white blood cells in four large aquares were counted on each side of the Neubauer haematocytometer and the average was multiplied by 50 to give the number of white blood cells per cubic mm.

Counting of Platelets. A modified method of Brecher & Cronkite (1950) was used.

i) 20 c.mm (0.02 ml) of freshly-drawn blood was added to 2 ml. fresh-filtered diluting fluid producing a 1 in 100 solution. The diluting fluid consisted of a 1% solution of ammonium oxalate which was stored in the refrigerator until required for use.

ii) The mixture was spun in a Matburn Mixer for 15 minutes.

iii) Both sides of a plain Neubauer Haematocytometer were filled. A thin $\frac{7}{8}$" x $\frac{7}{8}$" microslide coverslip was used.

iv) The haematocytometer was then placed in a petri-dish containing a piece of moistened blotting paper and left on the bench for 15 minutes.

v) All the platelets in five squares (i.e. 80 small squares) were counted a phase contrast microscope being used. Both sides of the chamber were counted and the total number divided by two to give the average which was multiplied by 5,000 to give the number of platelets per cubic millimetre.

4. Estimation of Haemoglobin (Hb) The haemoglobin content was estimated as alkaline haematin in a MRC Grey Wedge Photometer (Wootton et al, 1948).

i) 20 c.mm (0.02 ml) of well-mixed blood was added to 4 ml of N/150 Ammonium hydroxide in a test-tube.

ii) After mixing thoroughly by inverting several times the mixture was matched in the photometer a yellow-green filter (Ilford No. 625) being used.

iii) The haemoglobin was read as a percentage and the result was expressed as grammes of haemoglobin in 100 ml of blood, 100% being equivalent to 14.8 grammes per 100 ml blood.

5. Packed Cell Volume (PCV) or Haematocrit. This was estimated by employing the microhaematocrit method (McKinroy, 1954) and spinning for 10 minutes at 12,000 g.

6. Coagulation Time The simple method of Lee & White (1913) was adopted.

i) 1 ml of freshly-drawn blood was placed in a clean $2\frac{1}{2}$" x $\frac{3}{8}$" tube and a rubber bung inserted.

ii) The tube was kept in a water bath warmed to 37 degrees centigrade.

iii) Every 30 seconds the tube was inverted until a firm clot was formed.

iv) The time was then noted as the coagulation time. A control was used on each occasion.

7. Clot Retraction

v) The tube containing the clot was placed in an incubator at 37 degrees centigrade and examined for the presence of clot retraction at 1, 2, 4, 6 and 24 hours.

vi) The result was recorded as at 24 hours as follows :

50% and over retraction . . . . . GOOD

25% retraction . . . . . FAIR

5% to 10% retraction . . . . . POOR

No retraction . . . . . NIL

8. Estimation of the Fragility of Red Blood Cells. A method based on Creed's (1938) technique was employed. Osmotic fragility of rbcs was measured in three stages after standing for 24 hours as follows :

i) The first tube showing total haemolysis was noted and the NaCl concentration recorded as the "absolute corpuscular fragility", abbreviated to A.C.F.

ii) The tube showing 50% haemolysis was recorded as the "median corpuscular fragility" which was abbreviated to M.C.F.

iii) The first tube to show a trace of haemolysis was also noted and the NaCl concentration recorded as the "initial corpuscular fragility" abbreviated to I.C.F.

Concentrations of NaCl differed by steps of 0.04% and this allowed an in-between estimated reading of 0.02%.

Method

i) A stock 1% solution of sodium chloride (NaCl) in distilled water was prepared from chemically pure NaCl (Analar) which had been dried to constant weight by heating and allowed to cool in a desiccator.

ii) A series of 12 tubes, size $3\frac{1}{2}$" x $\frac{3}{8}$", was set up in a rack.

iii) With a teated dropping pipette, distilled water and 1% NaCl solution were mixed according to the following table :

Tube	1	2	3	4	5	6	7	8	9	10	11	12
Drops of Dist. Water	16	15	14	13	12	11	10	9	8	7	6	5
Drops of 1% NaCl	9	10	11	12	13	14	15	16	17	18	19	20
Conc.	.36	.4	.44	.48	.52	.56	.6	.64	.68	.72	.76	.80

iv) One drop of oxygenated blood was added to each tube and the tubes inverted ten times.

v) The tubes were then centrifuged at 4,000 rpm for 10 mins. to throw down non-haemolysed cells.

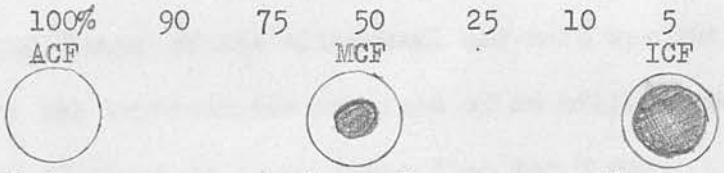
vi) They were subsequently placed in an incubator at 37 degrees centigrade and read at 2 hours and 24 hours. The first reading was done by assessing the degree of haemolysis by viewing the depth of pink or red colour in the supernatant. This reading has not been used.

vii) The recorded reading was done at 24 hours as follows :

a) An assessment of the degree of haemolysis was first made as before. This was then checked with a more easily measureable procedure as follows:

b) Each tube was inverted over a white enamelled dish.

c) The amount of haemolysis was estimated by recording the amount of "deposit" to be seen at the bottom of each tube :



Complete haemolysis no deposit ..... varying colour of supernatant & increase of deposit ..... Clear supernatant much deposit

Interpretation

viii) The first tube giving complete haemolysis was noted as the ACF.

The tube giving an estimated 50% haemolysis was recorded as the MCF.

The tube showing the first signs of haemolysis was put down as the ICF.

9. Vital Staining of cells was carried out as necessary by placing one (1) drop of alcoholic solution of 1% cresyl blue on a clean slide and adding one (1) drop of freshly-drawn blood. This was covered with a cover-slip and examined.

10. Erythrocytic Indices were calculated from the following formulae:

i) Mean Corpuscular Haemoglobin (M.C.H.) =

$$\frac{\text{Hb. in g/100ml x 10}}{\text{RBC count in millions/c.mm}}$$ in micro-micro-grammes ($\mu\mu$g)

ii) Mean Corpuscular Haemoglobin Concentration (M.C.H.C.) =

$$\frac{\text{Hb. in g/100ml x 10}}{\text{PCV as per cent}}$$ as a percentage (%)

iii) Mean Corpuscular Volume (M.C.V.) =

$$\frac{\text{PCV as per cent x 10}}{\text{RBC count in millions/c.mm.}}$$ in cubic microns (cu. μ)

11. Erythrocytic Sedimentation Rate

Wintrobe haematocrit tubes were used. The blood was loaded into a clean tube within half-hour of its withdrawal and held upright in a frame. The fall in the erythrocytes was read of in millimetres at 1 hour, 24 hours, and at every 24 hours thereafter for 7 days.

I. BONE MARROW Examinations Details of the technique employed have been published (vide Appendix VI Wilkins, 1962).

Sternal bone marrow aspirations were carried out on goats with a Salah needle; smears were stained with three stains in the same manner as blood smears; the differential counts were made on one thousand cells.

Method

i) The goat was immobilised with a chemical restraining and analgesic agent (either phencyclidine alone or combined with pentobarbitone or thiopentone) (vide Appendix II Wilkins, 1964). The animal was then lifted on to the special tubular metal cradle and placed on its side on a soft "sorbo" rubber mat with its head flopping over the side to allow saliva, if any, to drip away. An assistant held fore and hind legs. The hair was clipped from the sternal region and the underlying skin cleansed with ether.

ii) Just before aspiration a previously sterilised Salah needle 50 mm to 70 mm in length, 16 BWG with a bore of 1.5mm and a 10 ml or 20 ml syringe with eccentric nozzle was washed out with sterile 3.8% sodium citrate solution to prevent clotting.

iii) The needle with closely-fitting stylet was pushed through the sternal skin at the pre-determined site. Subcutaneous tissue was easily penetrated until the hard sternal bone could be felt. Then with a gentle screwing motion penetration of bone was achieved to a depth of about ½cm. As soon as the needle "gave" the marrow cavity had been reached and pressure was immediately stopped. (Photo 27).

iv) The stylet was then withdrawn and the syringe attached. The plunger was pulled out gently but firmly to about the 5 to 10 ml mark by applying steady suction all the time.

v) Bloody marrow fluid usually entered the syringe but often nothing was visible at all. In the latter case it was almost invariably found that sufficient marrow material was to be found in the lumen of the needle to enable smears to be made. Not more than 1 ml was withdrawn.

vi) The syringe was then detached and the marrow material was dropped on to a shallow watch glass or directly on to clean glass slides, when smears were made immediately in the usual manner.

vii) "Flecks" of marrow material were lifted off the watch glass with a platinum loop, transferred to clean slides and crushed gently by means of another glass slide held at right angles. At least twelve (12) smears and "spreads" were made from each bone marrow aspirate.

viii) Staining

a) the smears and spreads were dried in air and defatted by pouring chloroform over them and allowing to evaporate until dry.

b) four slides were then stained with Leishmans stain, four with Jenner-Giemsa and the remainder with May-Grunwald-Giemsa stain. The method of staining was identical as for blood smears (q.v.)

Counting of Bone Marrow Cells Cell identification and nomenclature was based on Whitby and Britton (1957). The slide was first scanned with the low power of the microscope and a good area selected depending on the relative amounts of cells, fat and reticulum. Then the smear was studied under the 1/6 objective and the 1/12 oil immersion objective. One thousand cells were usually differentiated in at least three slides and often more depending upon the efficiency of the spread and the staining. Sometimes "flecks" of marrow were not available and one had to rely on a tedious examination and count of a marrow-blood smear. Occasionally, especially in the older and fatter goats, smears were nothing more or less than fat smears consisting of reticulum, a few marrow cells and a preponderance of fat. In all such cases, repeat aspirations were carried out.

Bone Marrow Sections Marrow flecks were also fixed in du Boscqs-Brazil medium and stained sections examined. These were not so satisfactory for the cells were very much more difficult to differentiate although the actual structure of the marrow substance was more easily appreciated. Another objection to the section was the length of time between obtaining the specimen and the examination, which could be weeks afterwards when details had faded slightly from the examiners mind.

Sperm Physiology of Goats. Details of the techniques employed have been published (Wilkins 1963) and are attached in the Appendix (VII) The methods used in the studies of the normal random sample of goats were identical to that employed in the later weekly examinations of the irradiated and control animals.

J. Morbid Anatomical and Histological Methods. Complete post-mortem examinations were performed on all goats which died. The technique employed was as follows :

i) The carcase was first weighed and a thorough inspection made noting any abnormalities in its general external appearance. This included condition of nutrition, deformities, appearance of skin, eruptions, rigor mortis, and signs of decomposition.

ii) The skin was incised from the symphysis of the mandible to the brim of the pelvis and reflected. The state of the subcutaneous tissue and the musculature was noted. The mammary glands with their associated lymph nodes in females were removed and examined by incision. The umbilicus was examined.

iii) The abdominal wall was incised from the xiphoid cartilage to the pelvis and a transverse incision was made in one flank from this longitudinal incision to the lumbar region. The two resulting flaps of the abdominal wall were removed. The presence and the nature of fluid or other abnormality in the abdominal cavity was noted and specimens taken as required. The undisturbed contents were then inspected.

iv) The sternum was removed intact by cutting through the costo-chondral junctions of the ribs. The undisturbed contents of the thoracic cavity were examined and any fluid or other abnormality noted and specimens taken as required.

v) The abdominal organs were now removed. The rectum and the duodenum were ligatured in two places and incised. The intestines were removed and examined. The state of the blood vessels and portal vein were noted. The oesophagus was ligatured and the stomachs were removed. The peritoneum was examined for subserous haemorrhages and inflammation. The liver was removed, weighed and examined by making multiple incisions into its substance noting appearance, colour and consistency. A specimen was taken. The bile ducts were opened and the presence of flukes noted. The diaphragm was examined for subserous haemorrhages. The kidneys and adrenals were removed and examined for subcapsular haemorrhages. Each kidney was weighed and sectioned by a longitudinal incision; the cut surfaces were then examined. The capsule was stripped from each kidney and any adherence of the capsule was noted. Each adrenal was sectioned. Specimens of kidney and adrenal were taken.

The pelvic symphysis was cut through and the pelvic organs examined as necessary. In the female, ovaries, uterus, vagina and bladder were removed together and examined. Specimens were taken as necessary.

vi) The tongue was separated from the mandible and was removed together with the larynx, trachea, lungs and heart. These organs together with the regional lymphatic nodes were examined. Heart and lungs were weighed, incised and specimens taken as necessary.

vii) The gastro-intestinal tract was next examined. The rumen, reticulum and omasum were incised, examined, contents noted and specimens taken as required. The abomasum was incised along its greater curvature and its contents emptied into a container. The mucosa was washed under a running water tap and the mucous membrane closely examined. Parasitic helminths were noted and a smear from the mucous membrane was examined for the smaller helminths. The intestine was examined and detached from its mesentry. The associated regional lymphatic nodes were noted and specimens taken. The intestine was opened throughout its length, the contents and mucosa examined and specimens taken at intervals or as necessary. The mucosa was now washed under running water and closely inspected. Smears were examined from parasitic helminths.

viii) The carcase was examined and lymph nodes incised and specimens taken as necessary. The fifth rib was cut off and, with a "monkey wrench", bone marrow was squeezed out of one end directly into a specimen jar containing du Boscqs-Brazil fixative medium.
A representative piece of rib was taken for histological examination.

ix) The cranium was opened by sawing through the bones forming the vault of the skull and then raising the various portions with a lever, completing the operation with bone forceps. The brain was then removed for examination and specimens taken.
The spinal cord was exposed by slipping a sharp scalpel between the bodies of the vertebrae on the ventral side and gently manipulating the spinal cord out after bending the column. Specimens could be thus be taken from various levels as required.

Histological Techniques. Representative portions of tissue as required were fixed in 10% neutral buffered formalin and sections were routinely stained with haematoxylin and eosin for microscopic examination. Other fixing agents employed included picric acid and du Boscqs-Brazil fixative. Special staining and mounting fluids were used as required.

Tissues taken as a routine measure for histopathological examination were as follows :-

Thyroid

Thymus whenever available which was very seldom

Tongue

Pharynx

Myocardium - ventricular septum

Atrium

Smear of heart blood when possible

Lung 1) from periphery

2) from hilus

Spleen

Adrenal

Bladder wall

Ovary in female

Testis in male

Gastro-intestinal Tract as follows:-

Rumen

Reticulum

Omasum

Abomasum 1) from cardiac end

2) from pyloric end

Gastro-intestinal junction

Duodenum from first part

Jejunum

Ileum

Caecum

Colon

Rectum

Pancreas

Liver

Lymph Nodes as follows:-

1) Mesenteric

2) Cervical

3) Suprascapular

Bone Marrow squeezed out from 5th rib into du Boscqs-Brazil medium

Rib

III EXPOSURE AND DOSIMETRY

According to Failla (1937) only the absorbed dose of radiation effects the biological response. In domestic animals owing to the large mass involved and the inhomogeneity of the tissues the mean absorbed dose is significantly effected by the geometry of the radiation exposure. Where a unilateral exposure is made attenuation, scatter and build-up ultimately decreases the mean absorbed dose and the distribution is not uniform. This lack of uniformity can be overcome to a large extent by administering one half of the total dose from each side of the animal. This procedure, known as "bilateral irradiation", produces a significant uniformity of depth dose pattern. (Bond et al, 1957).

Cage Apparatus Three kinds of cages were employed to confine the goats during the irradiation (photos 13 to 15):

i) A wire cage with 2" meshes

ii) A fibre glass 'coffin'

iii) A solid aluminium cage

Animals which were irradiated without any sedation were placed either in the wire-meshed cage or the aluminium crate; the aluminium crate was also used for some goats which were mildly sedated but most of the animals sedated were irradiated whilst lying in a specially constructed fibre glass coffin measuring 165 cm x 72 cm x 58 cm. To assist uniformity of dose distribution by providing side-scattered radiation 15 cm of a resinbonded pressed wood known as "Novabord" was placed inside the cage above, below and at each end of the recumbent goat which was thus confined to the centre.

The whole assembly i.e. the cage plus packing and goat, was placed on a special rubber-wheeled cart provided with removeable sides and of the requisite height off the ground viz 80 cms to come conveniently between the two X-ray tubes. (Photo 16).

Photo 14. Wire Goat Cage

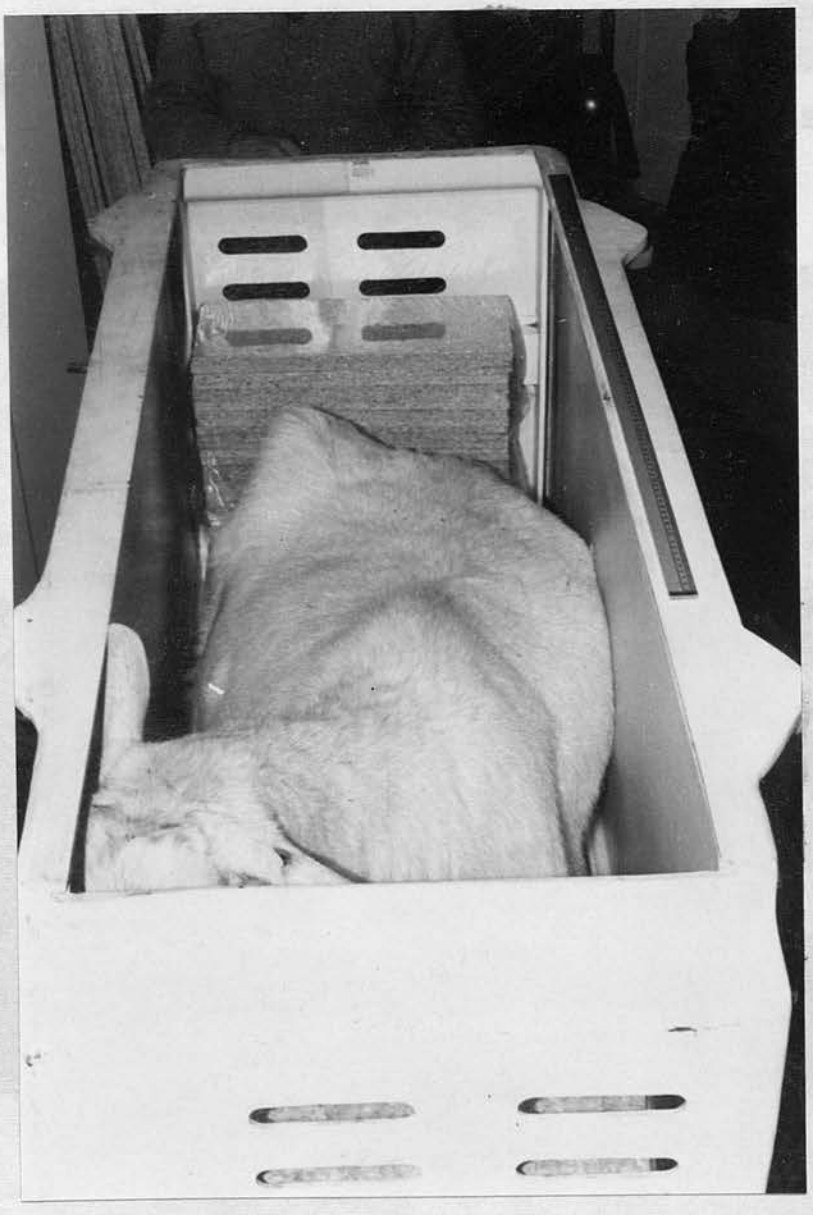


Photo 15. Fibre Glass Cage with goat in position

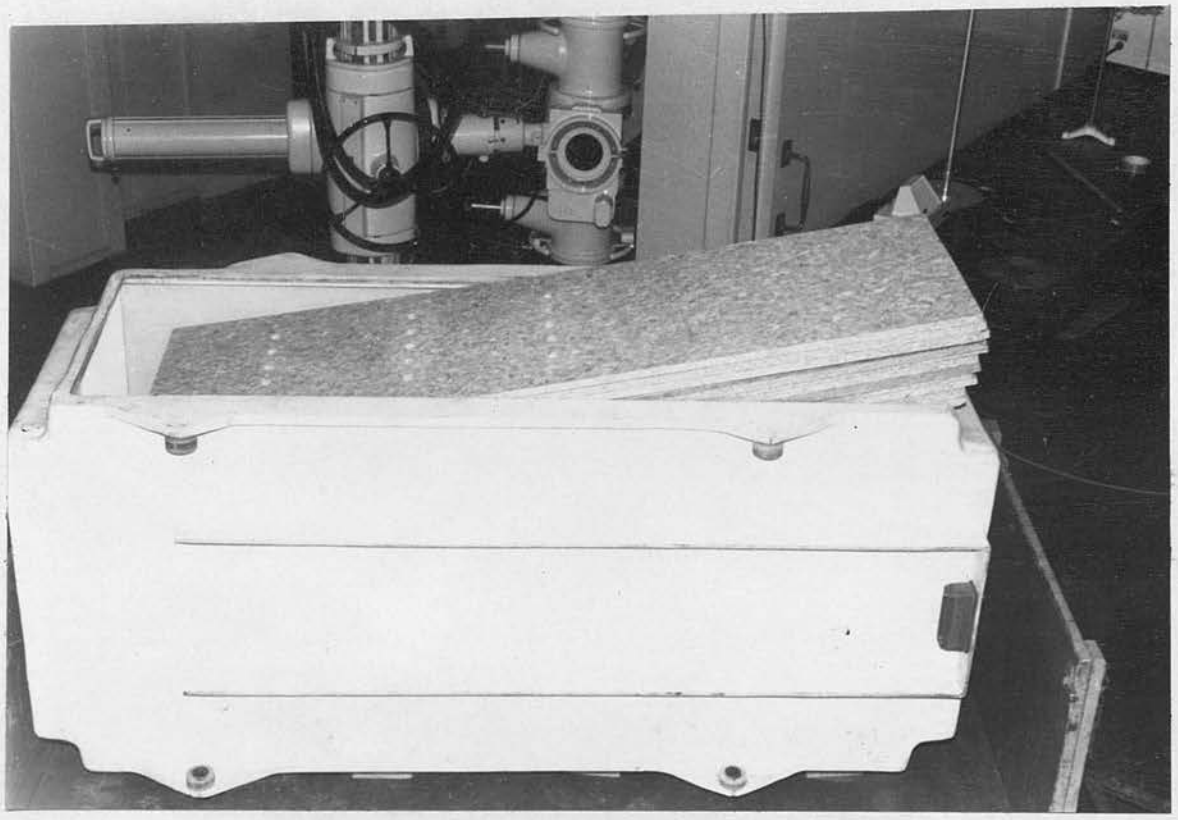


Photo 16. Fibre Glass Goat Cage showing "Novabord" Shield

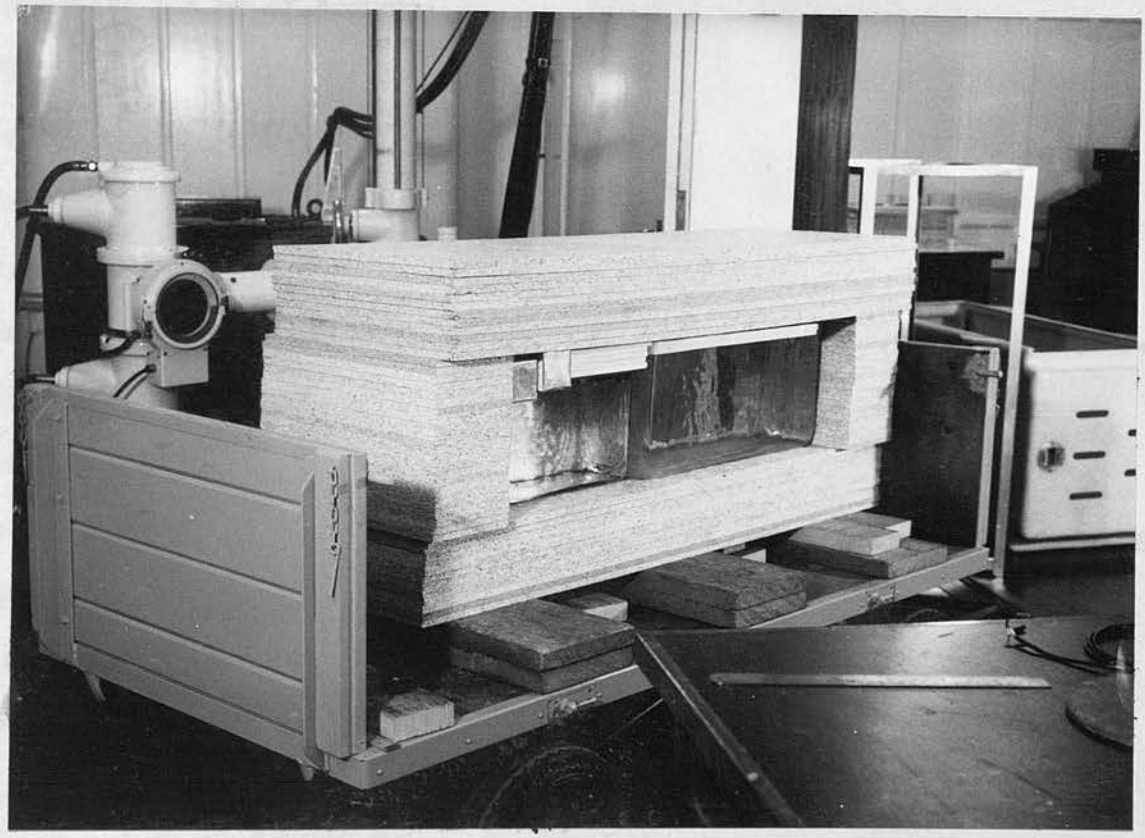


Photo 17. The Goat Phantom

Method of Exposure Goats were irradiated with two opposed X-ray beams from two 250 kV X-ray sets operated simultaneously. The tube shields were arranged so that the X-ray tube anodes were separated by a distance of 430 cms. A cone of radiation 140 cm, in diameter at a distance of 215 cm. i.e. the half way position between the two tube targets, was obtained with one X-ray machine and another cone of radiation of 125 cm. from the opposite machine. It was arranged that the goat should be irradiated at this mid-point within a circle of diameter 120 cm and with its sagittal plane at right angles to both X-ray beams: in other words, a bilateral irradiation was carried out. Each X-ray tube was operated at a constant potential of 250 kilovolts and a tube current of 14 milliamperes. Stepped copper filters were used to assist uniformity of the radiation field; these filters were of maximum thickness 0.25 mm and provided radiation of Half Value Thickness 1.2 mm of copper.

The combined dose rate in air at the middle position, i.e. 215 cm from each target, was 13 röentgens per minute. The quality of the radiation transmitted was the same from each tube and by both filters. Re-measurements throughout the eighteen month period of the experiment showed only the minor variation of $\pm$ 3%, which was expected (Corp 1957).

Dosimetry The doses quoted are "exposure" doses measured "free in air" in roentgens at the mid-point i.e. 215 cm from each X-ray tube, which was the position the middle of an animal would occupy during an irradiation. The instrument used was a Victoreen r meter calibrated at the National Physical Laboratory.

In the case of sedated animals the dose rate was measured at the centre of the empty fibre glass coffin. This allowed for absorption occurring in the thick fibre glass walls.

Some idea of the probable depth dose distribution in a goat was obtained from ionisation measurements in a rectangular water phantom which measured 108 cm x 32.5 cm x 29 cm. The phantom was surrounded by 15 cm

thickness of the resin-bonded pressed wood known as "Novabord" in an identical fashion to the goat (vide photograph). The measurement of the water phantom corresponded to that of the "average" goat when recumbent.

The ionisation current was measured with a 0.6 cc pencil-type chamber coupled to a D.C. Amplifier and calibrated against the Victoreen r meter. Holes were drilled in the "Novabord" to admit the chamber. Recordings were made from one "flank" of the phantom towards its middle in three representative planes viz:

1) in the middle
2) one-quarter way from one end
3) three cm. from the end of the phantom

This was done at two levels viz:

a) 2.5 cm below the surface
b) 14 cm below the surface

The recordings were made at the surfaces (flanks) (x1), $3\frac{3}{4}$ cm deep (x2), $10\frac{1}{4}$ cm (x3) deep and at the middle of the phantom (x4) (vide diagram Figure 2 for positions used). Because of the symmetry of the phantom dose rates and tissue dose distribution throughout the rest of the phantom could be reasonably inferred. (Figures 2, 3 and 4).

Results The results shown in the accompanying tables and graphs indicated that the tissue dose in roentgens as expected was highest on the two flanks of the phantom which were nearest to the X ray tubes, falling to a minimum on the mid-line.

The average dose to the whole phantom was approximately 75% of the exposure, or nominal, dose.

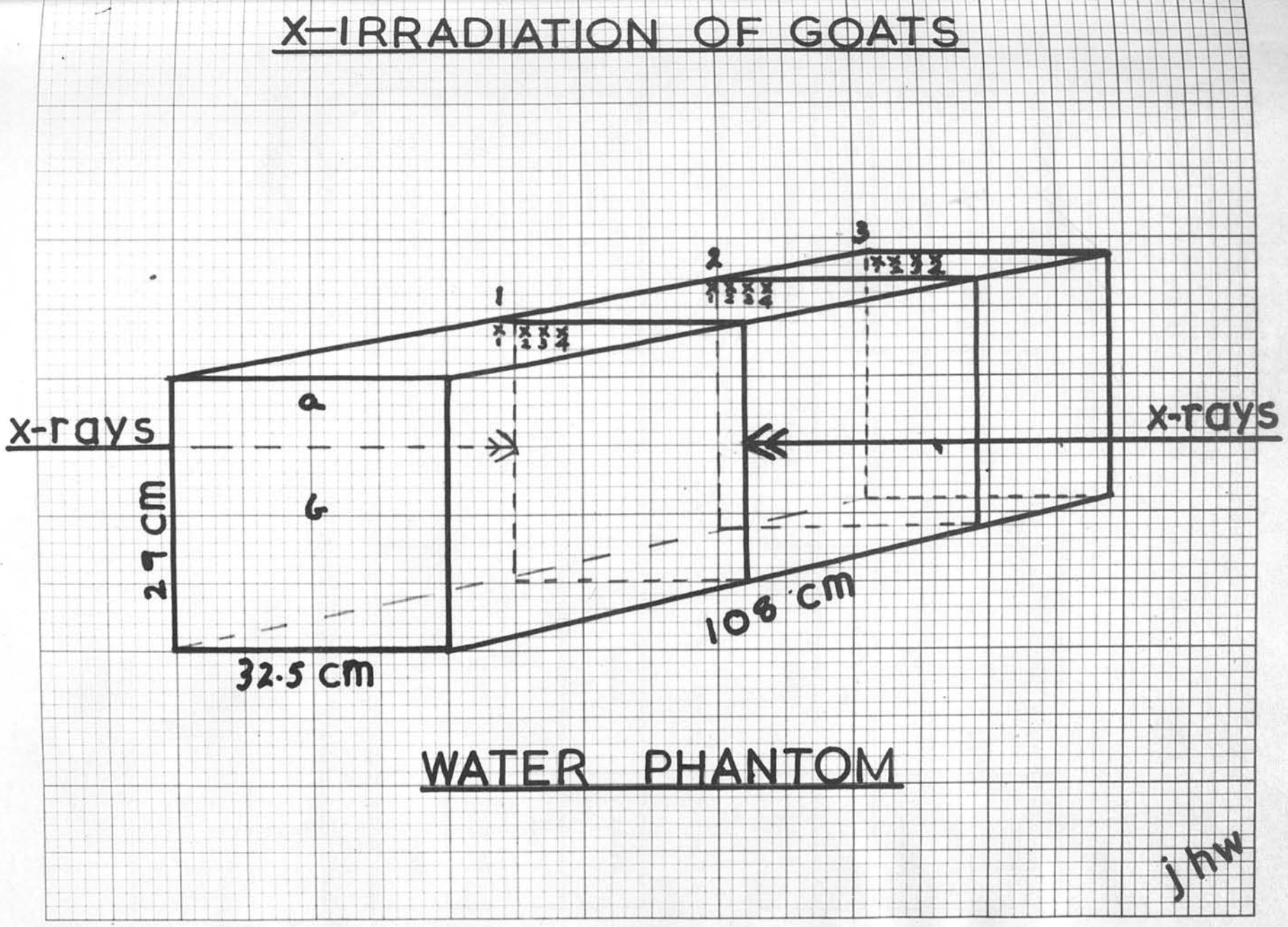

X-IRRADIATION OF GOATS
3
2
1
x-rays
a
b
x-rays
27 cm
32.5 cm
108 cm
WATER PHANTOM
jhw

Fig. 2

Table V.

Measurements 14 cms below the surface (Roentgens per Minute)

	Central Plane	Quarter-way Plane	End Plane
Entry Surface (Flank)	12.25	12.40	12.40
$3\frac{3}{4}$ cm deep	12.18	11.72	11.00
$10\frac{1}{4}$ cm deep	9.00	8.94	7.35
Mid-phantom (16.25 cm)	8.12	7.85	6.25

Measurements 2.5 cms below the surface (Roentgens per Minute)

Entry Surface (Flank)	12.60	12.62	12.76
$3\frac{3}{4}$ cm deep	12.18	11.82	11.36
$10\frac{1}{4}$ cm deep	9.34	9.21	7.55
Mid-phantom (16.25 cm)	8.65	8.13	6.46

Table showing dose rate (r/min) in rectangular water phantom bilaterally irradiated with 250 kV X rays.

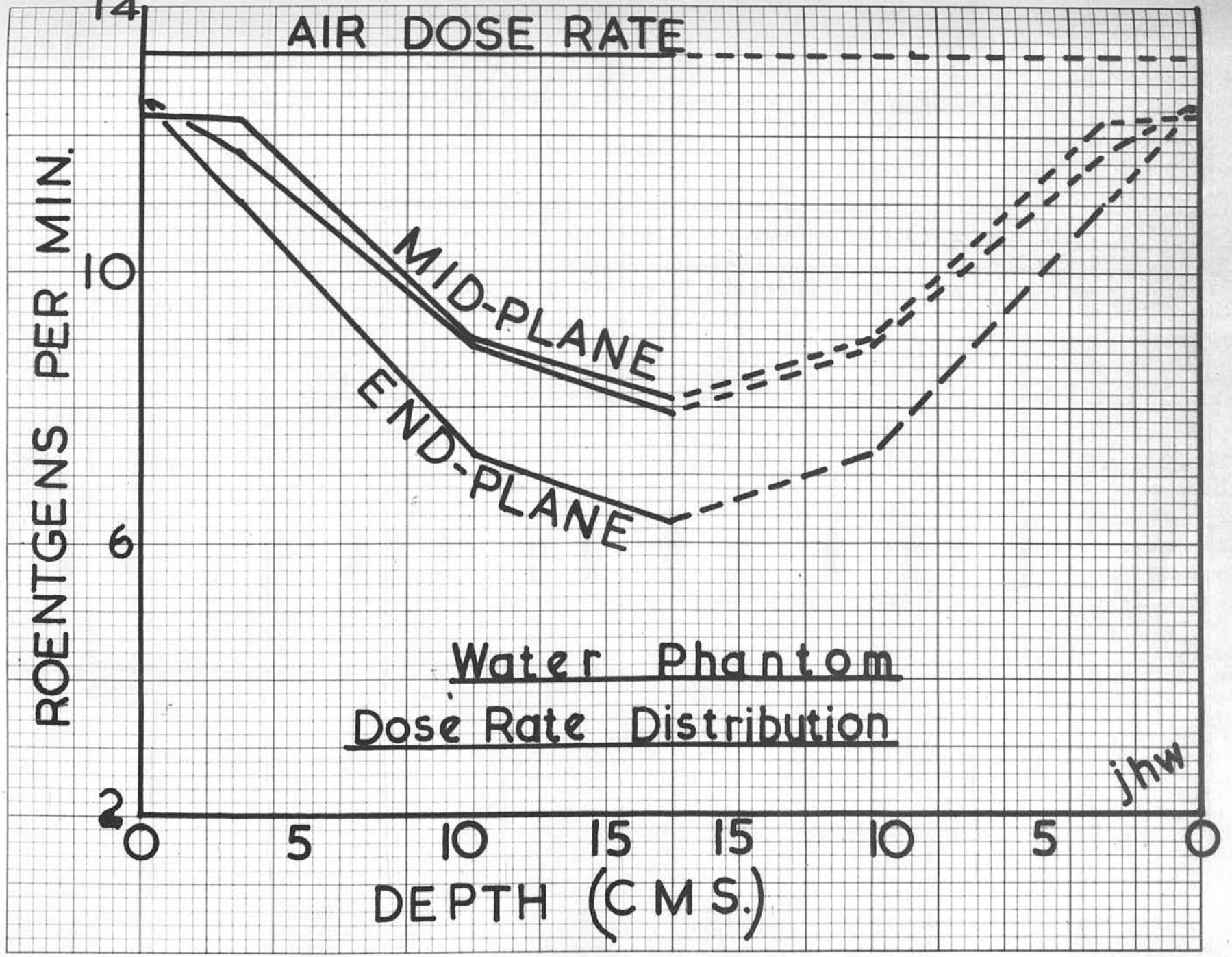

14
AIR DOSE RATE
ROENTGENS PER MIN.
10
6
2
MID-PLANE
END-PLANE
Water Phantom
Dose Rate Distribution
jhw
0
5
10
15
15
10
5
0
DEPTH (CMS.)

Table VI.

Table showing tissue dose distribution in rectangular water phantom bilaterally irradiated with 250 kV X Rays.

	Tissue doses (roentgens) expressed as a percentage of the "Exposure"dose					
	Central plane of phantom (1)		Plane $\frac{1}{4}$ way from end of phantom (2)		Plane 3 cm from end of phantom (3)	
	2.5 cm (dorsal) level (a)	14 cm (mid-line) level (b)	2.5 cm (dorsal) level (a)	14 cm (mid-line) level (b)	2.5 cm (dorsal) Level (a)	14 cm (mid-line) level (b)
Entry Surface(s) (flank) (x1)	97	94	97	95	98	95
$3\frac{3}{4}$ cm deep (x2)	94	94	91	90	87	85
$10\frac{1}{4}$cm deep (x3)	72	69	71	69	58	57
Mid phantom (x4)	66	62	63	60	50	48

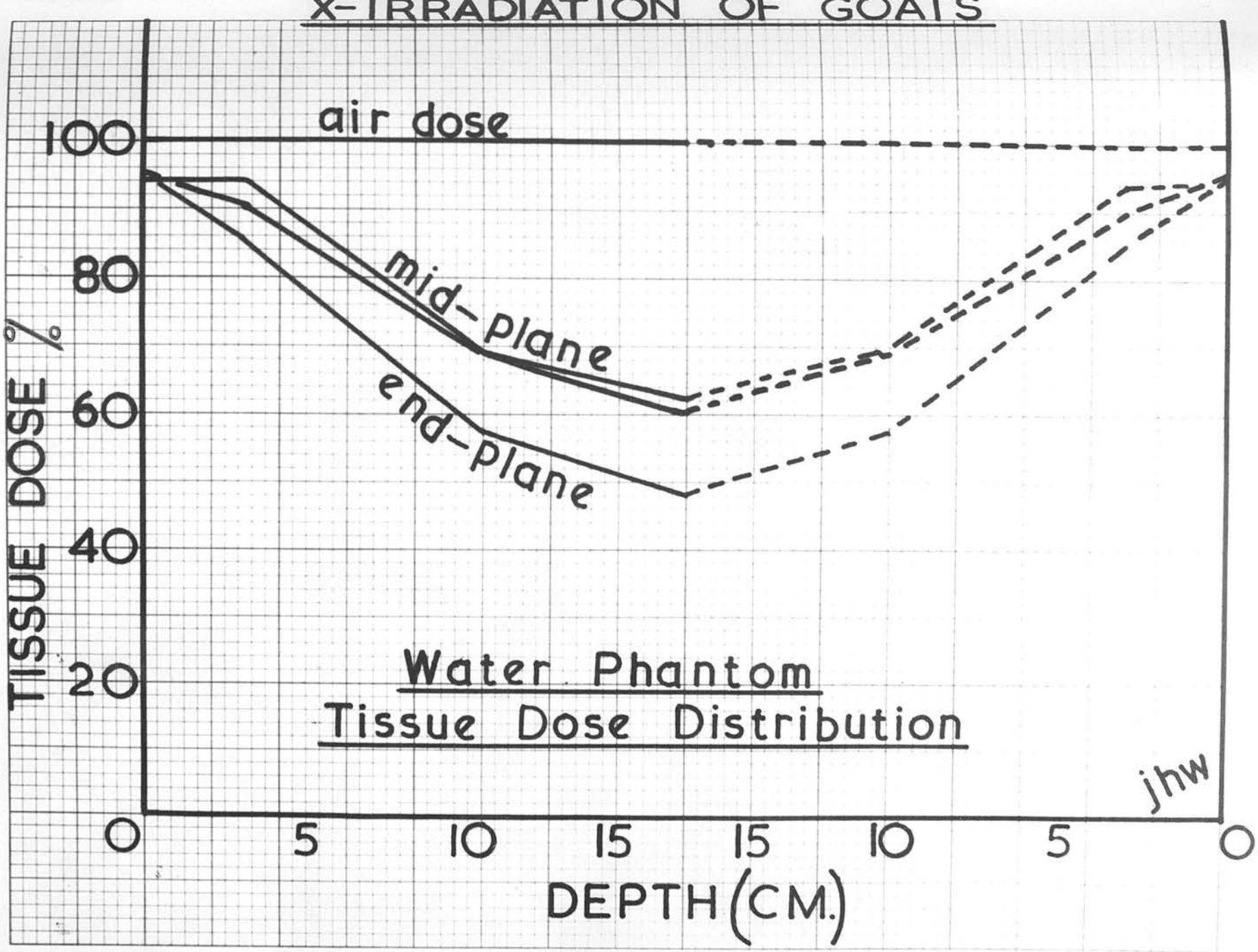

X-IRRADIATION OF GOATS
air dose
100
80
60
40
20
0
mid-plane
end-plane
TISSUE DOSE %
Water Phantom
Tissue Dose Distribution
jhw
0
5
10
15
15
10
5
0
DEPTH (CM.)

Fig. 4

Twenty adult goats of different breeds and sexes and of various ages were irradiated with single whole body bilateral air exposure doses of X-radiation varying from 400 r to 2000 r.

The calculations for the dose distribution in the bodies of the goats presented in Table VII were based on preliminary measurements under identical conditions made in the water phantom, and given at Table VI.

Table VII

Measured Air Exposure Dose (r)	Estimated Average Dose to Whole Animal (r) (75% of Exposure Dose)	Estimated Surface Dose (r) (94% of Exposure Dose)	Estimated Mid-Line Tissue Dose (r) (62% of Exposure Dose)
400	300	376	248
500	375	470	310
550	412	517	341
600	450	564	372
650	488	611	403
700	522	658	434
1000	750	940	620
2000	1500	1880	1240

Table showing estimated dose distribution in goats

IV RESULTS

a) LETHAL ASPECTS

The results of the investigations, dealt with under two main headings namely, mortality response and survival time are presented in Table VIII.

Mortality Response and Survival Time

Table VIII

Dose (r)	No. of goats	Died/ Total	Mortality %	Survival Time (Days)	Mean Survival Time (Days)
400	3	0/3	0	...	...
500	3	0/3	0	...	...
550	3	3/3	100	18 12 12	14
600	3	1/3	33.3	30	30
650	3	2/3	66.7	14 42	28
700	3	3/3	100	13 46 11	23
1000	1	1/1	100	8	8
2000	1	1/1	100	8	8
Total	20	11/20	55		19.5

Mortality Response

Such little weight could be attached to the observations at 2000r (1 goat), 1000r (1 goat), and 550r (which was out of range) that they were ignored in the probit analysis calculation carried out by the method of successive approximations (Finney, 1947). The calculations were based effectively on the observations at 700r, 650r, 600r, and 500r. The inclusion of the observations at 550r led to a negative value for the probit regression coefficient which was not meaningful. The results of the probit analysis are summarized in Table IX.

Table IX

LD/50	S.E.	95% Confidence Limits.	
621.73r	21.60	535.54	721.94

This result appears to be quite satisfactory with a reasonably small standard error and narrow confidence limits. The estimated midline LD 50 dose was 375.5r.

Survival Time

The mean survival time for 11 out of 20 goats irradiated with doses between 400 and 2000r and maintained until death was 19.5 days. The mean survival time for the 9 goats irradiated with doses between 550 r and 700r was 22 days.

Discussion

It is extremely difficult to compare the radiosensitivity of the goat with other animal species. The ruminant herbivore possesses peculiar individual idiosyncrasies of resistance and susceptibility. This is especially so in its adaptation to its environment and the husbandry obtaining.

The only other comparable experiment was carried out by Swift et al in 1946 at the University of Chicago. They used 11 Toggenburg goats, 6 males and 5 females of varying ages, and subjected them to single total-body exposure of 200 kV X radiation at 3.3 r/mins: three goats at 300r, four at 400r and three at 600r. (one goat was bitten by dogs and excluded).

They calculated an LD50 of approximately 350r and a mean survival time of 16 days for six (6) goats which died. The data from this study, where the animals were maintained under ideal laboratory conditions, indicate that adult goats in UK are not as sensitive to a single dose of X-radiation. However, the findings here are more in line with the LD 50's found by other workers using gamma radiation for ruminant herbivores in general, for example, 543r for adult cattle (Brown et al, 1961) and 524r for adult sheep with limits between 450r and 658r. (Trum 1955). Mean survival times of about 20 days are similar. Data from this study moreover indicate that adult goats are similarly radiosensitive to pigs which have an LD50 of 618r but more sensitive than the mature burro at 784r of gamma radiation (Rust et al,1954). Body size appears to be associated with radiosensitivity in the goat, sheep and pig but not so in the case of cattle and the burro. The pattern of deaths in goats in this study was mainly bimodal similar to that found in the burro and unlike that in cattle, sheep and pigs.

b) CLINICAL ASPECTS

Observations were made on the whole colony of goats which consisted of 119 animals of various breeds and ages; in addition, and more specifically, each of the 20 goats used in the actual irradiation experiments were observed as inidividual animals. This provided both group and individual controls and often enabled experimental results to be expressed as differences between group control and individual controls. This was important because there were commonly significant individual differences in the parameters investigated in normal goats.

1. Summary of the General Clinical Response (Photos 18 to 25)

Non-Survivors

Table X gives the major clinical signs observed and Table XI an approximate chronological sequence of their appearance. Irradiated goats varied in their clinical responses considerably. Initially animals appeared normal for varying periods of time during which muscular tremors were apparent. An early sign was a lessening, or complete loss, of water consumption which continued intermittently thereafter. Terminally animals often put their mouths into the water but appeared not to be able to swallow allowing the water to drool out. A reddening of the mucous membrane of the fauces with oedema and inflammation of the lingual lymphatic follicle was noted in some cases and could have been the cause of this.

Rectal temperatures remained essentially normal ($100^{\circ}F$ to $102.5^{\circ}F$) for most of the post-irradiation period although a higher normal plateau ($103^{\circ}F$ to $104^{\circ}F$) often occurred intermittently. Two to four days before death temperatures rose precipitously to between $106^{\circ}F$ and $110^{\circ}F$. On the day of death is usually dropped below normal ($98^{\circ}F$ to $99^{\circ}F$).

Anorexia and some apathy usually occurred soon after exposure but there was a quick return to normal.

Intermittent bouts of inappetence and non-rumination were observed for the following 14 to 21 days and continued in non-survivors until death. These goats spent a lot of time lying down and grinding their teeth indicating the presence of abdominal pain. An oedematous swelling of the subcutaneous tissues of the head especially around the buccal region gave some animals a characteristic appearance. Epilation was observed in some goats about the tenth day after irradiation and continued thereafter for varying periods. A "stripe effect" due to shielding was noted in some goats.

Intermittent cardiac arrhythmias and murmurs occurred in many goats. Some goats showed a transient irritability but a general depression combined with an apathetic fatigue was more common. Weakness of the hindquarters was often shown in the later stages but other animals showed jerky head movements, flicking at imaginary flies with the fore feet and other neuro-motor signs.

The passing of normal pelletted faeces was the rule and intermittent diarrhoea with some melaena the exception even at supra-lethal doses. During the terminal asthenic period some 2 to 3 days before death urination and defaecation usually ceased although a soiled anal region and tenesmus were often seen. Frank diarrhoea was uncommon. The temperature suddenly began going up in step-like rises to as much as 110°F, dyspnoea and tachypnoea occurred and a viscid discharge was sometimes observed from nose, mouth and eyes. The animal pushed its head into a corner and stood or lay in a forlornly apathetic fashion for hours on end. Death was sudden with little or no struggling. By and large, deaths occurred either between 8 and 14 days or between 30 and 46 days after irradiation.

Major Clinical Signs and symptoms

NON-SURVIVORS

Goat No.	C118	F20	C200	C315	F24	F14	F40	F27	C175	C234	C229
Breed	BS	BSA	BT	BS	BSA	BS	BS	BS	BA	BA	BS
Sex	CM	F	F	CM	F	F	F	F	F	CM	CM
Age (yrs)	4	5	4½	4	3½	4	4½	6	4	4	4
Dose (r)	550	550	550	600	650	650	700	700	700	1000	2000
Sedation	P+S	NIL	NIL	P+S	P	NIL	P+S	P+S	NIL	P	P+S
Survival Time (Days)	18	12	12	30	14	42	13	46	11	8	8
Haemoglobinuria	+	+	-	+	+	-	-	+	-	+	+
ANOREXIA											
Day of Onset	1st/15th	2nd/4th/10th	4th/11th	10th/24th	3rd/10th	4th/15th/40th	6th	45th	10th	3rd	1st
Duration (Days)	4/3	1/3/2	2½/1	4/6	5/4	5/4/2	1	1	1	5	8
APATHY											
Day of Onset	15th	10th	11th	27th	2nd/10th	5th/40th	10th	41st	10th	4th	1st
Duration	3	2	1	3	4/4	4/2	1	5	1	4	8
DIARRHOEA											
Day of Onset	18th	11th	-	-	13th	4th/41st	-	45th	-	4th	7th
Duration	1	1	-	-	1	4/1	-	1	-	3	1
PYREXIA											
Day of Onset	15th	11th	10th	27th	11th	3rd/8th/10th	12th	41st	10th	-	7th
Duration	3	1	2	3	3	2/4/2	1	5	1	-	1
EPILATION											
Day of Onset	-	5th	-	10th	8th	12th	8th	-	-	-	-
Duration	-	6	-	20	6	16	5	-	-	-	-
DISCHARGES											
Eye	-	-	-	-	-	+42nd	-	-	-	+	+
Nose	18th	-	-	-	-	+on 42nd	-	-	-	-	+
Mouth	-	12th	-	-	-	+on 42nd	-	-	-	-	+
CARDIAC SIGNS	+	+	-	+	+	-	+	+	-	+	+
RESPIRATORY SIGNS	+	+	-	+	+	-	+	+	-	+	+
NEURO-MUSCULAR SIGNS	-	-	-	+ Lame	-	-	-	+ Lame	-	-	-
Total WEIGHT(lbs)											
Loss -	NIL	-11	-13	-15	-17	-13	-3	-7	-8	-21	-20
Gain +		-	-	-	-	-	-	-	-	-	-
WATER Consumption	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced	Reduced

P = Phencyclidine

P+S = Phencyclidine combined with Sparine (Promazine hydrochloride).

Table XI

X-Irradiation of Goats

Summary of Chronological Sequence of Clinical Signs

Time after Irradiation	Clinical Signs
0 - 36 hrs	Muscular tremors and cardiac signs in some. Otherwise normal.
1 - 2 days	Anorexia. Malaise
2 - 7 days	Intermittent anorexia. Reduction or suppression of water consumption. Otherwise normal. Progressive loss of weight.
7 - 14 days	Progressive weight loss continues. Weakness and apathy: knuckling over in front. Ataxia. Death may occur. Terminal pyrexia, cardiac signs.
14 - 21	Signs continue. Occasional diarrhoea, epilation and discharge from eyes. Lying down grinding of teeth indicating abdominal pain. Swelling of head.
21 - 30 days	Intermittent discharge from eyes, nose and mouth. Diarrhoea in some. Intense apathy. Asthenia.
30 days & over	Survivors normal. Non-survivors remain normal until 2 to 3 days before death when cardiac arrhythmies and murmurs become intense. Terminal pyrexia.

Survivors

Table XII shows the major signs seen in survivors. Generally speaking clinical signs were conspicuous by their absence. Where they did appear however they resembled in milder form those observed in the non-survivors. Anorexia and apathy were seen in four goats but the duration of these signs was short. Two goats each had diarrhoea lasting for but one day and two showed signs of epilation. Cardiac and respiratory signs were shown by the majority and two goats showed late neuro-muscular signs: There was no reduction in water consumption in four goats, two showed only initial reduction within the first week and the three remaining did show a persistent intermittent reduction in consumption throughout the period of observation.

TABLE XII

Major Clinical Signs and Symptoms

SURVIVORS

Goat No.	W316	W302	M10	C240	M1	M3	F30	M9	F31
Breed	BA	BS	BS	BA	BT	BS	BS	BA	AN
Sex	F	F	M	CM	M	M	F	M	F
Age	4½	4½	4	4	4	6½	5½	3	7
Dose (r)	400	400	400	500	500	500	600	600	650
Sedation	NIL	P+S	S	P	S	NIL	P+S	S	NIL
Under Observation (Days)	213	225	172	584	179	190	239	164	157
Haemoglobinuria	-	+	-	-	-	-	+	-	-
ANOREXIA									
Day of Onset	1st/6th	-	37th	1st	-	-	-	-	1st/14th
Duration (Days)	½/1	-	1½	2	-	-	-	-	5/3
APATHY									
Day of Onset	-	-	37th	1st	164th	-	-	-	1st/14th
Duration (Days)	-	-	1½	2	1	-	-	-	1/3
DIARRHOEA									
Day of Onset	-	-	-	-	4th	-	-	-	3rd
Duration (days)	-	-	-	-	1	-	-	-	1
PYREXIA									
Day of Onset	-	-	-	-	-	-	-	-	-
Duration (Days)	-	-	-	-	-	-	-	-	-
EPILATION									
Day of Onset	-	-	-	-	-	-	-	20th	11th
Duration (Days)	-	-	-	-	-	-	-	14th	17th
DISCHARGES									
Eyes	-	-	-	-	-	-	-	-	-
Nose	-	-	-	-	-	-	-	-	-
Mouth	-	-	-	-	-	-	-	-	-
CARDIAC SIGNS	+	+	+	-	+	-	+	+	-
RESPIRATORY SIGNS	+	+	+	-	+	-	+	+	+
NEURO-MUSCULAR SIGNS	-	-	+ Lame 37th-45th day	-	+164th "Grand Mal" attacks	-	-	-	-
Total WEIGHT (lbs)									
Loss - Gain +	+3	-9	-36	+85	-36	-42	+29	-28	+12
Water Consumption Reduced -	No	Initially only	No	No	Reduced	No	Reduced	Initially only	Reduced

P = Phencyclidine

S = Sparine (promazine hydrochloride)

P+S = Combination of Phencyclidine + Sparine.

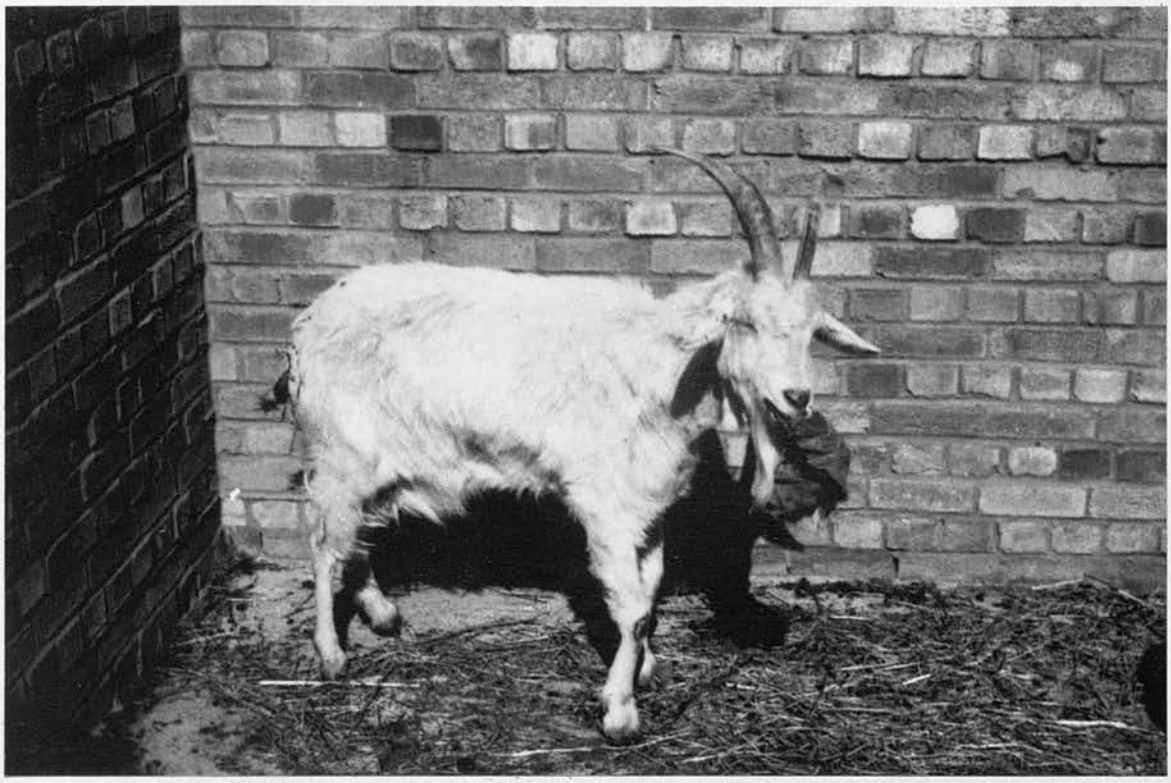


Photo 18. Knuckling-over in Front

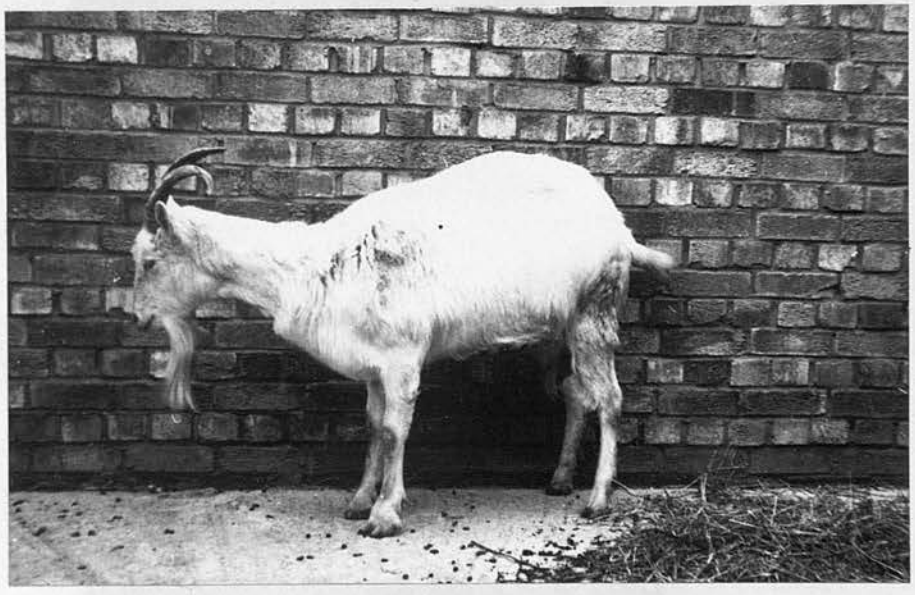


Photo 19. Apathy and Tucked-up

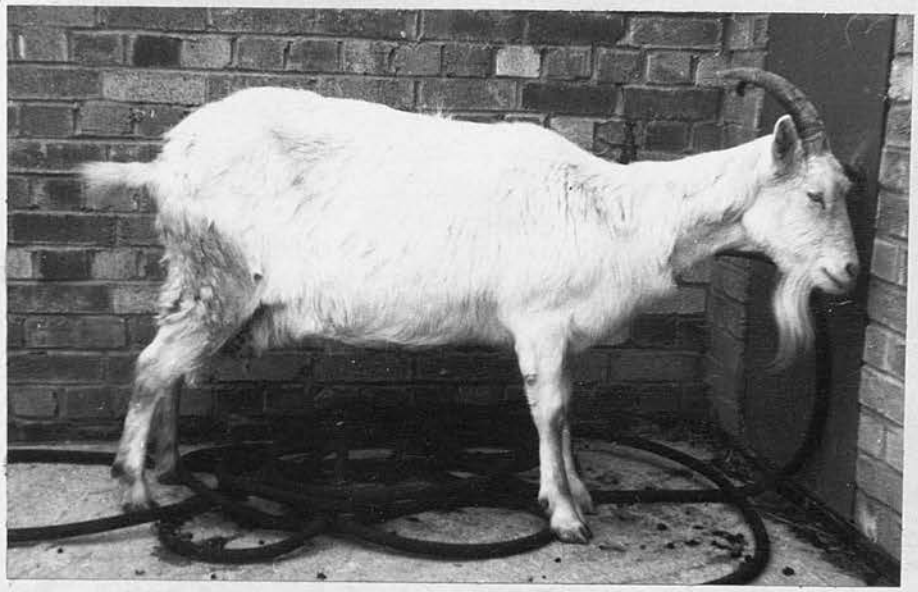


Photo 20. Apathy and Pushing the Head against a Wall

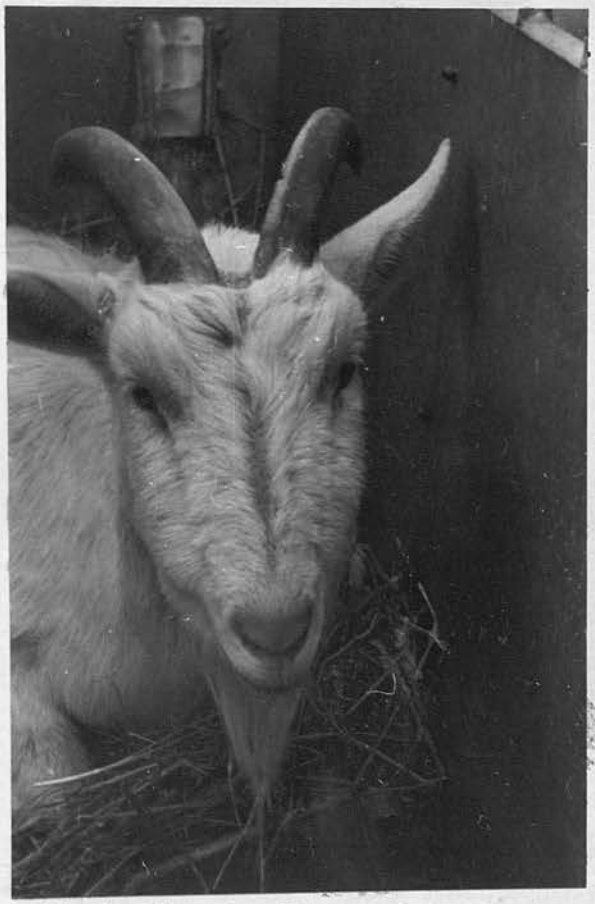


Photo 21. Swelling of the Head

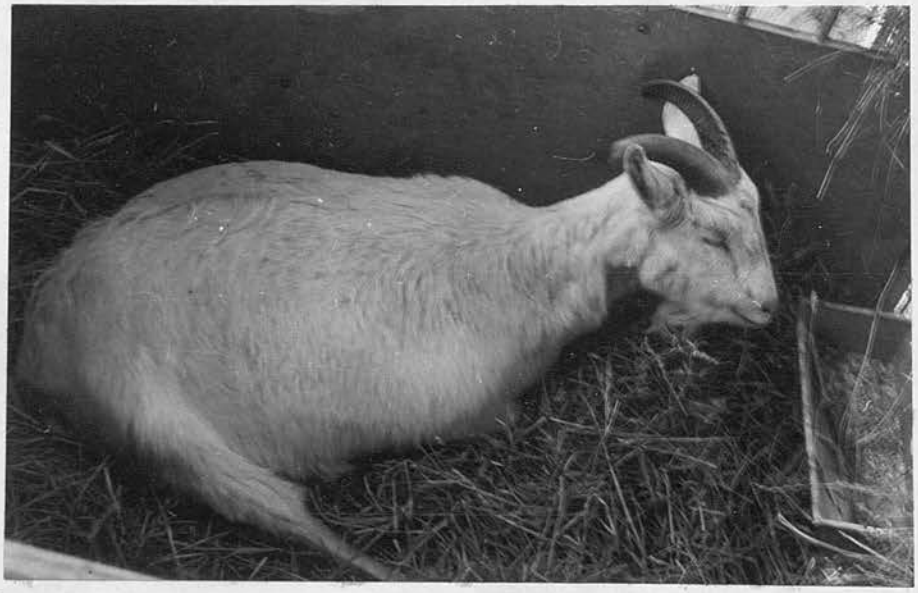


Photo 22. Lying Down and Grinding of Teeth indicating Abdominal Pain

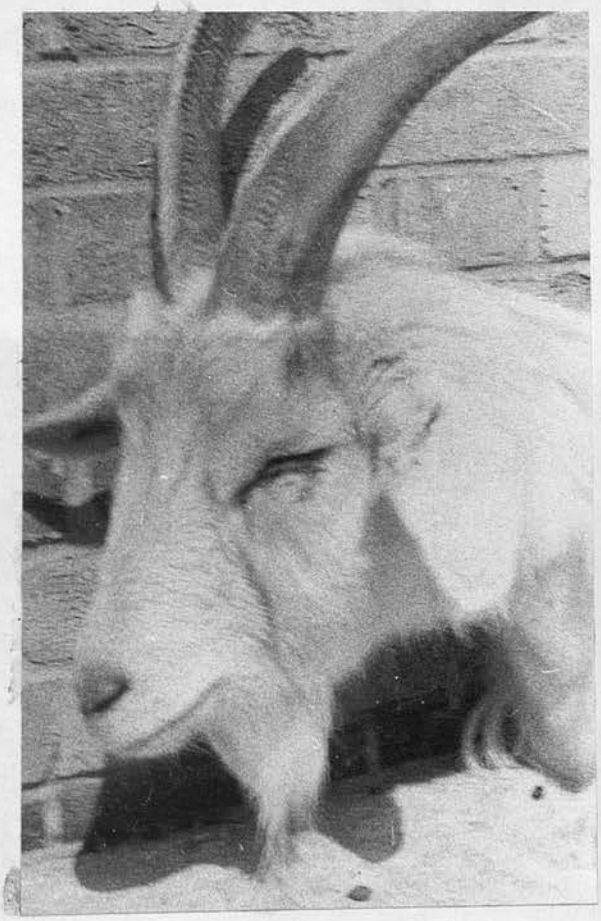


Photo 23. Orbital Oedema and Haemorrhage

Photo 24. Epilation

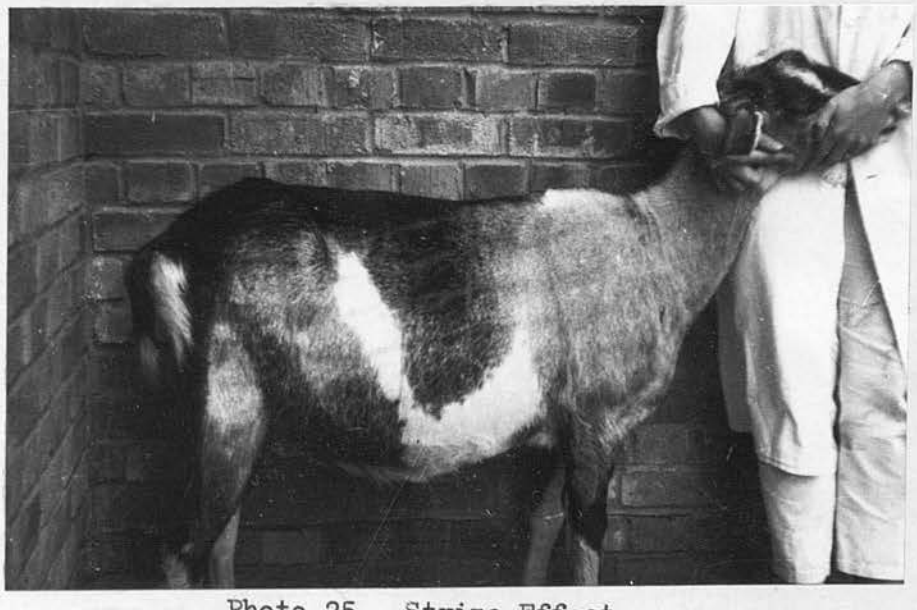


Photo 25. Stripe Effect

2. Investigations of Major Clinical Signs

The measurements of the 'average' adult British goat varied considerably but the following is the average of 7 adult goats :-

Height at withers . . . . . 75 cms

Length (poll to tail head) . . . . . 110 cms

Length of body (withers to tail head) . . 75 cms

Width of body at withers . . . . . 25 cms

Width of body at abdomen . . . . . 30 cms

Forearm (knee to elbow) . . . . . 23 cms

Gaskin (hock to stifle) . . . . . 23 cms

Cannon (fetlock to knee or hock) . . . . 15 cms

i) Body Weight

There were a few very large goats and a few rather small ones. The large animals weighed over 220 lbs (110 kg) and the small ones 80 lbs to 90 lbs (40 kg). The great majority of the goats weighed between 90 lbs (40 kg) and 180 lbs (80 kg).

Table XIII Distribution of the Colony of Goats by Weight and Sex

Sex	Total	Very Large (over 220 lbs)	Large 180 lbs to 220 lbs	Medium 130 lbs to 180 lbs	Small 90 lbs to 130 lbs	Very Small under 90 lbs
Male	64	--	1	31	29	3
Female	49	1	3	26	18	1
C.M.	6	2	1	3	--	--
	119	3	5	60	47	4

The increases in weights of 59 goatlings over a period of 18 months is presented in Table XIV.

Table XIV

Normal Goatlings

Extremes and Means of Body Weights in lbs

	8 Weeks old n = 59	6 months old n = 58	12 months old n = 57	18 months old n = 57
Range	18 to 41	40 to 94	72 to 159	85 to 175
Mean	25.7	65.1	107.9	132.1
S.D.	6.1	12.1	23.3	21.2
Mean % Increase	-	153.3%	65.7%	22.4%

The weight increases during the first four months were the greatest (153.3%) and substantial increases (65.75%) also occurred during the next six months but were reduced during the following six months to 22.4%. From this it was inferred that weight equilibrium in these goatlings was reached in approximately eighteen months to two years.

Comparable body weight data for adult goats given in Tables XV, indicate that body weight increases were greatest during the first six months after arrival at the Goat farm, after which they reverted to normal expected variations. Adult goats deprived of food lost no weight on the first day, 2.3% of their original weight on the second day, 4.4% on the third and 5.2% on the fourth.

Table XV

Normal Goats

Extremes and Means of Body Weights in lbs

	Wt on arrival n = 60	Wt after 6 months n = 58	Wt after 9 months n = 55	Wt after 12 months n = 49	Wt after 18 months n = 47
Range	71 to 196	81 to 240	86 to 240	96 to 240	110 to 250
Mean	126.1	145.8	150.5	153.6	153.1
S.D.	23.5	31.6	32	32.1	32.2
Mean % Increase	--	15.6%	3.2%	2.1%	NIL

Irradiated Goats

In this study it was observed that at 7 days after irradiation (Table XVI) non-survivors had lost about 6% and survivors about 3% of their original body weights. Both of these figures were within the expected variation and approximated to figures obtained in normal goats deprived of food. The intermittent dysphagia caused by the irradiation could therefore explain this loss. There was no consistent inter-relationship between dose and weight loss at 7 days although the greatest percentage losses tended to occur at the higher doses. A similar finding applied at death in non-survivors or at the end of observations in survivors (Table XVII).

Final weight changes are given in Table XVIII (Survivors) and Table XIX (Non-survivors). All non-survivors lost weight consistently averaging a decrease of 8.5% of the pre-irradiation control value. Four out of the nine survivors showed an increase of body weight at the conclusion of the observations. The other five showed decreases which in some exceeded quite abnormally the expected variation. The survivors as a whole showed an insignificant mean decrease of 0.38% at the end of

the observations. One survivor which, before irradiation, was a poor doer and losing weight began putting on condition almost immediately after irradiation and continued to do so over a period of 20 months when its weight was 51.5% greater than its pre-irradiation value. Graphical representation of body weight changes are given in Fig 5. (Non-survivors) and Fig. 6 (Survivor).

There was no apparent interrelationship between weight loss and clinical, neutrophilic or mortality response (Table XX).

The findings in this study are similar in some ways to those observed by Rosenfeld (1958) in calves and Brown (1962) in adult cattle. Rosenfeld (1958) found a progressive weight loss in irradiated calves beginning as early as the first day after exposure (with a weight loss of 5%) to the eleventh day with a weight loss of 16%. Brown (1962) observed that the weight loss in adult cattle exposed to lethal doses of Co^{60} gamma radiation was less than 10% but he included both survivors and non-survivors during the first 30 days. Rust el at (1954) did not find a weight-response relationship either in burros or swine but Thomas and Brown (1961) did find a weight relationship to both early neutrophil changes and to clinical response of burros to neutron-gamma radiation.

Table XVI

X-Irradiation of Goats

Body Weight Loss at 7 days post-Irradiation

Non-Survivors

Goat No.	Dose (r)	Control Wt. (lbs)	Wt. at 7 days (lbs)	Percentage Wt. loss at 7 days	Mean Percentage Wt. Loss	
					All Goats	400 - 700 r
C229	2000	210	190	9.5	)	
C234	1000	164	143	12.8	)	
C175	700	119	113	5.0	)	
F 27	700	111	104	6.3	)	
F 40	700	108	105	2.8	)	
F 14	650	96	86	10.4	)6.7	5.75
F 24	650	94	88	6.4	)	
C315	600	142	137	3.5	)	
C200	550	120	108	10.0	)	
F 20	550	136	126	7.4	)	
C118	550	235	235	NIL	)	
Survivors						
F 31	650	134	133	0.7	)	
M 9	600	174	174	0.0	)	
F 30	600	165	163	1.2	)	
M 3	500	190	182	4.0	)	
M 1	500	174	168	3.5	)2.9	
C240	500	165	162	1.8	)	
M 10	400	190	180	5.3	)	
W302	400	153	144	5.9	)	
W316	400	141	136	3.5	)	

Table XVII

X-Irradiation of Goats

Percentage Body Weight Loss at 7 days and at Death

or End of Observations in Survivors

Dose (r)	Percentage Loss of Weight	
	At 7 Days	At Death or End of Observations
2000	9.5	9.5
1000	12.8	12.8
700	4.7	5.3
650	5.8	7.6
600	1.6	3.0
550	5.8	6.3
500	3.1	Gain of 2.9
400	4.9	7.6

Table XVIII

Effects of X-Irradiation on Goats

Weight changes of survivors

Goat	Age (Years)	Breed	Sex	Dose (r)	Pre-Irradiation Control Weight (lbs)	Weight at end of Observations (lbs)	Survival Time at end of Observations (days)	% decrease (-) % increase (+) Total Wt. - Loss/ + Gain	Mean Wt. - Loss/ + Gain for Dose	Mean Wt. - Loss/ + Gain of all Goats	Mean Wt. - Loss/ + Gain for Dose range 400 r - 700 r
F31	7	AN	F	650	134	146	157	+ 9	+ 9		
F30	5½	BS	F	600	165	194	239	+ 17.6	+ 0.8		
M9	3	BA	M	600	174	146	164	- 16.1			
M3	6½	BS	M	500	190	148	190	- 22.1			
M1	4	BT	M	500	174	138	179	- 20.7	+ 2.9	- 0.38	- 0.38
C240	4	BA	CM	500	165	250	584	+ 51.5			
M10	4	BS	M	400	190	154	172	- 18.9			
W316	4½	BA	F	400	141	144	213	+ 2.1	- 7.6		
W302	4½	BS	F	400	153	144	225	- 5.9			

EFFECTS OF X-IRRADIATION ON GOATS

Weight Changes of Non-Survivors

Goat No.	Age (yrs)	Breed	Sex	Dose (r)	Average Control Weight (lbs)	Wt. at Death (lbs)	Survival Time (Days)	% decrease			
								Total Wt. Loss	Mean Wt. loss for dose	Mean Wt. loss of all goats	Mean Wt. Loss for dose range 400r - 700r
C229	4	BS	CM	2000	210	190	8	9.5	9.5		-
C234	4	BA	CM	1000	164	143	8	12.8	12.8		-
C175	4	BA	F	700	119	111	11	6.7			
F 40	4½	BS	F	700	108	105	13	2.8	5.3		
F 27	6	BS	F	700	111	104	46	6.3			
F 14	4	BS	F	650	96	83	42	13.5	15.8	9.0	8.5
F 24	3½	BSA	F	650	94	77	14	18.1			
C315	4	BS	CM	600	142	127	30	10.5	10.5		
C118	4	BS	CM	550	235	235	18	NIL			
C200	4½	BT	F	550	120	107	12	10.8	6.3		
F 20	5	BSA	F	550	136	125	12	8.1			

Table XX

X-Irradiation of Goats

Inter relationship between Body Weight

Clinical and Neutrophilic Response

Goat No.	Dose (r)	Final Weight % Loss (-) % Gain (+)	Clinical Response	Neutrophilic Response Maximum % Increase
C229	2000	- 9.5	Died	88
C234	1000	-12.8	Died	344
C175	700	- 6.7	Died	143
F 27	700	- 6.3	Died	161
F 40	700	- 2.8	Died	278
F 14	650	-13.5	Died	171
F24	650	-18.1	Died	140
C315	600	-10.5	Died	321
C118	550	NIL	Died	451
C200	550	-10.8	Died	170
F 20	550	- 8.1	Died	48
F 31	650	+ 9.0	Moderate	93
F 30	600	+17.6	Minimum	450
M 9	600	-16.1	Moderate	156
M 3	500	-22.1	Minimum	374
M 1	500	-20.7	Minimum	58
C240	500	+51.5	Minimum	501
M 10	400	-18.9	Minimum	102
W302	400	- 5.9	Minimum	576
W316	400	+ 2.1	Minimum	290

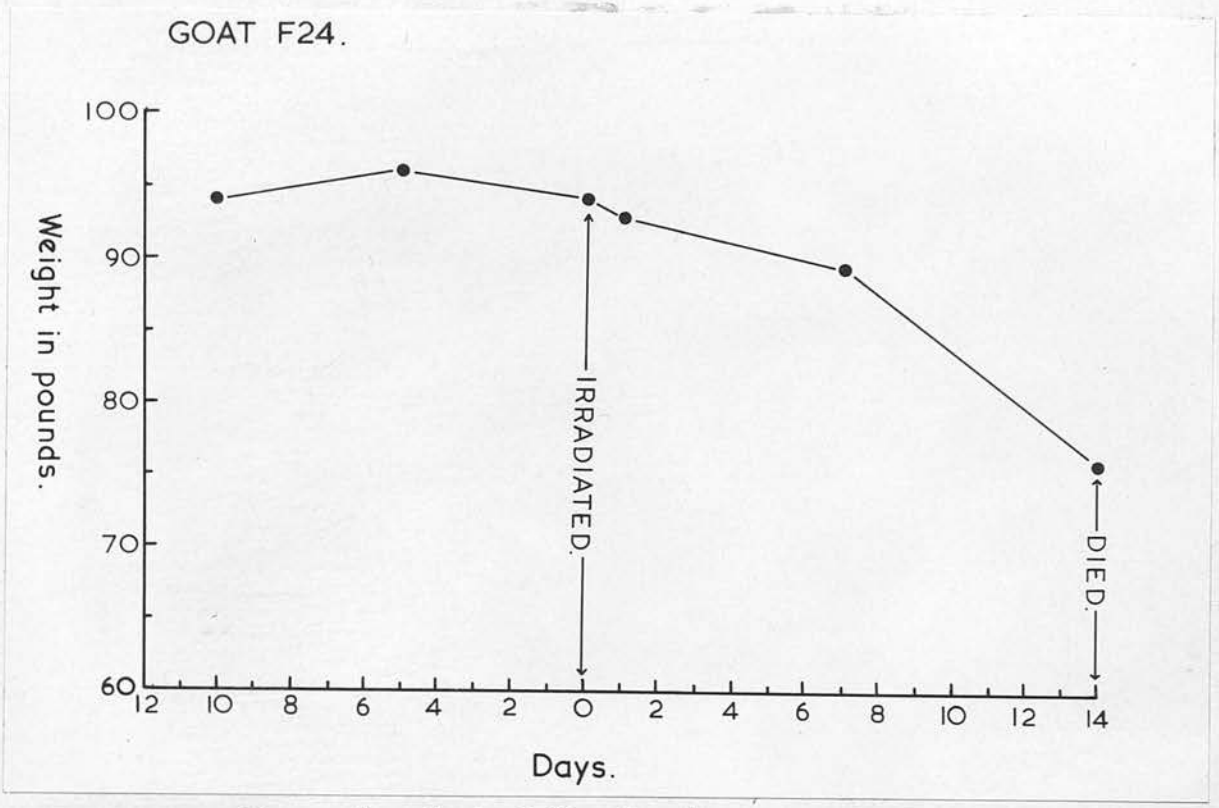


Figure 5. Typical Non-Survivor

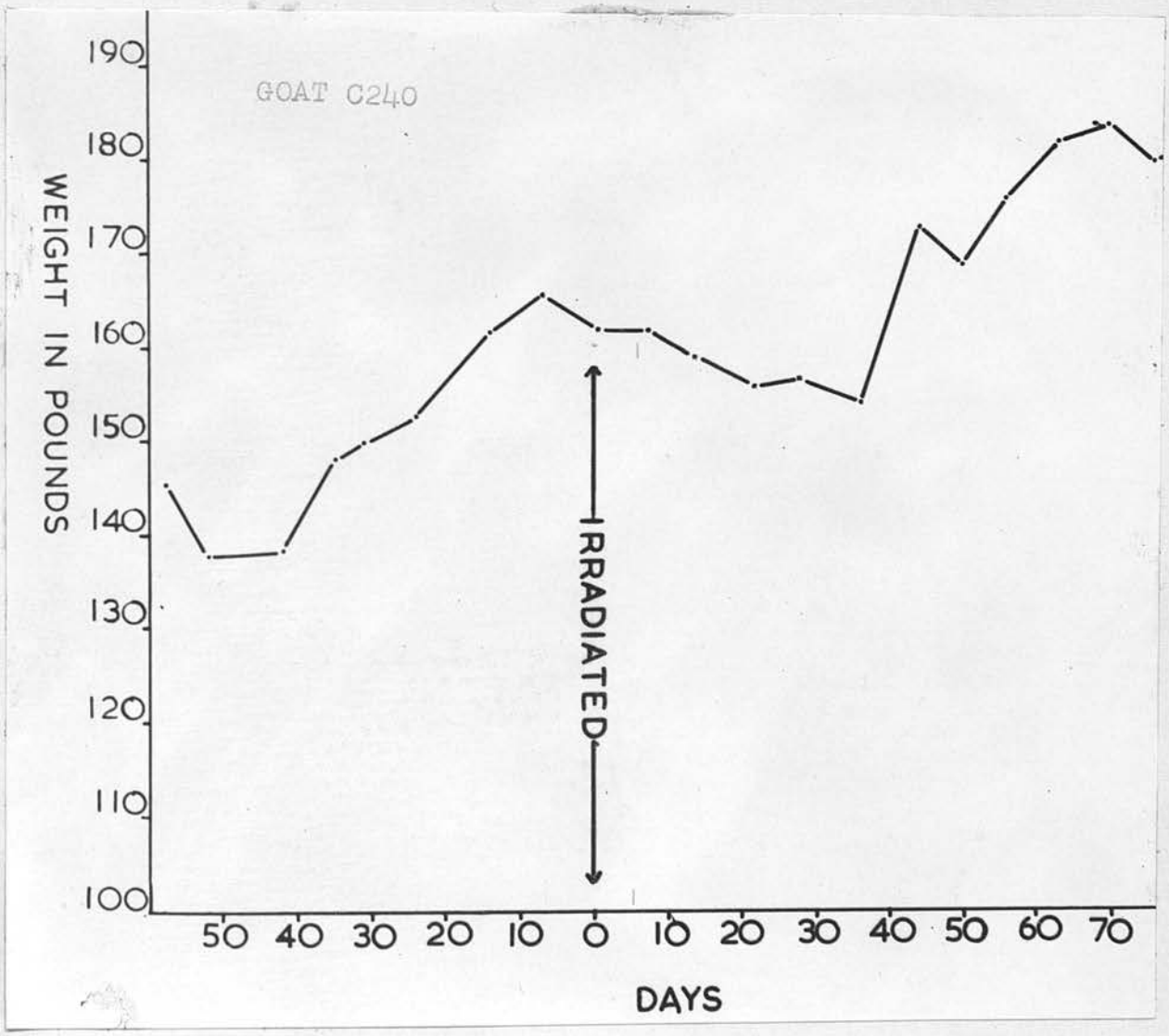


Figure 6. Typical Survivor

ii) Water Consumption

Normal Goats

The average daily consumption of water in adult goats was found to be 2070 ml . This was the mean value of 129 observations from 17 goats.

Daily Water Consumption of Normal Adult Goats

Range	250 ml. to 5500 ml.
Mean	2070
S.D.	1140
S.E.	99.8

Irradiated Goats

a) Non-Survivors

The daily water consumption of non-survivors after irradiation is given in Table XXI. Goats receiving 2000r and 1000r did not drink any water at all from the time of irradiation until they died 8 days afterwards. Consumption at other doses was adversely affected for varying periods after irradiation and ceased altogether from one to seven days before death. Table XXIa and Figure 7 present the average daily water consumption of non-survivors before death.

b) Survivors

Water consumption in survivors was not so severely affected as in non-survivors and the average daily consumption was usually within the expected variation (Table XXII).

Discussion

Water makes up about 70 per cent of the body weight of the average adult animal (Dukes, 1955).

The body fluids are in a state of dynamic equilibrium with constant

exchanges between intracellular water, which represents about 50 per cent of the body weight, and the extracellular water which consists of interstitial fluid (15 per cent) and blood plasma (5 per cent). As the animal body cannot store water for extended periods and as its total water content must be kept at a relatively constant level, it is essential that a continuous supply is provided. In this study it was found that the normal average adult goat drinks approximately 2000 ml. of water daily. This is in addition to the water in its food and the amount formed by its metabolism. It has been arbitrarily assumed that as much is needed as preformed and oxidative as in actual water drunk; thus the average goat in this work was considered to require about 4000 ml of water daily to maintain its water balance. As the water content of the body under normal conditions should be fairly constant the amount eliminated from the body should balance the water drunk plus the metabolic water. Water loss occurs principally through the lungs, skin, faeces and the urine. The obligatory loss of water through the urine in the normal average adult goat was measured to be about 1500 ml daily. The facultative loss was considered to be approximately equivalent to the oxidative requirement i.e. about 2000 ml daily, leaving some 500 ml to be eliminated by other means. Pulmonary ventilation and losses from faeces and skin account for a considerable amount so is the loss from the digestive tract which is several times greater than the total plasma volume (Dukes, 1955). Most of this is retrieved in the normal animal but in pathological conditions of the gut much of it is lost to the body.

In irradiated goats the average amount of water drunk daily was 425 ml in non-survivors and 1400 in survivors, and the daily urine output was 650 ml.

A common finding at post mortem was a spasm and obstruction of the pylorus together with mucosal denudation of the intestines. An osmotic imbalance was apparent between the different parts of the alimentary tract; often much fluid was seen in the forestomachs and none in the rest of the tract. At other times a frank dehydration was the general picture. Haemoconcentration often seen in general water depletion was observed in the goats irradiated with the highest doses (Fig. 20).

It is probable that the natural safeguards to water depletion in the animal body were adversely affected by radiation: for example, damage to the renal tubules may have affected the homeostatic liberation of the anti-diuretic hormone from the posterior lobe of the pituitary and the urge to drinking may have been indirectly affected.

In monogastric animals e.g. the dog, the diuretic response is not affected by the retention of water in the stomach but is affected by the absorption from the intestines combined with the effects of the anti-diuretic hormone (Klisiecki et al, 1932). In ruminants, which are poly-gastric, water absorption is slower than in non-ruminants (Dalton, 1964). Andersson (1955) did not observe any adverse effects in goats given water loads equivalent to 20% of their body weights other than a prolonged diuretic response. In addition, the goat possesses a capacious colon capable of minimising water loss and this may have been a factor in producing some of the inconsistent clinical and lethal responses observed after irradiation. In the horse the colon is even larger than in the ruminant herbivore and may well account in part for the notoriously anomalous and unpredictable clinical responses reported in that species.(Rust et al 1955).

Impaired water balance implies an electrolyte imbalance as well so that an abnormal decrease in bodily fluid (dehydration) involves both water and electrolyte - hormone relationships. In irradiated goats dehydration was caused by

1) reduced intake of water
2) severe losses from dyspnoea, fever, renal dysfunction and occasionally diarrhoea and
3) loss from haemorrhages.

It was considered that many of the more general clinical signs occurring after irradiation could be indirect manifestations of a water depletion: for example, the apathy, the general asthenia and weakness, and the cardiac signs could be attributed to a minor depletion whereas ataxia and collapse to a more severe deficiency.

Table XXI — The Daily Water Consumption of Goats (Non-Survivors) exposed to X irradiation. (ml).

Period after Irrad.	C229 2000r	C234 1000r	C175 700r	F40 700r	F27 700r	F14 650r	F24 650r	C315 600r	C118 550r	C200r 550r	F20 500r	Mean Daily Consumption
1 day	Nil	Nil	Nil	500	1650	1000	750	Nil	250	Nil	Nil	377
2 days	Nil	Nil	Nil	250	1500	Nil	Nil	500	Nil	Nil	800	277
3 days	Nil	Nil	Nil	500	1000	500	Nil	Nil	500	500	Nil	273
4 days	Nil	Nil	Nil	1100	Nil	Nil	500	Nil	1000	Nil	Nil	236
5 days	Nil	Nil	500	700	1200	Nil	Nil	600	500	700	750	460
6 days	Nil	Nil	500	1300	700	Nil	Nil	400	1000	500	600	455
7 days	Nil	Nil	500	400	1900	Nil	500	Nil	750	1000	Nil	460
8 days	Dead	Dead	500	1200	Nil	200	500	Nil	750	700	250	372
9 days	--	--	Nil	1400	1000	Nil	Nil	600	Nil	3000	300	700
10 days	--	--	Nil	100	1000	500	200	400	500	800	1500	555
11 days	--	--	Dead	500	1500	500	Nil	Nil	Nil	Nil	Nil	277
12 days	--	--	--	Nil	1600	Nil	100	Nil	Nil	Dead	Dead	212
13 days	--	--	--	Dead	2000	500	Nil	800	250	--	--	591
14 days	--	--	--	--	1800	1000	Dead	600	Nil	--	--	680
15 days	--	--	--	--	2000	4000	--	400	Nil	--	--	1600
16 days	--	--	--	--	1000	4000	--	200	Nil	--	--	1300
17 days	--	--	--	--	900	Nil	--	1100	Nil	--	--	500
18 days	--	--	--	--	3000	1000	--	Nil	Dead	--	--	1000
19 days	--	--	--	--	1500	1000	--	Nil	--	--	--	833
20 days	--	--	--	--	750	Nil	--	400	--	--	--	383
21 days	--	--	--	--	1500	3800	--	Nil	--	--	--	1767
22 days	--	--	--	--	500	1100	--	200	--	--	--	600
23 days	--	--	--	--	2000	2000	--	800	--	--	--	1600
24 days	--	--	--	--	1000	1000	--	Nil	--	--	--	667
25 days	--	--	--	--	1000	5000	--	Nil	--	--	--	2000
26 days					5000	2000		Nil				2333
27 days					1500	2500		Nil				1333
28 days					1500	Nil		Nil				500
29 days					1500	200		Nil				567
30 days					2000	500		Dead				833
31 days					1500	1000						1250
32 days					2200	400						1300
33 days					Nil	1000						500
34 days					100	800						450
35 days					750	1000						875
36 days					900	500						700
37 days					600	1000						800
38 days					2000	1500						1750
39days					1500	1000						1250
40 days					1200	1500						1350
41 days					500	Nil						250
42 days					250	Dead						125
43 days					1500							1500
44 days					500							500
45 days					400							400
46days					Dead							All Dead
Average Daily Water Consumption of each goat after Irradiation	NIL	NIL	180	600	1250	1000	180	230	300	600	350	

Table XXI a

Mean Daily Water Consumption of Non-Survivors before Death

DAYS BEFORE DEATH	WATER CONSUMPTION (ML)
On day of death	NIL
1 day	36
2 days	445
3 days	582
4 days	436
5 days	405
6 days	382
7 days	595
8 days	467
9 days	356
10 days	472
11 days	344
12 days	350
13 days	510
14 days	850
15 days	1225
16 days	1150
17 days	1888

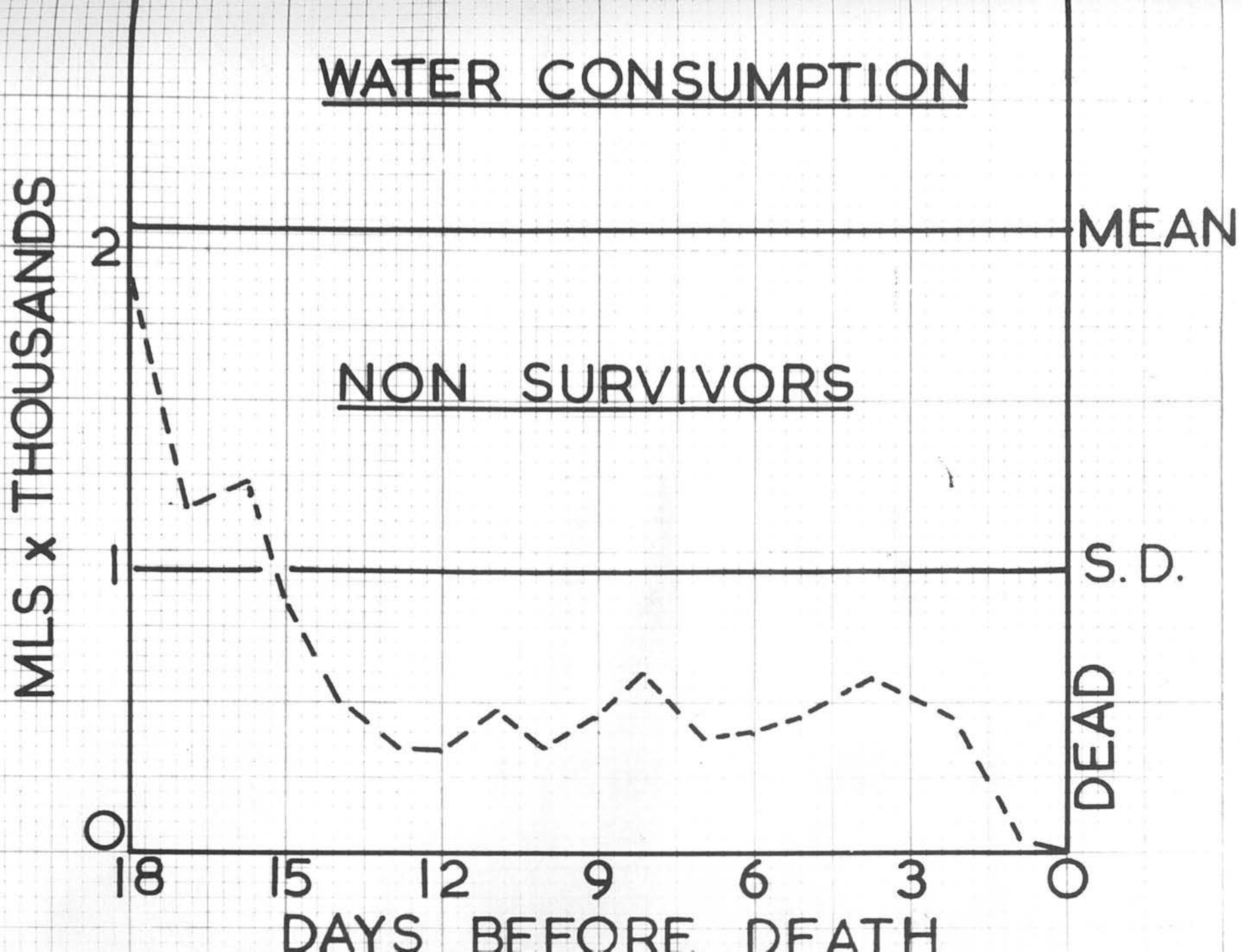

WATER CONSUMPTION
MEAN
NON SURVIVORS
S.D.
DEAD
MLS x THOUSANDS
2
1
0
18
15
12
9
6
3
0
DAYS BEFORE DEATH

Table XXII — The Daily Water Consumption of Goats exposed to X irradiation. (ml).

Survivors

Period after Irrad.	F31 650r	F30 600r	M9 600r	M3 500r	M1 500r	C240 500r	W316 400r	M10 400r	W302 400r	Mean Daily Consumption
1 day	2500	200	3000	4000	5000	2000	250	3500	Nil	2270
2 days	1900	Nil	2500	4500	200	Nil	500	3500	500	1510
3 days	1000	Nil	200	3000	2000	250	1000	3000	250	1190
4 days	Nil	Nil	500	500	1250	1000	500	3500	1000	920
5 days	250	300	1000	4000	2500	750	500	3000	1500	1533
6 days	Nil	200	100	4000	2500	1000	500	3000	600	1322
7 days	Nil	Nil	2500	3700	Nil	1000	3000	4000	500	1633
8 days	500	Nil	3000	3200	750	800	500	2500	500	1305
9 days	Nil	100	200	4500	Nil	1500	1000	2500	1000	1200
10 days	Nil	200	250	4500	250	1000	500	2000	1500	1133
11 days	500	500	Nil	4000	Nil	750	1000	3500	1250	1277
12 days	1500	500	Nil	5300	750	2000	1000	2000	1300	1595
13 days	1500	1000	1000	5000	1000	1500	1500	1500	1000	1667
14 days	150	2000	500	2500	1000	1500	1000	3000	800	1383
15 days	Nil	650	Nil	500	500	1000	1500	2500	Nil	739
16 days	500	Nil	Nil	650	800	1000	2000	2000	2000	995
17 days	700	250	800	800	1000	850	1800	1500	1000	966
18 days	Nil	1600	1000	1500	2000	1500	2000	1750	Nil	1260
19 days	500	1000	500	2000	1000	1000	1400	2000	2000	1265
20 days	200	500	150	3000	1000	2000	1100	2000	500	1160
21 days	800	400	800	4000	2000	2500	1000	1500	1500	1610
22 days	500	500	1500	4000	1500	3000	500	3000	2000	1833
23 days	200	1000	1000	3500	750	2000	1400	3500	1000	1595
24 days	700	700	1000	3000	1000	2000	2000	3000	1000	1600
25 days	1500	800	1500	1500	2000	800	1500	2000	1750	1483
26 days	1650	1000	750	1500	200	1000	500	2000	1500	1122
27 days	1500	1500	1000	800	800	2000	1000	1500	1000	1233
28 days	1000	1000	800	1000	1000	2500	2000	1600	1500	1378
29 days	800	Nil	700	500	500	3000	1000	1750	1600	1095
30 days	1000	1000	1000	1000	500	2000	1500	2500	2500	1333
31 days	2000	Nil	1500	1000	500	1500	500	2250	1700	1217
32 days	Nil	500	Nil	2000	Nil	1000	500	2000	1000	778
33 days	1500	400	2000	2000	1000	800	1000	1500	900	1233
34 days	500	500	2000	4000	2000	900	1000	1500	2000	1378
35 days	1000	1000	1500	2500	2000	2000	500	2000	1750	1583
36 days	1000	1500	1000	3000	3000	1500	1500	3000	1300	1867
37 days	Nil	1000	1000	4000	2500	600	650	3500	2000	1706
38 days	750	1500	750	3000	2500	2500	2000	3000	Nil	1778
39 days	2250	2000	2000	2000	1000	3000	650	4000	1000	1989
40 days	Nil	1500	Nil	2000	1000	2000	1000	1500	1500	1167
41 days	200	1800	750	750	750	2000	1000	1750	1650	1183
42 days	200	2000	200	1000	800	800	1500	2250	1800	1172
43 days	500	2000	500	850	900	1000	1000	2000	2000	1195
44 days	450	3000	500	800	1000	1500	1500	2000	2000	1417
45 days	900	500	2000	1000	2500	1500	1250	3000	1500	1572
46 days	1300	1500	1500	3000	2000	2000	1500	3000	2000	1978
Average Daily Water Consumption of each goat after Irradiation	740	820	965	2475	1240	1475	1130	2450	1230	

iii) Rectal Temperatures

Normal Goats

The average normal temperature of the colony of goats was 101.7°F. The mid day figure was slightly lower (100.9°F) than either morning or evening. The figures given in Table XXIII are those calculated from 363 readings from 26 adult goats.

Table XXIII

Rectal Temperature of Adult Goats

Statistic	Morning	Mid-Day	Evening
Range	100.5 103.8	100.2 103	100.4 103.2
Mean	101.7	100.94	101.7
S.D.	0.71	0.63	0.56
S.E.	0.065	0.043	0.051

Irradiated Goats

Non-Survivors

A series of graphs are presented in Figures 8 to 12 to depict the general effects on the rectal temperature in goats irradiated with various doses of X irradiation.

In all cases (except the goat irradiated with 1000r) the temperature rose to precipitous heights on the last day or two before death; temperatures of up to 110.8 degrees Fahrenheit were recorded.

Temperatures generally were slightly above the normally expected variation after one day and often continued at this higher plateau until the terminal rise occurred.

Survivors

Survivors also sometimes showed increased temperatures slightly above the expected mean soon after irradiation but these rises were intermittent and were never followed by any sudden abnormal elevation as in the goats destined to die (Fig 9).

Discussion

Normal Goats

Dukes (1955), quoting Damant (1916), gives the normal temperature range of American goats as between 101.7°F and 105.3°F with an average of 103.8°F. Jha et al (1961) again in the USA found the average of 4 goats to be 103.4°F in accord with both Dukes (1955) and Spector (1956). In this country a similar figure is given in most text books. It was a surprise therefore to find that in this study the range was between 100.2°F and 103.8°F with a mean of 101.7°F. None of the many conditions capable of causing normal variations in the body temperature (such as age, sex, season, time of day, environmental temperature, exercise, digestion and water consumption) could be considered as valid causes of this observed discrepancy. The explanation perhaps lies in the broader basis from which the figures in this work are drawn.

After Irradiation

The mean temperature immediately after irradiation was 102°F, six hours later it was 101.2°F. and at 24 hours 102.9°F, all of which were within the expected variation. This finding is in contrast to that of Brown et al (1961) who observed that 80% of irradiated cattle showed a temperature rise of between 1 to 3 degrees above normal at the end of the irradiation period.

Trum et al (1952) found that in the burro (donkey) temperatures, for the most part, remained normal except during a short interval before death. A few were subnormal (95° - 98°F) and some were above normal (101 - 107°F). Thomas and Brown (1961) found a similar response in burros to neutron-gamma radiation. However they noticed a rise, which did

not exceed 103.7°F (normal temperature being 99° - 100°F), 4 to 8 hours after exposure but it returned to normal at 12 hours. The non-survivors had a subnormal temperature prior to death.

Following exposure to 600r, the temperature of dogs remained within the normal range for the first 5 to 6 days and, thereafter, the mean temperature progressed to about 106°F until death. After 400r (an approximate LD 90 in dogs) the onset of the temperature increase was later and more gradual (Cronkite and Brecher, 1955).

Within 24 hours post irradiation most of the goats in this study showed temperatures within the normal range (101° - 102.5°) but a few, especially at the highest doses, remained at a higher plateau (102° - 104°). Rosenfeld (1958) noted a significant rise in the rectal temperatures of calves on the fifth day post irradiation as a reliable prognostic indication of early or delayed death. Once the temperature of a calf rose to around 105°F (normal about 102°F) death usually supervened within 3 or 4 days. He also observed that in those animals dying after the tenth day, the diurnal rectal temperature often varied 1 or 2 degrees but remained elevated at least 3 degrees above normal at all times and a sharp decline was noted immediately before death. Although the last observation was similar to that found in this study on goats (i.e. a sudden drop to subnormal temperatures before death) the pattern generally in goats was different from that observed in calves. Normality was quickly achieved and continued in the majority of non-surviving goats until a sudden precipitous rise became a reliable prognostic sign of death within 24 hours.

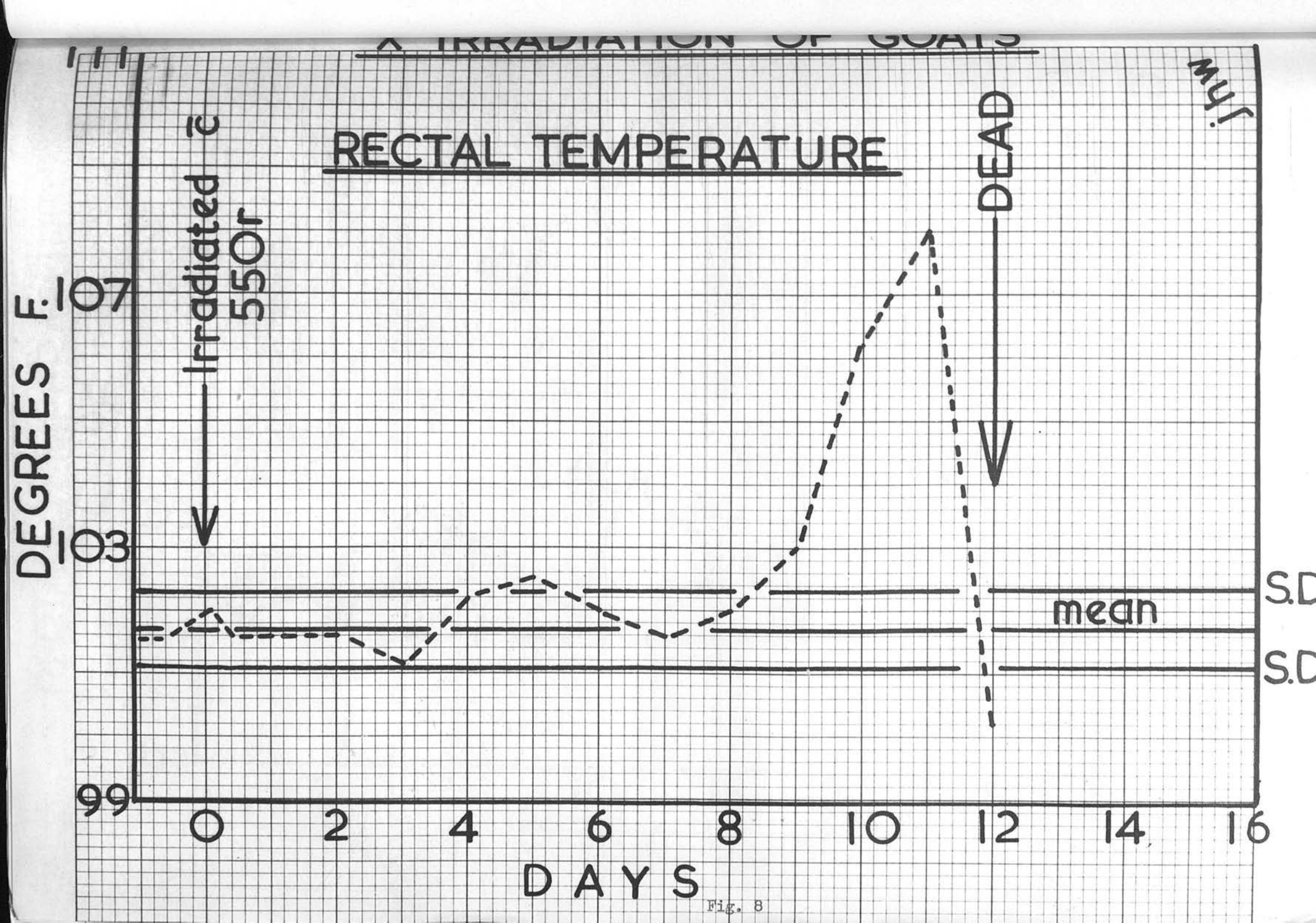

X IRRADIATION OF GOATS
RECTAL TEMPERATURE
DEGREES F.
111
107
103
99
Irradiated
550r
DEAD
mean
S.D
S.D
0
2
4
6
8
10
12
14
16
DAYS

Fig. 8

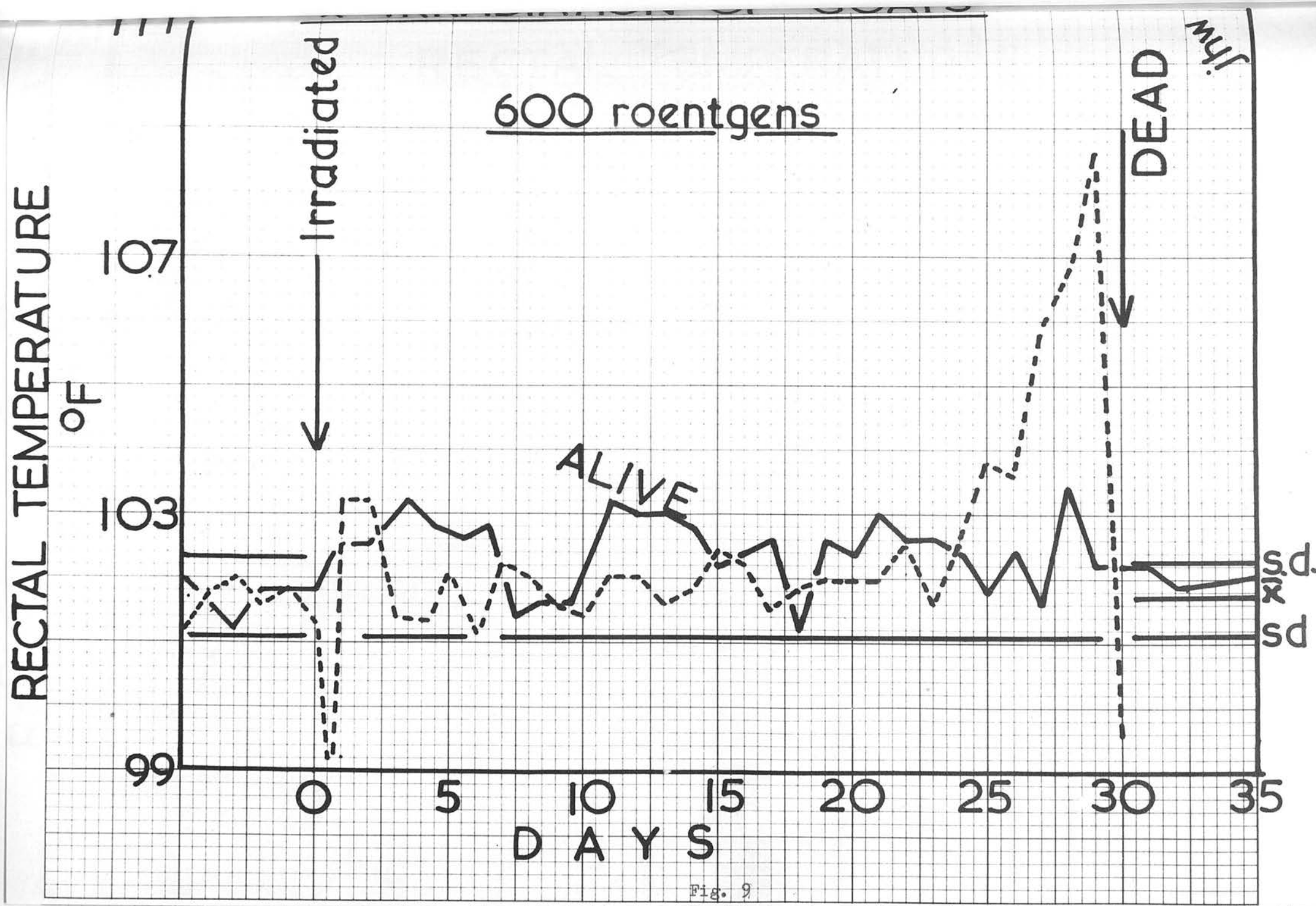

600 roentgens
Irradiated
DEAD
ALIVE
RECTAL TEMPERATURE °F
107
103
99
0
5
10
15
20
25
30
35
DAYS
s.d.
x
sd

Fig. 9

Fig. 11

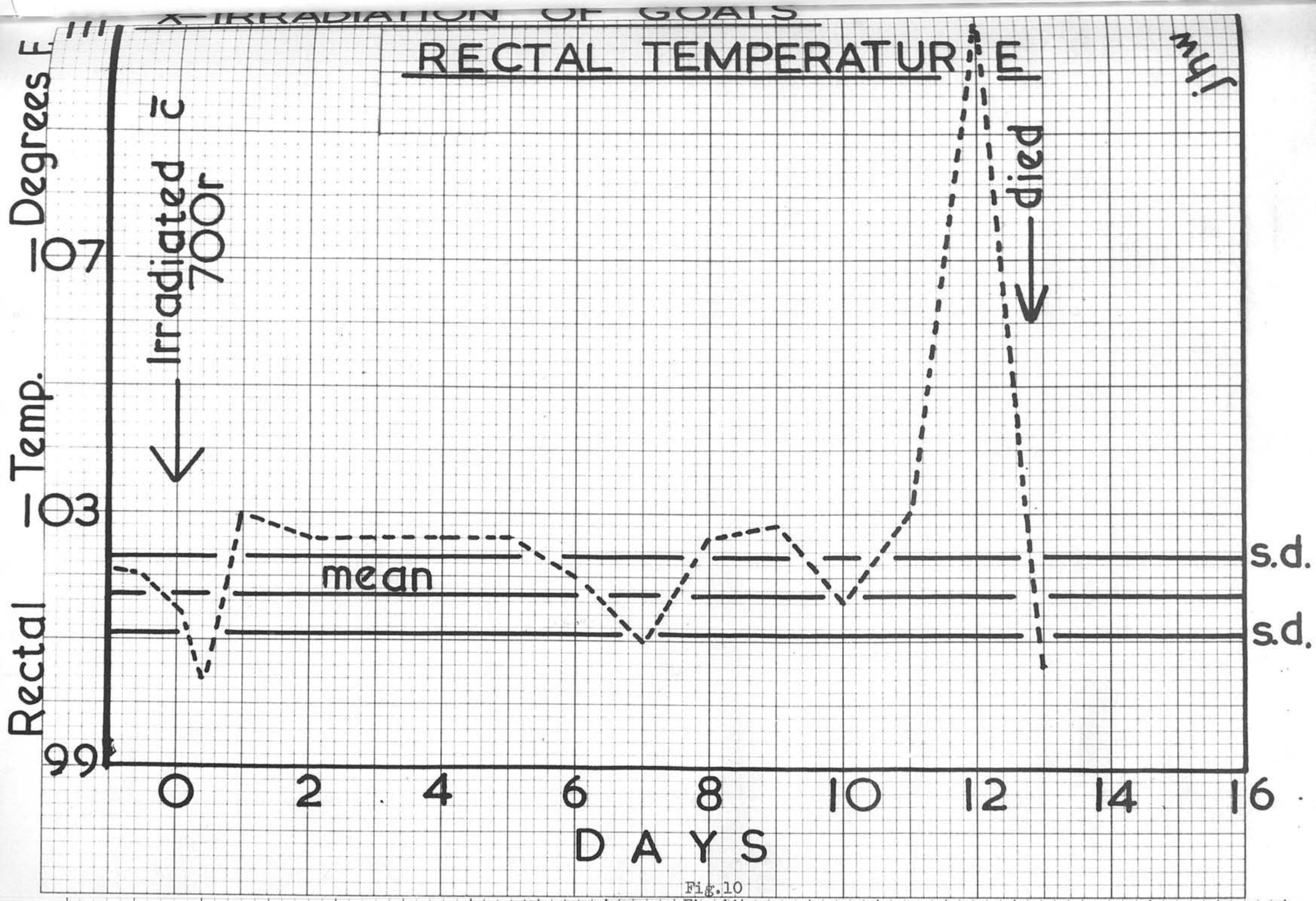

X-IRRADIATION OF GOATS
RECTAL TEMPERATURE
Jan.
Degrees F.
111
107
103
99
Rectal Temp.
Irradiated 700r
died
mean
s.d.
s.d.
0
2
4
6
8
10
12
14
16
DAYS

Fig.10
Fig.11

X IRRADIATION OF GOATS

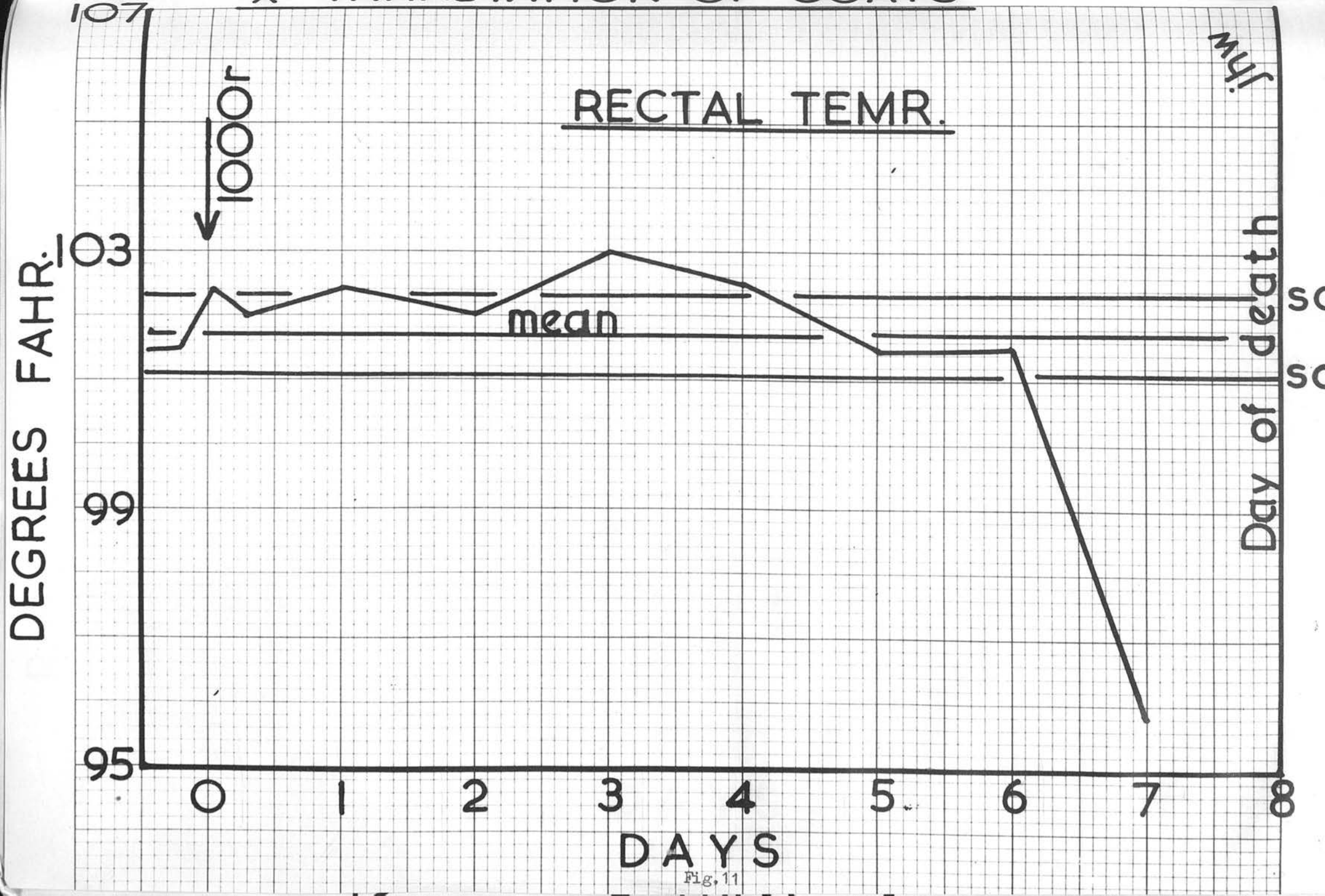


Fig. 11

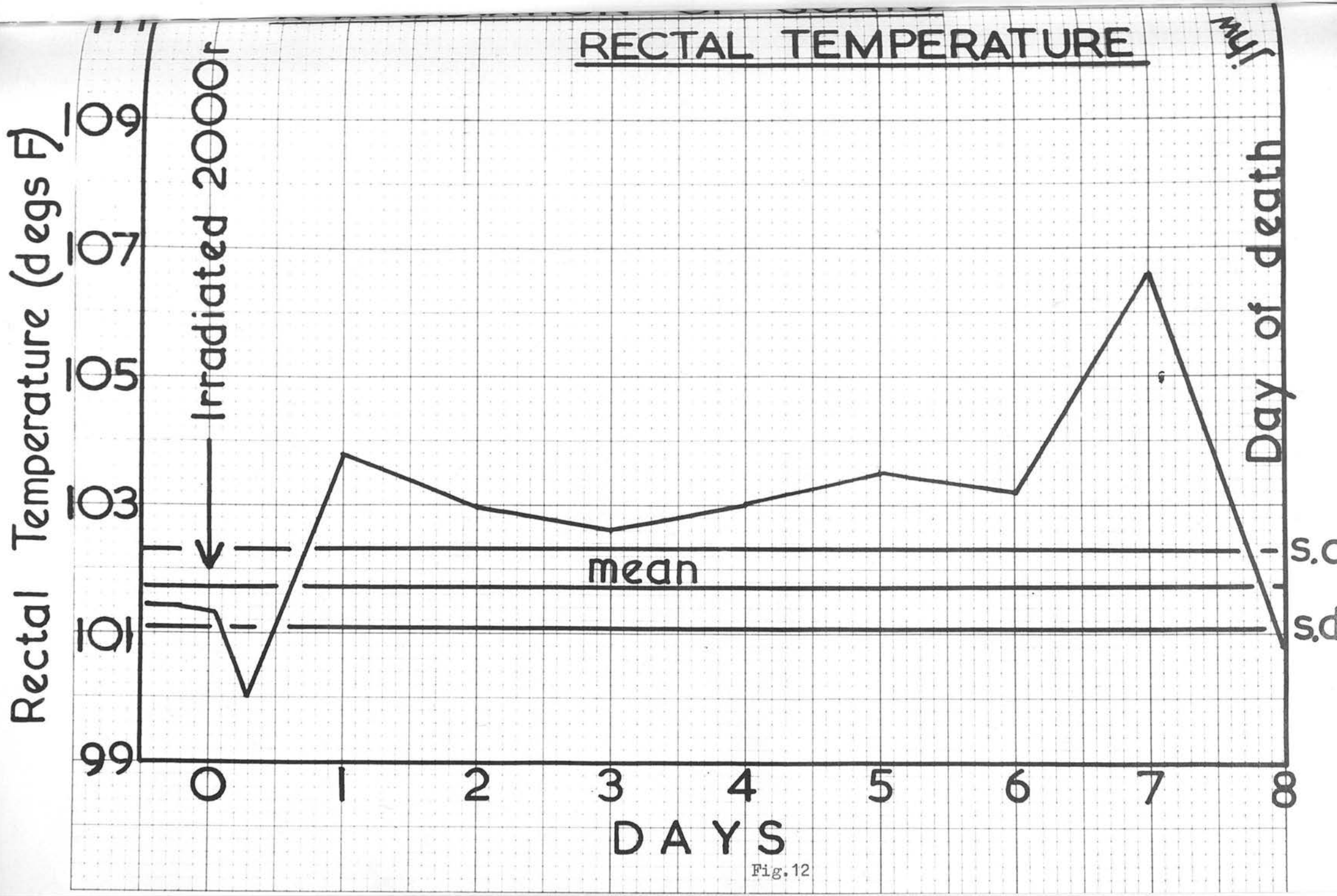

RECTAL TEMPERATURE
Rectal Temperature (degs F)
109
107
105
103
101
99
Irradiated 2000r
mean
s.d
Day of death
0
1
2
3
4
5
6
7
8
DAYS

Fig.12

The Inter relationship of the Rectal Temperature and the Granulocyte Count

The development of fever in goats was studied and it appeared to be a consistent precursor of death as illustrated in the graphs. In addition the development of fever was well correlated with the absolute depression of the granulocytes and the rate at which the count fell. The mean temperature and granulocyte counts for nearly a fortnight before death are given in Table XXIV and in Figure 13. This gives a clear impression that a gradual step-like rise of the temperature above the normal range starts 7 days before death and continues to its peak at the penultimate day after which it fell abruptly to subnormal values.

On the other hand, the mean granulocyte count began falling 10 days before death, rallied slightly for a few days after which they suffered a dramatic reduction just prior to death.

It is probable that this correlation is due to the susceptibility to infection brought about by the granulocytopenia. This bacterial infection was probably one mainly of Clos. Welchii judging from the clinical signs and symptoms shown and the subsequent morbid anatomical and histopathological findings. Similar factors i.e. bacterial infection consequent upon a depression of granulocytes and fever, were evident in studies with dogs (Cronkite and Brecher (1952)).

Table XXIV

X-Irradiation of Goats

The Mean Granulocyte Count and Rectal

Temperature of Non-Survivors

Days before Death	Granulocytes (Cells/mm^3)	Mean Rectal Temperature (^oF)
12	5234	101.6
11	4605	101.4
10	2794	101.8
9	2565	101.9
8	3130	101.5
7	2684	102.2
6	1439	102.4
5	401	103.1
4	121	103.2
3	60	104.0
2	29	104.8
1	14	106.4
0 (DAY OF DEATH)	19	100.2

DEGREES °F
107
106
105
104
103
102
101
100
DEATH
TEMPERATURE
GRANULOCYTES
0
4
8 DAYS
12
6
5
4
3
2
1
0
THOUSANDS PER CU. MM.
CELLS

Fig. 13

Respiratory Frequency, Heart Rate and Heart Sounds

NORMAL GOATS

Respiratory Frequency

The average number of respirations per minute in normal adult goats was observed to be between 21 and 22. This was the mean of 104 observations on 25 goats.

Range	14 to 30
Mean	21.59
S.D.	3.51
S.E.	0.35

Heart Rate

The average number of heart beats per minute in normal adult goats at rest was observed to be between 108 and 109. This was the mean of 222 observations on 40 goats.

Range	80 to 160
Mean	108.58
S.D.	16.40
S.E.	1.11

Heart Sounds

Only two main heart sounds were recognized as follows:

i) LUB ... when the atrio-ventricular valves closed

ii) DUPP .. when the semi-lunar valves closed.

It was occasionally possible to recognize a splitting of each of these sounds due to asynchronous closure of the valves (Figure 14). Unfortunately no electrocardiographic nor phonocardiographic studies could be carried out.

The anatomical and the physiological areas of auscultation were conveniently divided as follows:

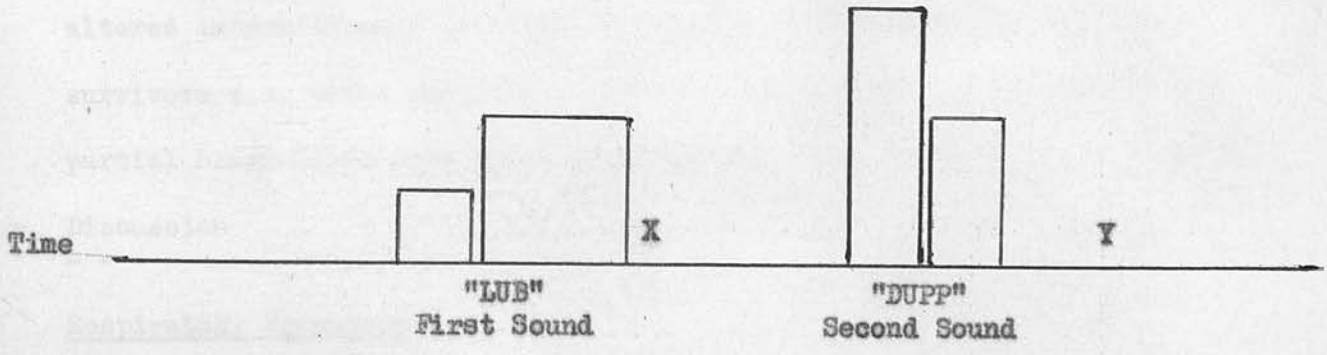
i) The Mitral Area, in which the first sound was heard on the left side of the thorax between the 5th and 6th intercostal space.

ii) The Aortic Area, in which the second sound was best heard between the 3rd and 4th left intercostal space. Whenever there was an abnormal difference between the physiological findings and the expected anatomical areas then a cardiac atrophy or hypertrophy was suspected.

Cardiac arrhythmias and murmurs were the principal dysfunctions and turbulences noted:

i) Arrhythmias were either atrial fibrillations, heart block (partial or complete) or ventricular extra systoles

ii) Murmurs were classified by their duration, quality, location and loudness. Regular systolic and diastolic murmurs were considered to be pathological whereas cardiorespiratory or occasionally intermittent murmurs were taken as non-pathological.



X - area of systolic murmurs
Y - area of diastolic murmurs

Fig. 14

Heart Sounds in Goats

IRRADIATED GOATS

Figures 14aand 15 present in graphical form the details of the daily observations in a non-surviving and a surviving goat (dose 600r) as an example of the effects of X irradiation on the heart rate and respiratory frequency.

Non-Survivors

The respiratory frequency increased progressively with time after irradiation in most goats reaching a positive dyspnoea prior to death. The heart rate was increased soon after irradiation and also terminally. The character of the beat was altered intermittently throughout the post irradiation period: the lub-dupp was usually more pronounced, often there was an arrhythmia usually atrial fibrillation with a partial heart block super-imposed. Crescendo-decrescendo systolic murmurs and decrescendo diastolic murmurs were common.

Survivors

The respiratory frequency was little affected, being slightly increased intermittently but more usually normal throughout the period of observations.

The heart rate was increased slightly initially soon after irradiation but settled to within the normal range in a day or two and remained so thereafter in the main. The character of the beat was sometimes altered intermittently in a similar manner as described for the non-survivors i.e. sinus arrhythmia, atrial fibrillation and incomplete and partial heart-block with occasional murmurs, both systolic and diastolic.

Discussion

Respiratory Frequency

Dukes (1955) gave the range of the frequency in normal mature goats as between 12 and 20 per minute. Jha et al (1961) found that respirations ranged from 21 to 35 per minute with a mean for 8 goats of 28 per minute.

The findings in this work, viz 21 to 22 per minute, was in accord more with Dukes (1955) than with the Jha et al (1961) or Spector (1956). It must be borne in mind that the respiratory frequency is subject to such variations as body size, age, exercise, excitement, environmental temperature, pregnancy and the degree of the fullness of the digestive tract especially of the rumen. In addition many pathological conditions involving fever will affect the frequency. It was obvious from this study that the X irradiation was sufficient to produce a combination of cell injury, oedema of the lungs and an accompanying febrile state to cause a clinically observable tachypnoea. This was not alluded to by Swift et al (1946) in their study on X radiation on goats. In fact few workers allude to respiratory frequency in their studies of the effects of radiation on the various animal species. Trum et al (1952) noted that respiratory movements in burros in a semi-comatose condition after gamma irradiation were slow and shallow: other animals, which were believed to be suffering from an anaemic anoxia had an increased respiration rate and pulse. In adult cattle subjected to Co60 gamma radiation dyspnoea, which was associated with an oedema of the larynx and lungs, usually progressed from a mild to a pronounced state over a period of 3 to 4 days (Brown et al 1961): this was similar to findings in this study in goats. Rosenfeld (1958) reported marked respiratory distress with much salivation in calves for 24 hours before exitus. In this work goats which died showed a gradually developing hyperpnoea, which later became rapid shallow and panting in type (polypnoea) and a day or so before death it developed into a laboured phase (dyspnoea). The finding of oedema of the larynx, trachea and lungs together with facial oedema at post-mortem together with haemorrhagic congestion and pneumonic consolidation adequately explained the manifestation of these clinical signs.

Heart Rate and Sound

Jha et al (1961) observed that the heart rate for 8 goats ranged from 90 to 150 cycles per minute, with a mean of 105. This was in close agreement with the findings in this work of between 108 to 109 cycles per minute but both are higher than that reported by Dukes (1955) who gives a figure of between 70 to 80 beats per minute. Unfortunately it was not possible to carry out any electrocardiographic or phonocardiographic studies which would have been all important in confirming the clinical findings by auscultation of the disturbances caused by the irradiation. These, as we have seen, consisted essentially of an intermittent increase in rate and a change of character mainly in rhythm and conduction. It was subsequently gratifying to encounter on the post mortem table common evidence of massive subendothelial haemorrhages most noticeable in the ventricles especially around the papillary processes of the heart. Further, on histological examinations of heart sections, petechial haemorrhages were often observed around Purkinje fibres and His's bundle; in addition polysaccharide PAS-positive globules were also seen in these sites (Photo 49). Sufficient evidence it was thought to conclude that the nature of the cardiac changes clinically observed was due to alterations in conduction probably brought about by the collection of foreign material around the nervous tissue of the heart. Rust el at (1955) carried out electrocardiographic studies in burros and noticed similar aberrations. They concluded that disturbances in the heart originated peripherally and not from the CNS.

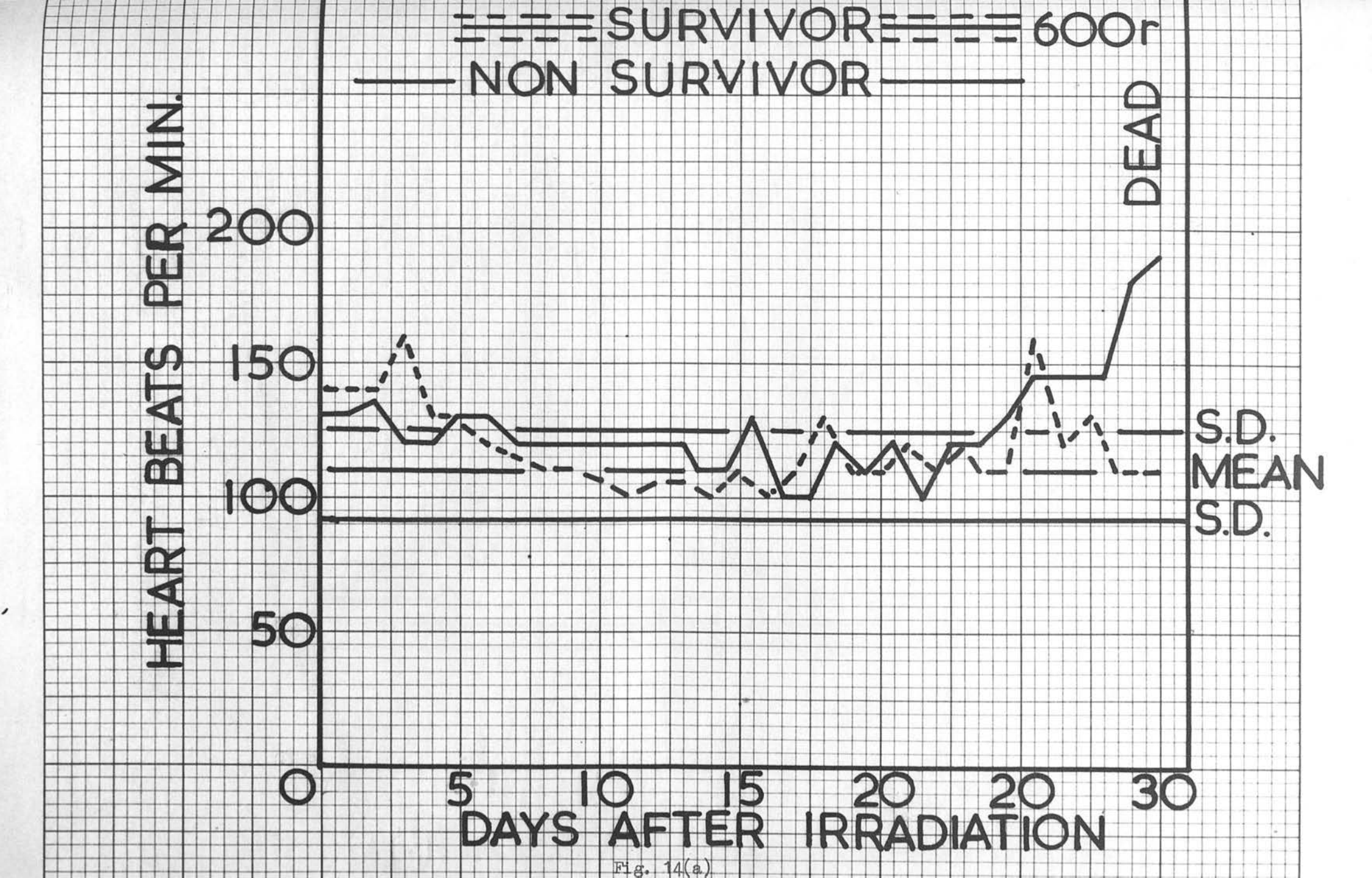

SURVIVOR 600r
NON SURVIVOR
DEAD
HEART BEATS PER MIN.
200
150
100
50
0
S.D.
MEAN
S.D.
5
10
15
20
20
30
DAYS AFTER IRRADIATION

Fig. 14(a)

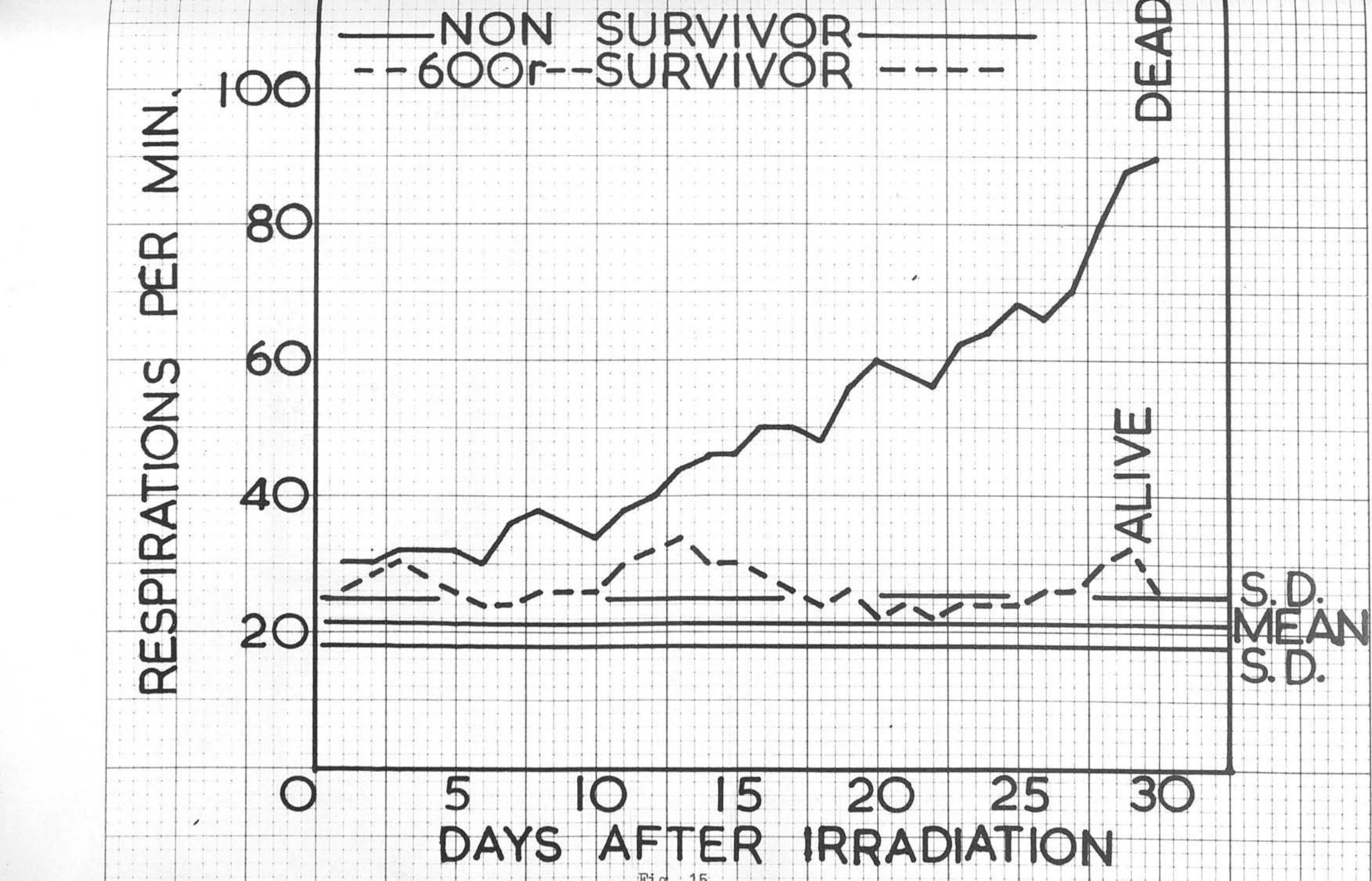

NON SURVIVOR
600r SURVIVOR
RESPIRATIONS PER MIN.
100
80
60
40
20
0
5
10
15
20
25
30
DAYS AFTER IRRADIATION
DEAD
ALIVE
S. D.
MEAN
S. D.

Fig. 15

v) Urine Examination

The average clinical findings of normal and irradiated goats are given in Table XXV. Detailed results are attached at Appendix III.

Table XXV

Urinalysis

Constituent	Normal Goats	Irradiated Goats
Number of Samples (Goats)	52 (52)	39 (20)
Appearance	Limpid and Pale Yellow	Varied from normal to dark, red brown and turbid.
Specific Gravity		
Range	1001 to 1060	1003 to 1102
Mean	1013	1037
S.D.	12.9	7.9
S.E.	1.8	1.25
Average Daily Amount passed	1500 ml	650 ml
Reaction	Alkaline	Alkaline (2 acid)
Protein	Nil	Yes in 67%. Usually in significant quantities within $\frac{1}{2}$ to 3 hours post irradiation.
Blood	Yes in 9.6%	Yes in 56% Haematuria in 60%
	Haemoglobinuria NIL	Haemoglobinuria in 45%
Sugar	NIL	Yes in 38%
Bilirubin	Nil	Yes in 18%
Bile Salts	Nil	Nil
Acetone	Nil	Nil
Deposits	Yes	Yes

Discussion

Appearance: It was soon discovered that the appearance of the urine in irradiated goats, with the exception of those which contained large quantities of blood or blood pigment (Photo 26), was often misleading.

Specific Gravity: The increase of the specific gravity in irradiated goats reflected the observed oliguria.

Reaction: Alkalinity was expected in the goat as in other herbivorous animals. The two samples which were acid were collected during a period of increased rectal temperature.

Protein and Acetone: In this study acetone was never found in the urine either in normal or irradiated goats. 67% of the samples i.e. 26 out of 39, were positive for albumin. These were usually first observed within $\frac{1}{2}$ to 3 hours but were more consistently detected on and after the 6th day post irradiation. There appeared to be no correlation with temperature. Brown et al (1961) in their work on cobalt 60 gamma radiation in cattle reported finding albumin and acetone in the urine in significant quantities: these were first detected 12 to 14 days post irradiation and in general were associated with increased temperature.

Trum et al (1952) did not find any clinical changes suggestive of kidney damage in the urine of burros exposed to gamma radiation.

Sugar and Other Constituents

38% of the samples of urine of irradiated goats showed the presence of reducing substances. As none of the urine of the 52 normal goats examined showed any sugar this glycosuria in irradiated goats was taken to be due to a hyperglycaemia and not a physiological renal glycosuria.

The presence of bile pigment in 18% irradiated goats indicated a pre-renal condition probably stemming from the liver, blood stream or alimentary canal.

The deposits were either inorganic (phosphates and carbonates) or organic (rbcs, leucocytes and renal epithelial cells and casts) and were common in both normal and irradiated goats.

Blood

Out of 39 urine samples tested from the 20 irradiated goats, 22 samples showed a haematuria and of these 11 also showed a haemoglobinuria. The 22 samples came from 12 different goats and the 11 urines showing a haemoglobinuria came from 9 goats. (Table XXVI).

Table XXVI

Goat No.	Dose (r)	Haematuria	Haemoglobinuria	Number of Samples of Urine with Time of Collection after Irradiation.
NON-SURVIVORS (16 positive samples)				
C229	2000	Yes	Yes	1 (1 hour)
C234	1000	Yes	Yes	2 (2 days; 5 days)
F 27	700	Yes	Yes	2 (3 hours; 4 hours)
F 40	700	Yes	No	1 ($\frac{1}{2}$ hour)
F 24	650	Yes	Yes	1 (1 hour)
C315	600	Yes	Yes	2 (4 hours; 11 days) 2 (29 days and 30 days (day of death))
F 20	550	Yes	Yes	1 (4 days)
C118	500	Yes	Yes	4 ($2\frac{1}{2}$ hours; 1 day, 2 days; 16 days)
TOTALS		8 Goats	7 Goats	16 Samples
SURVIVORS (6 positive samples)				
F 30	600	Yes	Yes	1 (2 hours)
C240	500	Yes	No	1 ($\frac{1}{2}$ hour)
W302	400	Yes	Yes	1 ($\frac{1}{2}$ hour)
W316	400	Yes	No	2 ($\frac{1}{2}$ hour) 1 (6 hours)
TOTALS		4 Goats	2 Goats	6 Samples
GRAND TOTALS		12 Goats	9 Goats	22 Samples of urine.

The haemoglobinuria shown in 9 goats (7 in non-survivors and 2 in survivors) appeared without obvious pattern or correlation although the numbers of non-survivors affected (64%) was significantly greater

than the survivors (22%): however, in both groups the appearance of the haemoglobinuria usually occurred a few hours after irradiation but seldom thereafter. Only 5 samples of normal goats urine (9.6%) showed any red blood cells and of these only one (2%) could be diagnosed clinically as haematuria, none manifested haemoglobinuria. The difference in irradiated goats as compared to normal goats is too great to be ignored; it can be explained by abnormal renal damage as a result of the direct or indirect effects of irradiation reinforced possibly by the presence of a haemolytic factor causing intravascular haemolysis leading to a haemoglobinaemia (which was often seen on spectroscopic examination) and subsequent haemoglobinuria. It is conceded that haemolysed plasma is more often caused by erythrocytic trauma during blood taking than by disease but, the number of irradiated goats showing this sign appeared to be quite significant as compared to normal goats undergoing similar hazards from the same experimenter. In addition, the mean red blood cell fragility of the irradiated goats, both survivors and non-survivors, was slightly greater than that of normal goats although not significantly so. It is possible that a haemoglobinaemia was commoner than was either observed or suspected judging from the haemosiderinuria and the not uncommon finding of a haemosiderosis in many organs seen at post-mortem. The threshold value for the excretion of haemoglobin depends largely upon the extent to which the cells of the tubules of the kidney can split it: when the amount of haemoglobin is larger than can be dealt with a haemoglobinuria is the result. Where the renal tissue is damaged in any way, e.g. possibly by irradiation, the threshold will be lower. Haematuria, on the other hand, although an important sign sometimes, occurs in very many more groups of conditions e.g. injuries,

many circulatory disturbances, in inflammatory states such as nephritis and lesions of bladder or ureter. The ruminant herbivore is particularly susceptible to certain toxic factors which lead to intravascular haemolysis: for example, the feeding of marrow-stem kale in cattle causes a syndrome characterised by gross anaemia and haemoglobinuria (Rosenberger, 1939; Clegg and Evans, 1962) as does the excessive feeding of white cabbage (Schubert, 1954). Gordon et al (1940) reported a haematuria-haemoglobinuria in sheep artificially infected with Clos. welchii, a common intestinal pathogen in goats. Similar syndromes to those seen in goats receiving MLD doses of X radiation occur in other diseases of ruminants, for example bracken poisoning and the enterotoxaemias (see Differential Diagnosis). Potter (1958) reported that haemoglobinuria occurred in sheep anaesthetized by an intravenous injection of pentobarbitone sodium (Nembutal) containing propylene glycol, which is a common menstruum. The possibility therefore that either some ingredient of the foodstuffs fed to the goats or the narcotic agent used in sedating the animals might have been the cause of the observed haemoglobinuria-haematuria was carefully considered. The food could be quickly eliminated as both normals and irradiated goats received the same, and as for the narcotic agents none contained propylene glycol and the menstruum used for the phencyclidine did not produce the signs when injected into goats separately. As haemoglobinuria had in fact occurred in one irradiated goat which had not been sedated it was reasonable to exclude this source. It was therefore tentatively concluded from the observable evidence that the haemoglobinuria-haematuria seen in irradiated goats was probably in part due to renal damage caused by irradiation and the indirect effect of a bacterial toxaemia. However as the condition was confined to the time just after irradiation other factors such as manipulation cannot be excluded altogether. This aspect warrants further research.

26. Specimen of Urine from Irradiated Goats showing Different degrees of Haematuria - Haemoglobinuria

vi) Faeces Examination for Internal Parasites

Normal Goats

Table XXVII presents a summary of the results of the examinations of faeces from 58 adult goats on entry to the goat colony.

Table XXVII

Internal Parasites of Goats

Type of Infestation	No. of Goats affected	Percentage affected	Range (Ova per gms.)
Strongyloid spp.	30	52	100 to 1500
Nematodirus	13	22	100 to 1200
Fasciola hepatica	8	14	50 to 200
Trichuris	6	10	100 to 350
Moniezia spp.	3	5	50 to 200
Lungworms	3	5	--
Coccidia	18	30	--

Thirty out of the 58 adult goats bought in at the commencement of the experiment showed evidence on arrival of infestation with strongyloids: of these 16 goats had egg counts of between 500 to 1500 per gramme of faeces.

Thirteen goats showed signs of nematodirus infestation with egg counts varying between 100 and 1200 eggs per gramme of faeces; six had evidence of trichuris, eight of liver fluke, three of tape-worms, three of lung-worms and eighteen of coccidia.

Irradiated Goats

All irradiated goats showed negative or very low egg counts and were free from lungworm larvae throughout the period of observation. Because of the regular anthelmintic treatment before irradiation these negative findings could not be taken per se as the effect of the irradiation.

Diarrhoea was uncommon as a general rule in irradiated goats. In 7 non-survivors it occurred a day or two before death but was seldom observed during the course of the actual radiation syndrome. In some of these cases the faeces was of a treacly consistency and dark red to purplish in colour. Only two survivors showed any signs of abnormality in faeces which were semi-solid and not pelletted. This occurred a few days after irradiation and soon cleared up. Swift et al (1946) reported similar findings but Cronkite (1950) observed continuous and severe diarrhoea in goats exposed at Bikini.

The diarrhoea encountered in this study was both intestinal and colonic in origin, but one or the other form usually predominated. The main causes of the diarrhoea were considered to be mucosal as a direct result of irradiation brought about by epithelial denudation, together with bacterial infection and intoxication. Intraluminal causes, such as parasitism, were ruled out because of the effective anthelmintic treatment carried out before irradiation and already referred to above.

vii) Miscellaneous Clinical Manifestations

Epilation

Epilation occurs in human beings approximately two weeks after irradiation. Delayed epilation has been reported to occur in burros after neutron-gamma radiation (Thomas et al, 1961) but not after lethal or sub-lethal doses of gamma radiation alone (Trum et al 1952) nor in cattle, calves, sheep or dogs (Brown et al 1961; Rosenfeld 1958; ORO-145 USAEC, 1955).

In this study on goats, a total of 7 goats (35%) showed signs of abnormal shedding of hairs: five of these were non-survivors, and two were survivors. There was some variation in individual goats both in time of onset of epilation and the type. Generally speaking, it appeared in non-survivors sooner (between the fifth and twelfth days after irradiation) than in survivors (between the eleventh and twentieth days post-irradiation). Duration of epilation continued for an average time of ten days in non-survivors and fifteen days in survivors.

Loosening of hair occurred on both sides of the body, belly and neck; the long outer hair was shed but the soft under-coat remained intact in most cases. Those goats which were irradiated in the wire cage showed a characteristic stripe effect (see photos 24 and 25) due to the long outer hairs still remaining on the parts of the body which were shielded by the wire mesh. Normal hair regrowth with no change in colour occurred in survivors after five to six months.

Other Skin Conditions

Hyperaesthesia of the skin was apparent for about a week after irradiation but then disappeared suddenly. Four non-survivors showed subcutaneous oedema of the facial region of the head (photo 21) during the last few days before death. Three of these goats also showed

swellings of the orbital cavity (photo 23) which on post mortem were seen to be haematomata. None of the survivors showed oedema of the face but a few manifested petechial haemorrhages in the conjunctiva and mucous membranes of the mouth and nasal cavities: all non-survivors showed some signs of petechiae in the mucous membranes of mouth, nose and eye. One non-survivor (F20) suffered from a pustular dermatitis all over its body after irradiation. No survivor was thus affected. Trum et al (1952) observed hyperaesthesia and facial oedema in burros irradiated with large doses of external whole body gamma irradiation. After MLD and lower doses of radiation infection of all bodily tissues is much more prevalent (Chrom, 1935; Lawrence and Tennant, 1937); this phenomenon was also observed in animals exposed to the atomic bomb (Tullis 1949). Brecher and Cronkite (1951) reported a clinical infection in dogs showing a cellulitis of the lips, neck and skin of the legs together with oropharyngeal and anal ulceration. However, these signs have not been observed in experiments on ruminants before (Rosenfeld, 1958), although Brown (1962) reported occasional swelling of legs of cattle exposed to gamma radiation.

Discharges

Only two goats showed discharges from nose, eyes and mouth on the day of death. The discharge was viscid in both cases but one was also bloody. Three other non-survivors showed a viscid discharge from the mouth only just prior to death. None of the survivors exhibited discharges of any kind at any time.

viii) Differential Diagnosis.

The clinical signs and general pathological findings in the acute radiation syndrome in ruminants resemble those of a number of other conditions. Differential diagnosis is therefore important although it may be difficult. Perhaps one of the most dangerous diseases with which it may be confused is anthrax, in the acute form of which there is pyrexia, cerebral signs, cardiac and respiratory distress followed by a quick death. In the not so acute case rumination ceases, there are bloody discharges and subcutaneous oedematous swellings may occur. Examinations of a blood smear stained with McFadyeans methylene blue easily differentiates anthrax in which an amorphous purplish material is noted between the bacteria. Other conditions and diseases which show similarities to the radiation syndrome include purpura haemorrhagica, haemorrhagic septicaemia, tick fever, various kinds of poisons (bracken, warfarin, heavy metals and TCESOM or tri-chloroethylene-soyabean-meal) and the enterotoxaemias. Table XXVIII details some of the major signs of the more important diseases to emphasise the similarities.

Haemorrhagic septicaemia of goats and sheep caused by Pasteurella sp. usually take the pneumonic form. The affected animals suffer from abnormally high temperatures (108^{o}F or even higher) and show dyspnoea but haemorrhages are not a prominent feature. Purpura haemorrhagica on the other hand is manifested by multiple haemorrhages and subcutaneous oedematous swellings but fortunately is a rare condition. (Hagan et al, 1957).

An insidious haemorrhagic disease similar in some ways to the radiation syndrome can occur in animals which eat toxic quantities of spoiled sweet clover or silage, in which the harmless natural courmarins are converted into toxic dicouramins. Warfarin, a most effective rat poison, is a closely related synthetic chemical. The ingested dicouramins inhibit

the production of prothrombin by the liver by antagonising Vitamin K. All the clinical signs shown are referable to the haemorrhages resulting from faulty blood-clot formation due to hypoprothrombinaemia. (Garner, 1957).

Hughes (1950) has observed that pyruvate oxidation in tissue is depressed by a deficiency of vitamin B1 (thiamine) and that there was a similarity in the lesions found in heavy metal poisoning (e.g. by arsenic, gold, mercury and ~~the~~ thallium) to those seen in this avitaminosis. An interesting observation by Lane et al (1955) was that blood pyruvates in cattle and burros were elevated when the respiratory quotients were depressed after whole body irradiation. This was previously noted in mice by Hevesy and Forrsberg (1951). In bracken poisoning of horses an athiaminosis occurs leading to death from congestive heart failure with nervous complications whereas in ruminants a marked thrombocytopenia occurs together with severe depression of bone marrow activity and death is due to anaemia resulting from multiple petechial haemorrhages (Evans et al 1951, 1954). The fundamental haemorrhagic lesions in bracken poisoning and the radiation syndrome in equidae and cattle appear to arise from the same profound metabolic disturbances: probably this is because of the extreme sensitivity of cellular enzymes to both ionizing radiation and the thiaminase plus aplastic anaemia factor present in bracken fronds. In di-chlorovinyl-cysteine (or TCESOM) poisoning the clinical signs and lesions are those of an aplastic anaemia and closely resemble those of bracken poisoning: the only difference is that whereas in bracken poisoning there is a granulocytic and thrombocytic aplasia, in TCESOM poisoning the erythrocytic series is also affected (Pritchard et al, 1956). It is reasonable to presume that bracken is a naturally-occurring, and TCESOM an artificially-produced, radiomimetic substance.

Basically a common factor is an interference with carbohydrate metabolism. In ruminants this is also the case in many enterotoxaemias caused by several types of Clostridium welchii. These bacteria are widely distributed in nature and are to be found in the intestinal tract of animals where normally they do not cause disease. However, under certain circumstances they multiply rapidly in the lumen of the intestine and produce very potent toxins. Bullen et al (1953) investigated the factors which might influence the growth of Clos. welchii in the gut. They found that under normal conditions the rate of bacterial multiplication was not sufficient to produce disease in ruminants. However, whenever digestive disturbances occurred partially digested food rich in carbohydrates passes from the proventricles (rumen, reticulum, omasum and abomasum) into the intestine. Ruminant herbivores depend upon the ruminal bacteria for the efficient digestion of cellulose; incomplete breakdown quickly leads to digestive disturbances. When incompletely digested food rich in carbohydrates passes into the intestine the latter becomes abnormally filled with starch grains. This is the ideal medium for the propagation of Clos. welchii (which grows at fulminating rapidity in a starchy medium) producing very large amounts of toxin. As the concentration of the toxin increases so there is a corresponding increase of the permeability of the intestinal mucosa. Large quantities of the toxin are quickly absorbed into the circulation causing a toxaemia.

It is probable that total body irradiation at MLD doses causes a sufficient dysfunction of the gastro-intestinal tract to bring about the necessary digestive disturbances in goats to trigger off a similar chain of events. Certainly the observed clinical, haematological and pathological findings in irradiated goats in this study show a remark-

able resemblance to those to be found in the enterotoxaemic diseases of ruminant herbivores. Similar blood and post-mortem changes were described by Gordon et al (1940) following intravenous inoculation of sheep with culture filtrates of Clos. welchii. The diagnosis of the enterotoxaemic syndrome in this study was based on the following criteria:

i) The clinical signs shown by a number of irradiated goats

ii) The incidence of a glycosuria in 38% of irradiated goats (Bullen and Batty 1957)

iii) The post mortem and histological lesions observed

iv) The detection of typical Cl. welchii organisms in smears made from the intestinal contents and visceral organs. These were gram-positive short rods and usually dominated every microscopic field (Rowlands 1957)

v) The presence of enterotoxin lethal to mice in the gut contents (Wilsdon, 1931)

Table XXVIII

SUMMARY OF MAJOR SIGNS IN DISEASES AND CONDITIONS RESEMBLING THE ACUTE RADIATION SYNDROME IN RUMINANTS

ENTEROTOXAEMIA	BRACKEN POISONING	TCESOM POISONING	SWEET CLOVER POISONING	HEAVY METAL POISONING	PURPURA HAEMORRHAGICA
Cause	Cause	Cause	Cause	Cause	Cause
Toxins of Clos. welchii Types A and D	Aplastic anaemia factor and avitaminosis B1	Di-chloro-cysteine (Tri-chloroethylene-extracted-soya-bean-oil-meal)	Dicoumarol: the active principle insilage & spoiled sweet clover, warfarin	Arsenic, lead, thallium gold etc.	Virus
Clinical Signs	Clinical Signs	Clinical Signs	Clinical Signs	Clinical Signs	Clinical Signs
Pyrexia Cerebral signs Haemoglobinuria Dyspnoea Cardiac signs Diarrhoea Glycosuria	Pyrexia Cerebral signs Haemorrhages Dyspnoea Cardiac signs Oedema of larynx Mucus discharges from mouth and eyes Aplastic anaemia	Pyrexia Abdominal pain Haemorrhages Dyspnoea Cardiac signs Blood in faecs Asthenia Anorexia & Apathy Aplastic anaemia	Anorexia Apathy Lameness due to subcutaneous haemorrhages Bloody diarrhoea Haemorrhages Epistaxis Anaemia	Pyrexia Cerebral signs Haemoglobinuria Dyspnoea Cardiac signs Bloody diarrhoea Abdominal pain Epilation Haemorrhages	Pyrexia Petechial haemorrhages Dyspnoea Epistaxis Bloody diarrhoea Subcut. oedem
Pathology	Pathology	Pathology	Pathology	Pathology	Pathology
Transient agranulocytosis followed by neutrophilia Lymphopenia Haemolytic anaemia Petechial haemorrhages in all viscera Pyrexia correlated with infection and leucopenia	Leucopenia Thrombocytopenia Defective clot retraction and time Bone marrow aplasia Purpuric haemorrhages Pyrexia not correlated with infection	Pancytopenia Thrombocytopenia Aplastic anaemia Petechial haemorrhages in all viscera Pyrexia not correlated with infection	Leucopenia Thrombocytopenia Normocytic anaemia Delayed clotting time and defective retraction Petechial haemorrhages in all viscera Pyrexia not correlated with infection	Anaemia Coagulation of blood at death Petechial haemorrhages in all viscera Liver necrosis Pyrexia not correlated with infection	Anaemia Thrombocytopenia Increased clotting time Purouric haemorrhages in all viscera Heart v. abn. Pyrexia correlated with infection and leucopenia

c) CLINICAL PATHOLOGY

Haematological Response

Data from 160 blood examinations taken from 66 normal healthy goats revealed considerable differences between individual goats (Appendix V (i) Wilkins and Hodges, 1962). This finding necessarily implied that the dividing line between any physiological and pathological variation would be difficult to assess unless local criteria of normality were set up. When considering the 41 pre-irradiation and 311 post-irradiation blood examinations made on the 20 adult goats used in the X irradiation experiments it was therefore decided to express the results in two main ways, namely (i) as differences between survivors (alive) and non-survivors (dead) and (ii) as differences between the mean pre-irradiation values and the post-irradiation experimental findings over arbitrarily specified time periods.

The normal range and standard deviations in the 20 adult goats used in these experiments are given in Table XXIX. Mean values post-irradiation are presented in Table XXX. These values are the mean of the findings over similar time periods for both survivors and non-survivors alike. The general pattern of recovery of the major blood elements in survivors is presented in Table XXXI. Graphic representation of the haematological response is presented for one typical survivor (Figures 16 and 17) irradiated with a dose of 500r and two non-survivors exposed to 650r and 1000r respectively (Figures 18 to 20). The control values given are in all instances the average of data obtained from blood examinations carried out $\frac{1}{2}$ hour to 15 days before irradiation.

Each set of findings has been grouped and discussed under three general headings as follows:

i) The Leucocytic Picture

ii) The Erythrocytic Picture

iii) The Thrombocytic Picture

	LEUCOCYTIC PICTURE				
	Total Leucocytes (Cells/mm^3)	Polymorpho-nuclears (Cells/mm^3)	Eosino-phils Cells/mm^3)	Lymphocytes (Cells/mm^3)	Monocytes (Cells/mm^3)
RANGE	4300 11300	688 7006	0 750	2279 6743	0 649
MEAN	7216	2455	222	4254	271
S.D.	1600	1380	51	742	147
S.E.	260	223	8	120	24

	ERYTHROCYTIC PICTURE									THROMBOCYTIC PICTURE		
	RBCs (Cells/mm^3) in millions	PCV (%)	Hb (gm/ 100ml)	MCHC (%)	MCH ($\mu\mu g$)	MCV ($cu.\mu$)	ICF (% NaCl)	MCF (% NaCl)	ACF (% NaCl)	Platelets (Cells/mm^3) in Thousands	Coag-ulation Time (mins)	Clot Retract-ion (%)
RANGE	10.80 19.35	23.5 41.5	9.6 16.4	34.75 44.64	7.08 10.40	16.22 26.70	0.54 0.72	0.40 0.60	0.32 0.48	150 650	3 8	50 50
MEAN	14.88	32.9	12.7	38.67	8.63	22.43	0.64	0.50	0.40	354	4.7	50
S.D.	2.43	4.6	1.65	2.13	1.1	2.85	0.0461	0.0454	0.0416	138	1.18	--
S.E.	0.395	0.747	0.266	0.344	0.178	0.461	0.007	0.007	0.006	22	0.184	--

Table XXX

THE MEAN BLOOD VALUES IN GOATS AFTER EXPOSURE TO X IRRADIATION (DOSE RANGE 400r to 700r)

TIME AFTER IRRAD. (DAYS)	NO. OF BLOOD SAMPLES	ERYTHROCYTIC PICTURE									LEUCOCYTIC PICTURE					THROMBOCYTIC PICTURE		
		RBC (Cells/mm3) in millions	PCV (%)	Hb. (gms/ 100ml)	MCH (μμg)	MCHC (%)	MCV (cu.μ)	ICF % NaCl	MCF % NaCl	ACF % NaCl	TOTAL WBC (Cells/ mm^3)	POLY-MORPHS (Cells/ mm^3)	EOSINO-PHILLS (Cells/ mm^3)	LYMPHO-CYTES (Cells/ mm^3)	NONO-CYTES (Cells/ mm^3)	PLATELETS (Cells/mm^3) in Thousands	COAGULATION TIME (Mins)	CLOT RETRACTIO (%)
									NON-SURVIVORS									
½ hr	9	12.28	27.28	10.72	8.8	39.38	22.38	0.70	0.55	0.44	6686	3510	104	2876	193	306	5.8	40
1	9	14.70	34.28	12.79	8.8	37.34	23.47	0.71	0.56	0.45	7239	6948	62	212	35	348	6.0	30
2-5	18	14.07	32.55	12.34	8.9	37.84	23.56	0.69	0.55	0.44	2818	2430	49	257	51	350	6.8	20
6-9	22	14.72	33.18	12.96	9.0	39.20	22.86	0.69	0.54	0.43	956	635	17	288	20	248	8.7	15
10-14	16	14.46	31.62	12.14	8.5	38.47	22.21	0.70	0.56	0.44	680	288	3	364	26	164	12.9	5
15-19	11	12.34	31.41	12.33	10.0	39.35	25.48	0.70	0.55	0.44	542	191	6	328	16	55	22.4	3
20-24	8	13.61	30.69	11.89	9.0	39.49	22.87	0.72	0.53	0.41	1062	502	119	407	34	130	10.3	7
25-34	7	11.93	26.14	10.47	8.7	39.81	21.98	0.72	0.55	0.45	1132	318	187	595	33	177	9.8	0
35-45	5	10.18	18.40	8.48	8.3	43.10	19.82	0.72	0.54	0.42	415	68	17	323	7	124	14.2	0
									SURVIVORS									
½ hr	9	15.64	32.22	12.48	8.1	38.67	20.84	0.65	0.52	0.43	8472	5273	308	2704	186	412	4.8	40
1	9	16.27	34.05	13.14	8.2	38.73	21.09	0.66	0.52	0.41	8371	7669	117	520	65	342	5.2	25
2-5	18	15.19	33.28	12.74	8.5	38.39	22.18	0.66	0.52	0.40	2890	2178	79	547	85	284	4.8	40
6-9	16	16.09	33.84	12.58	8.1	37.29	21.63	0.68	0.54	0.43	1916	1324	46	484	31	338	5.1	30
10-14	18	15.06	32.00	12.55	8.4	39.42	21.40	0.68	0.54	0.43	1454	876	32	515	31	219	5.8	20
15-19	14	13.78	29.93	11.68	8.6	39.04	22.10	0.66	0.52	0.41	1463	550	31	846	36	140	6.1	10
20-24	12	14.33	29.00	11.18	7.9	38.52	20.52	0.67	0.53	0.42	1543	718	73	710	42	148	5.8	15
25-34	15	12.55	28.50	10.73	8.8	37.82	23.18	0.64	0.52	0.41	1986	1064	79	772	70	225	10.3	20
35-45	12	12.34	28.58	10.86	8.9	38.11	23.43	0.65	0.52	0.40	3253	1899	135	1121	98	195	5.6	30
46-60	13	12.01	28.23	10.80	9.1	38.48	23.99	0.67	0.53	0.42	3669	2633	135	1235	110	142	6.3	20
61-75	10	13.23	28.50	11.50	8.9	40.73	21.88	0.69	0.54	0.41	4380	3385	61	1925	165	142	6.8	30
76-90	9	14.31	30.72	12.00	8.5	39.26	21.82	0.68	0.54	0.44	4422	2946	137	1628	116	157	6.6	35
91-110	11	14.18	29.59	12.40	8.8	41.95	21.05	0.67	0.54	0.44	4361	3184	202	1563	101	176	7.1	35
111-145	20	15.51	30.66	12.70	8.3	41.61	20.00	0.69	0.55	0.44	4999	3310	133	1593	175	239	7.1	25
146-210	20	14.70	31.19	13.10	9.0	41.83	21.64	0.68	0.54	0.44	4018	2391	222	1638	138	333	5.7	30

Table XXXI

Pattern of recovery of Main Blood Elements in surviving goats after exposure to X irradiation

(Dose Range 400r to 700r)

Blood Element	Time to reach normal control value (Days)	Recovery in 210 Days (%)
Total WBC	More than 210 Days	65
Granulocytes	46 - 60 Days	100
Mononuclears	More than 210 Days	39
Total RBC	100 - 150 Days	100
Hb	100 - 150 Days	100
PCV	150 - 210 Days	100
Platelets	210 Days	100

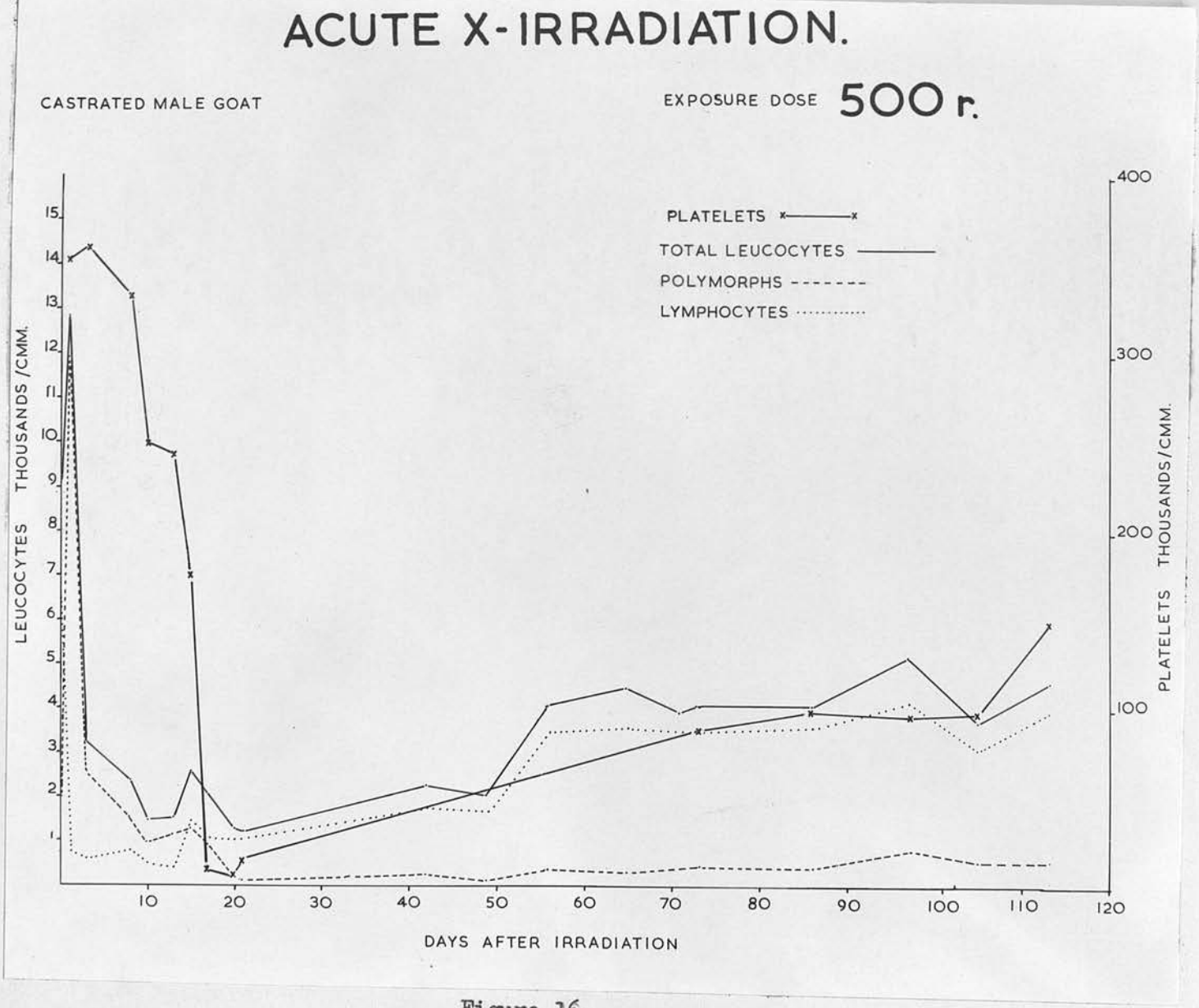

ACUTE X-IRRADIATION.
CASTRATED MALE GOAT
EXPOSURE DOSE 500 r.
PLATELETS
TOTAL LEUCOCYTES
POLYMORPHS
LYMPHOCYTES
LEUCOCYTES THOUSANDS /CMM.
PLATELETS THOUSANDS/CMM.
DAYS AFTER IRRADIATION
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
100
200
300
400
10
20
30
40
50
60
70
80
90
100
110
120

Figure 16.

TYPICAL SURVIVOR SUB-LETHAL DOSE

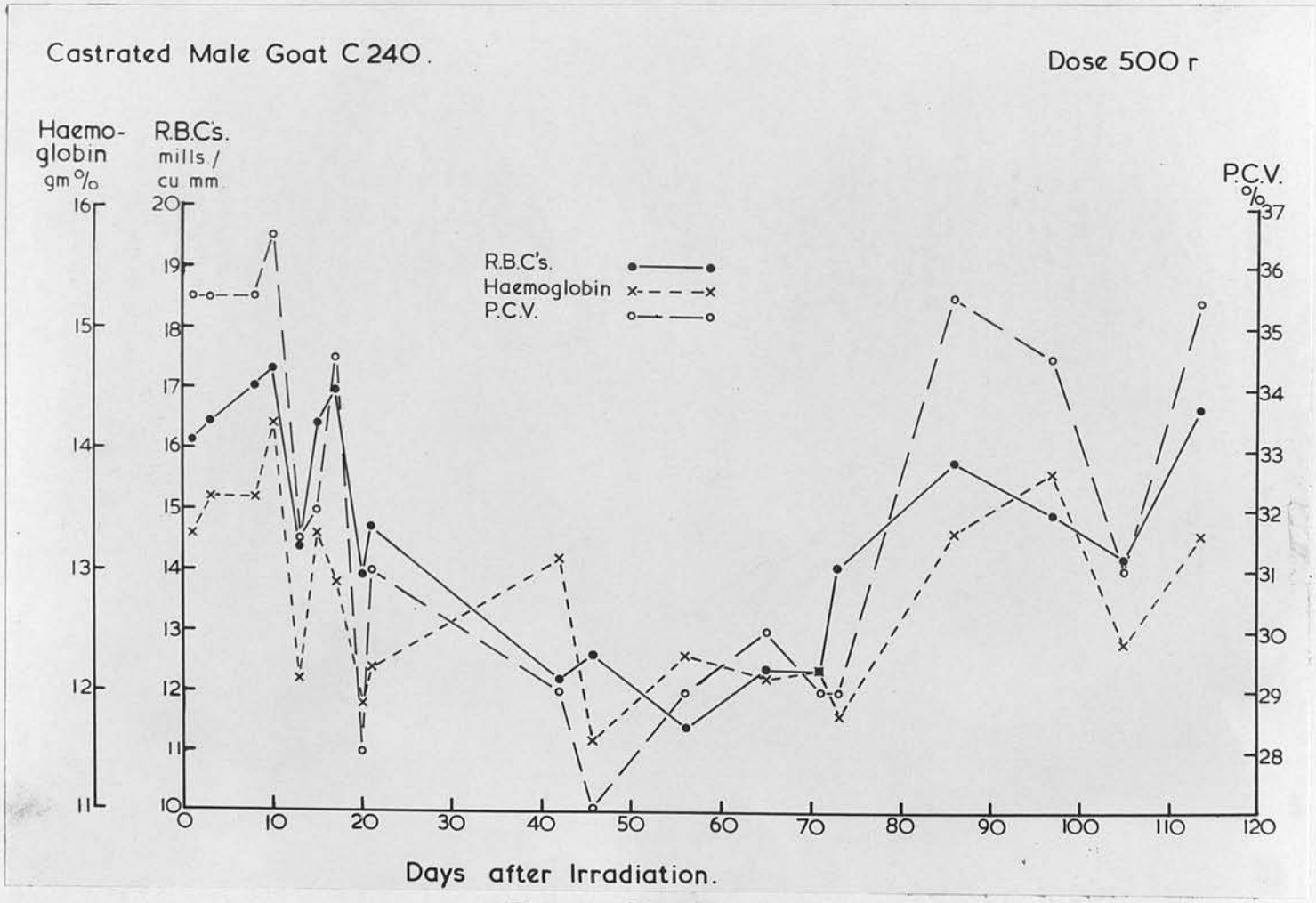


Figure 17.

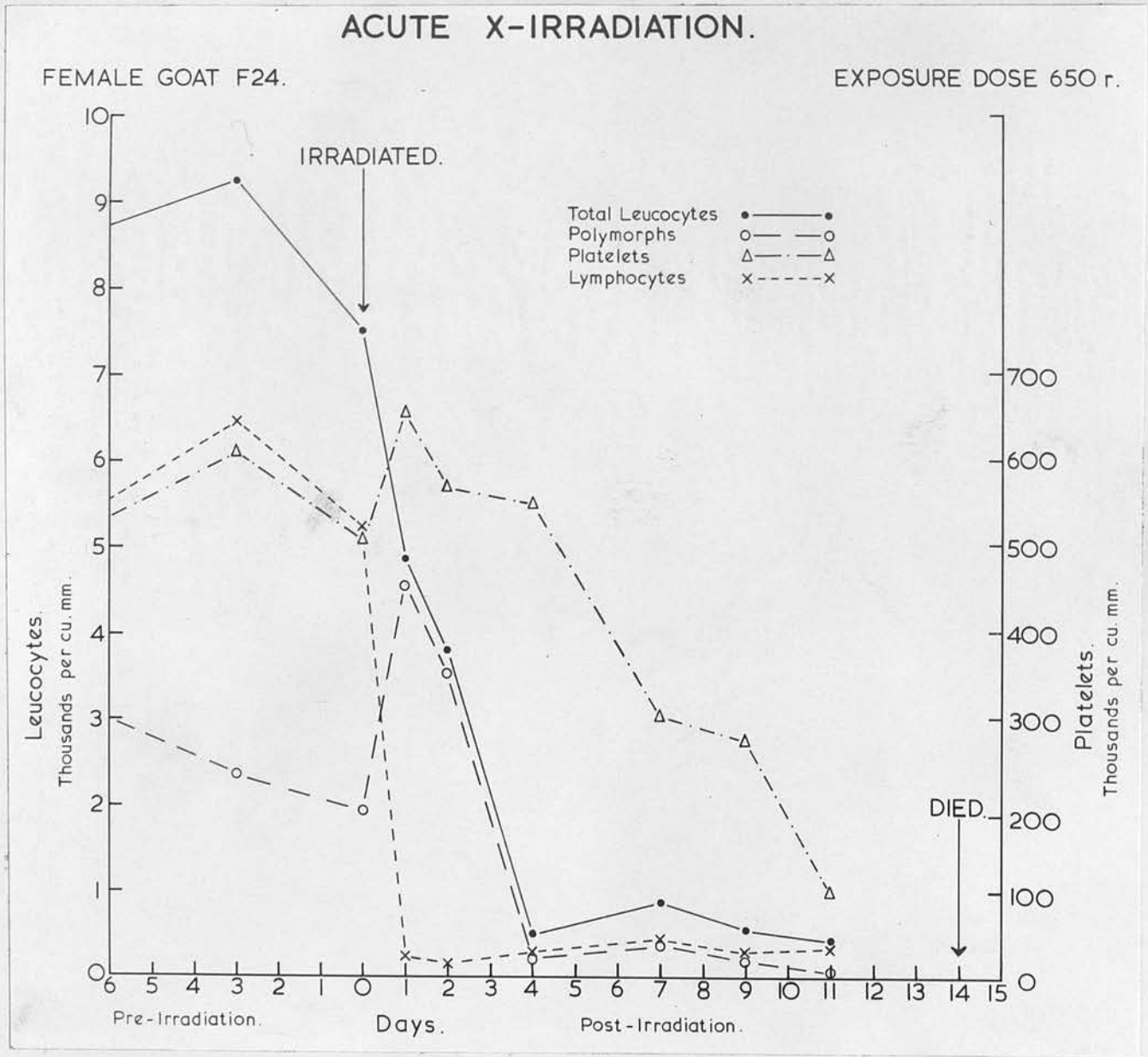

ACUTE X-IRRADIATION.
FEMALE GOAT F24.
EXPOSURE DOSE 650 r.
IRRADIATED.
DIED.
Total Leucocytes
Polymorphs
Platelets
Lymphocytes
Leucocytes.
Thousands per cu. mm.
Platelets.
Thousands per cu. mm.
Pre-Irradiation.
Days.
Post-Irradiation.

Figure 18.

TYPICAL NON-SURVIVOR MEDIAN LETHAL DOSE

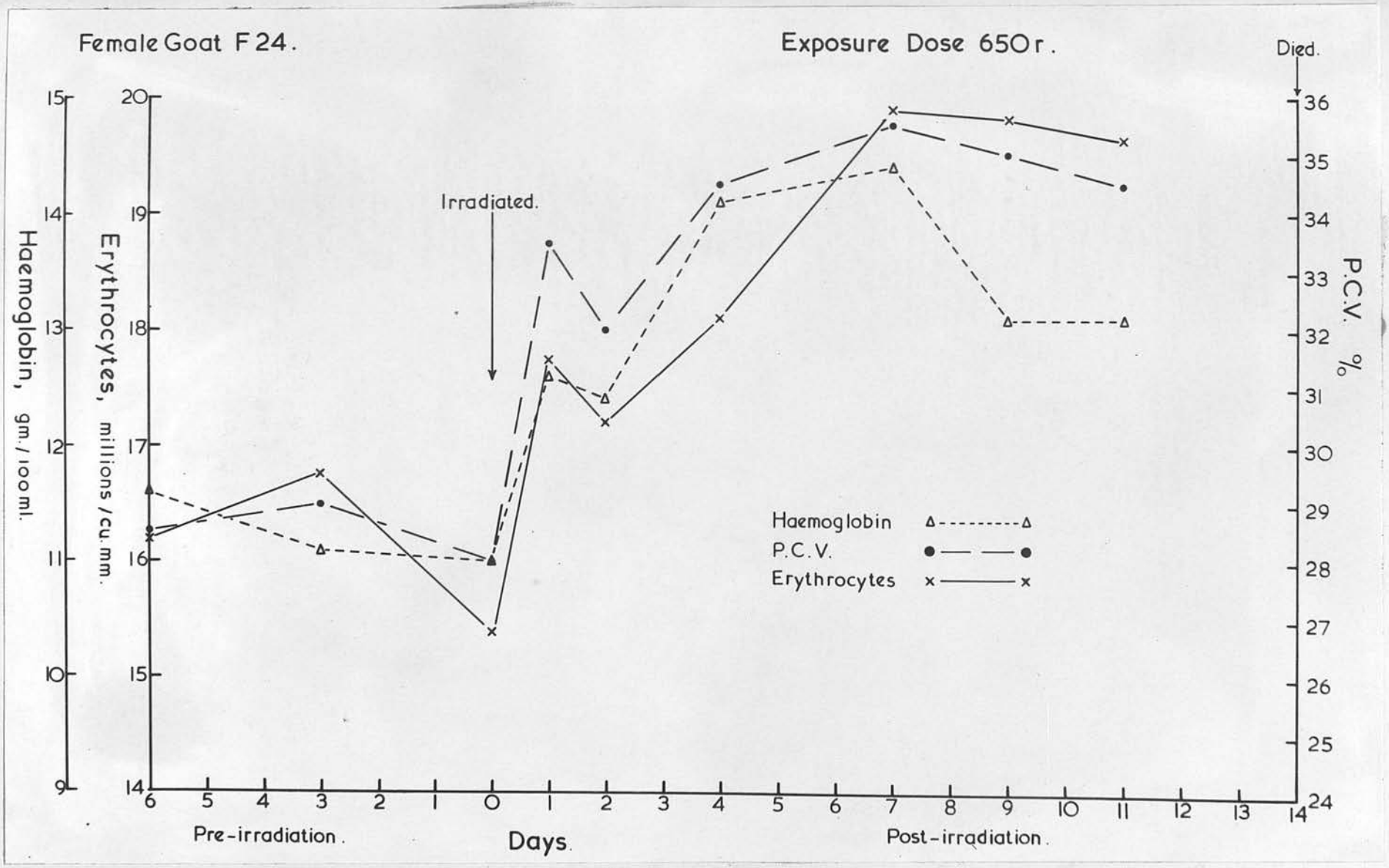


Figure 19.

HAEMATOLOGY

SUPRA LETHAL DOSE

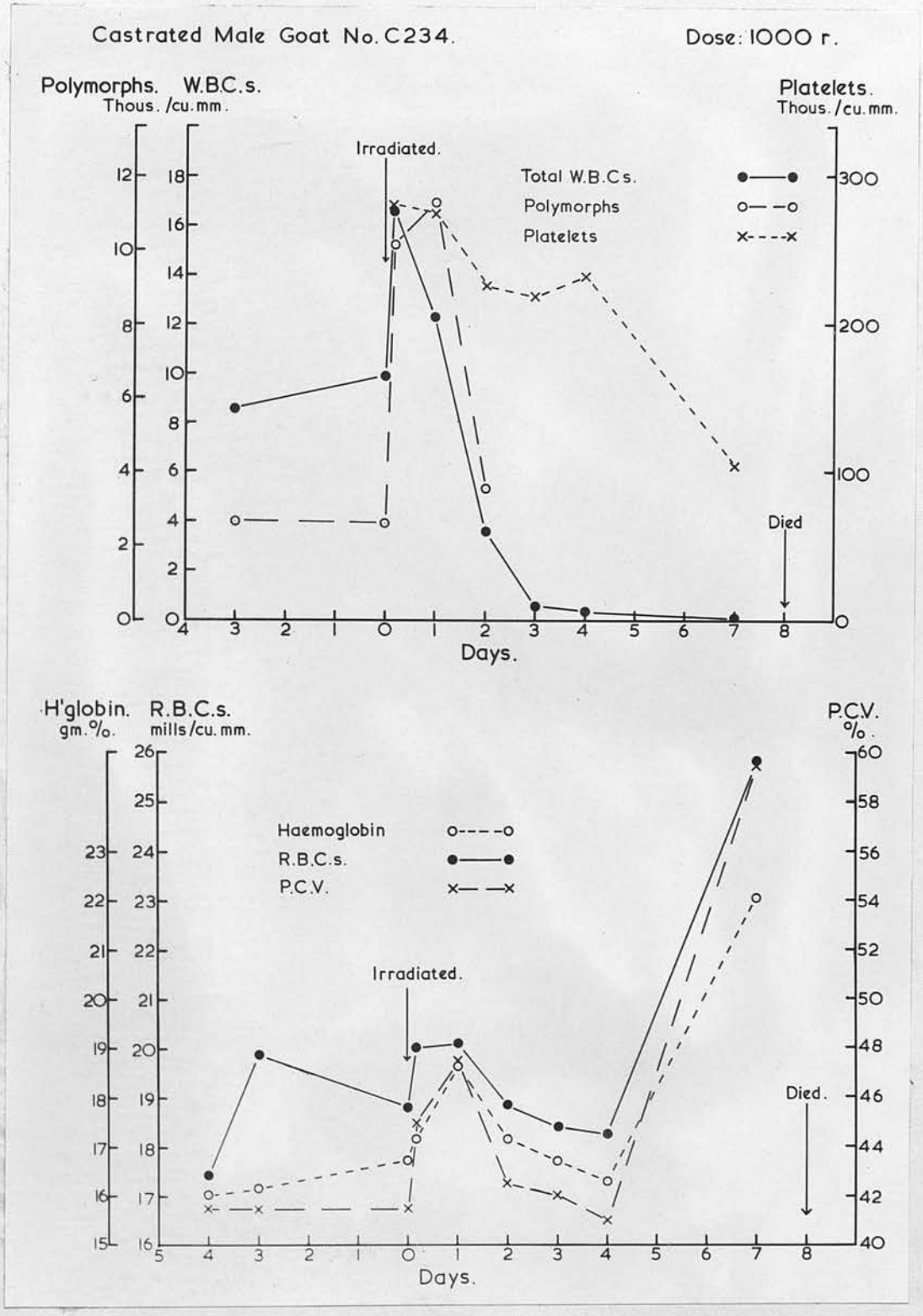


Figure 20.

The Leucocytic Picture

Changes in the white blood cells were measured by total cell and differential counts.

i) Total Leucocytes (Fig 21)

a) Maximum and Minimum Values (Tables XXXII and XXXIII)

There was an initial leucocytosis in all irradiated goats; in survivors there was an increase over control counts of 31% and in non-survivors a lesser increase of 13%. These increases consisted essentially of a neutrophilia which reached maximum values both in survivors and non-survivors in approximately the same time, viz 21 hours. (Table XXXVI). The maximum per cent decrease of the leucocyte count was 97% in non-survivors and 90% in survivors. Recovery in survivors became apparent between the 24th and 35th day after irradiation. Progress, however, was very gradual being still 30% to 40% below normal control values at 7 months due mainly to a continuing lymphopenia.

b) Changes in the Leucocyte Count as a function of Time, Survival and Dose.

Table XXXIV presents a summary of the mean cell counts and percentage changes in survivors and non-survivors.

At ½ hour following irradiation the total leucocyte count in non-survivors was depressed some 11% below mean control values whereas that of the survivors was nearly 20% above. At one day whilst survivors remained approximately at the same level as at ½ hour the non-survivors count rose slightly to -3.6% of control. Between 2 to 7 days the values of both groups were severely depressed, non-survivors to some 72% and survivors to nearly 62% below the control. Thereafter non-survivors progressively deteriorated until death supervened whereas survivors, after a further slight depression to nearly 77% below, slowly began to recover being some 46% below controls between 31 to 60 days after irradiation some 36% below at 7 months and still 20% below controls at 18 months post irradiation, due mainly to a continuing lymphopenia.

Table XXXII

Effects of X-Irradiation on Goats

Total Leucocytes (Maximum Values)

Goat No.	Dose (r)	Control Mean WBC Count (cells/mm^3)	Maximum WBC Count recorded (cells/mm^3)	Hrs/ post-irradiation	% Increase: Maximum	% Increase: Average for Dose	% Increase: Average for all goats	% Increase: Average for Dose Range 400 - 700r
				Non-Survivors				
C229	2000	5225	5500	½ hr	+ 5	+ 5		
C234	1000	9900	16600	3½hrs	+68	+68		
C175	700	8100	9200	½ hr	+14			
F 40	700	6830	8800	24	+29	+24		
F 27	700	5710	7300	3 hrs	+28			
F 14	650	8525	7350	½ hr	-14		+17	
F 24	650	7550	4850	24	-36	-25		+13
C315	600	7630	8700	24	+14	+24		
C118	550	5370	8550	24	+59			
C200	550	7110	8200	24	+15	+28		
F 20	550	10750	11750	½ hr	+ 9			
				Survivors				
F 31	650	8750	9650	½ hr	+10	+10		
F 30	600	6110	7200	½ hr	+18			
M 9	600	9425	13200	24	+40	+29		
M 3	500	5450	8800	½ hr	+61			
M 1	500	7050	7500	½ hr	+ 6	+36	+31	+31
C240	500	9100	12850	24 hr	+41			
M 10	400	5900	6050	½ hr	+ 3			
W316	400	6400	10300	½ hr	+61	+34		
W302	400	5435	7500	24	+38			

Mean Time to reach Maximum

Non-Survivors - All Goats - 11.7 hours

Dose Range 400-700r - 13.8 hours

Survivors 8.3 hours

Table XXXIII

Total Leucocytes (Minimum Values)

Goat No.	Dose (r)	Control Mean WBC Count (cells/mm^3)	Minimum WBC Count (cells/mm^3)	Days Post-irrad.	% Decrease: Minimum recorded	% Decrease: Average for dose	% Decrease: Average for all goats	% Decrease: Average for Dose Range 400 - 700r
				Non-Survivors				
C229	2000	5225	NIL	7	100	100	-	-
C234	1000	9900	50	7	99.5	99.5	-	-
C175	700	8100	200	10	97.5	98	97.7	97.3
F 40	700	6830	100	9	98.5			
F 27	700	5710	125	44	98			
F 14	650	8525	100	13,41	99	97		
F 24	650	7550	425	11	94.5			
C315	600	7630	175	29	97.5	97.5		
C118	550	5370	200	17	96	96.8		
C200	550	7110	250	11	96.5			
F 20	550	10750	250	10	98			
				Survivors				
F 31	650	8750	1100	17	87.5	87.5	90	90
F 30	600	6110	200	14	96.5	97		
M 9	600	9425	290	22	97			
M 3	500	5450	750	12	86	87		
M 1	500	7050	800	17	88.5			
C240	500	9100	1275	21	86			
M 10	400	5900	110	30	98	89.5		
W316	400	6400	900	13	86			
W302	400	5435	800	15	85			

Mean time to reach Minimum

Non-Survivors	(all goats)	15.3 to 17.8. days
Non-Survivors	(400-700r range)	17.1 to 20.2 days
Survivors		17.9 days

There was little or no interrelationship discernible between the response and the doses used either at ½ hour or 1 day following irradiation but thereafter a correlation was observed in both non-survivors and survivors in that the greater dose produced the deeper ultimate depression.

Table XXXIV

Total Leucocytes

Mean Cell Counts and Percentage Changes

(Dose Range 400r to 700r)

Period	Non-Survivors		Survivors	
	Cells/mm^3	Percentage of Control	Cells/mm^3	Percentage of Control
Pre-Irradiation Control	7508	-	7069	-
Post-Irradiation ½ hour	6687	- 10.94	8472	+ 19.85
1 Day	7239	- 3.58	8371	+ 18.42
2-7 Days	2098	- 72.06	2709	- 61.68
8-30 Days	619	- 91.76	1635	- 76.87
31-60 Days	651	- 91.33	3835	- 45.75
61-210 Days	-	-	4507	- 36.24
8-12 Months	-	-	5767	- 18.42
13-18 Months	-	-	5567	- 21.25

ii) Granulocytes (Fig 22)

a) Changes in the Granulocyte Count as a function of Survival and Time

Table XXXV gives a summary of the mean cell counts and percentage changes in survivors and non-survivors.

Some non-survivors showed a transient drop in numbers of granulocytes ½ hour after irradiation, which made their overall increase (31.6%) significantly less than in the survivors (107%). This tendency continued during the ensuing hours so that at 1 day post-irradiation

the survivors manifested a percentage increase of granulocytes above control values of 189% whereas the non-survivors had shortened the previous gap to an increase of 155% above controls. From the second day onwards both groups showed a decrease in cell counts but the speed of this decrease was more rapid in the non-survivors than in the survivors. In non-survivors the decrease was progressive until death supervened whereas in survivors the general depression continued up to approximately the thirtieth day after which there was a gradual recovery to normal between $1\frac{1}{2}$ to 2 months post-irradiation.

Table XXXV

Granulocytes

Mean Cell Counts and Percentage Changes

(Dose Range 400r to 700r)

Period	Non-Survivors		Survivors	
	Cells/mm^3	Percentage of Control	Cells/mm^3	Percentage of Control
Pre-Irradiation Control	2749	-	2697	-
Post-Irradiation $\frac{1}{2}$ hour	3617	+ 31.58	5582	+ 106.97
1 Day	7010	+ 155.00	7786	+ 188.69
2-7 Days	1781	- 35.21	2133	- 20.91
8-30 Days	370	- 86.54	936	- 65.30
31-60 Days	139	- 94.94	2614	- 3.08
61-210 Days	-	-	2797	+ 3.71

Changes in the Granulocyte Count as a function of Dose.

At the lowest doses i.e. 400r and 500r, the granulocytic response was more definitely dose dependent showing a diphasic variation as follows:

400r

Maximum depression (46% below controls) was reached between 8 and 30 days with recovery overshooting normal control values by between 25%

to 35% for periods in excess of 7 months.

500r

More profound mean decreases (58% below controls) were reached than the 400r group in 8 to 30 days followed by a slower recovery to normal values in 2 to 7 months.

600r

Survivors showed over 80% depression between 8 and 30 days after irradiation but their recovery, although very much slower, paralleled the 500r group in the later stages reaching normality in 7 months.

Polymorphonuclear Leucocytes (Fig 23)

a) Maximum and Minimum Values (Tables XXXVI and XXXVII)

There was a maximum per cent increase above control values of 209% in non-survivors and 289% in survivors in approximately the same time (21 hours) after irradiation. By the time of death polymorphs had almost completely disappeared from the circulating blood in non-survivors (99% decrease) whereas in survivors minimum values were reached about 18 days after irradiation and were only slightly less (91% decrease). Their general decline in all irradiated goats paralleled the total leucocyte count but their recovery was comparatively slow.

b) Changes in the polymorphonuclear leucocyte count as a function of Time, Survival and Dose

At the highest doses (2000r and 1000r) i.e. over the LD50 doses, the mean percentage increases initially and depression terminally were the greatest. In the range of the LD50 dose and below (700r to 400r) survivors showed an initial neutrophilia lasting 24 to 48 hours whereas many non-survivors showed an initial neutropenia followed quickly by a neutrophilia for an overall similar period of time. This accounted for the lower mean increase ½ hour after irradiation in non-survivors as compared with survivors.

Table XXIV

Polymorphonuclear Leucocytes (Maximum Values)

Goat No.	Dose (r)	Control Mean Count Cells/mm^3	Maximum Count Recorded Cells/mm^3	Hours Post Irradiation	% Increase: Maximum Recorded	% Increase: Average for Dose	% Increase: Average for all Goats	% Increase: Average for Dose Range 400-700r
					Non-Survivors			
C229	2000	1945	3665	½	88	88		
C234	1000	2574	11,439	24	344	344		
C175	700	2605	6345	24	143			
F 40	700	2255	8536	24	278	194		
F 27	700	2300	5996	24	161			
F 14	650	2485	6732	24	171			
F 24	650	1910	4583	24	140	155	211	209
C315	600	2025	8526	24	321	321		
C118	550	1490	8208	24	451			
C200	550	1922	5207	½	170	223		
F 20	550	6307	9311	24	48			
					Survivors			
F 31	650	4248	8202	24	93	93		
F 30	600	1032	5676	24	450			
M 9	600	4656	11,946	24	156	303		
M 3	500	1605	7610	24	374			
M 1	500	3576	5656	24	58	311	289	289
C240	500	1984	11,925	24	501			
M 10	400	1590	3207	½	102			
W316	400	1998	7787	24	290	323		
W302	400	1048	7088	24	576			

Mean Time to reach Maximum

Non-Survivors

All Goats 19.6 hours

Dose Range

400 to 700r 21.3 hours

Survivors 21.3 hours

Polymorphonuclear Leucocytes (Minimum Values)

Goat No.	Dose (r)	Control Mean Count Cells/mm^3	Minimum Count recorded Cells/mm^3	Days Post Irr.	Survival Time Days	% Decrease			
						Min.	Average for dose	Average for all goats	Average for dose range 400-700r
Non-Survivors									
C229	2000	1945	NIL	4	8	100	100		-
C234	1000	2574	50	7	8	99.8	99.8		-
C175	700	2605	8	10	11	99.7			
F 40	700	2255	7	9	13	99.7	99.5		
F 27	700	2300	21	44	46	99.1		99	
F 14	650	2485	NIL	41	42	100			
F 24	650	1910	47	11	14	97.5	98.7		98.7
C315	600	2025	4	29	30	99.8	99.8		
C118	550	1490	8	17	18	99.5			
C200	550	1922	25	11	12	98.7	99.3		
F 20	550	6307	27	10	12	99.6			
Survivors									
F 31	650	4248	792	17		81.4	81.4		
F 30	600	1032	23	11		97.8	97		
M 9	600	4656	171	22		96.3			
M 3	500	1605	166	15		89.7			
M 1	500	3576	260	17		92.7	91.9	90.5	90.5
C240	500	1984	134	21		93.2			
M 10	400	1590	47	30		97.0			
W316	400	1998	344	16		82.8	87.7		
W302	400	1048	176	15		83.2			

Mean Time to reach minimum

Non-Survivors

All Goats 17.5 days

Dose Range

400 to 700r 20.2 Days

Survivors 18.2 Days

Both survivors and non-survivors showed a massive neutrophilia at 24 after irradiation. Thereafter both groups decreased to approximately the same level and at similar rates for the next four days. Non-survivors subsequently showed rapidly decreasing cell counts until they had almost completely disappeared from the circulating blood at the time of death. The decrease in survivors was less in degree and more gradual reaching minimum values between the 15th. and 19th. days; thereafter there was a gradual recovery until normal cell counts were observed between the 46th and the 60th days post irradiation. (Some survivors (Fig 16) showed consistently low counts for many months). Generally speaking, the depression of polymorphonuclear leucocytes in the circulating blood of non-survivors paralleled closely the pattern observed in the total leucocytes. Immature cells corresponding to the classical "invasion phase" of Schilling were very evident in the initial neutrophilia of both survivors and non-survivors. There was in general a more rapid disappearance of this neutrophilic reaction in non-survivors than in survivors.

Eosinophilic Leucocytes. (Fig 24)

After irradiation an eosinopenia was usually observed within 24 hours indicating a severe general leucocytic response. Subsequent reactions paralleled those of the polymorphs. Some goats showed an increase in the numbers of eosinophils although there was no clinical helminthiasis detectable and this manifestation was considered to be an actual or abortive recovery phase.

Basophilic Leucocytes

Basophils were almost completely absent.

Atypical and Abnormal Forms.

At very high doses (i.e. 2000r and 1000r) a large number of stab and non-lobulated forms (myelocytes and metamyelocytes) were seen in smears taken ½ hour after irradiation; at one day most granulocytes seen were

non-lobulated. Cells were very scanty in numbers on the second day and after the third day it was almost impossible to make a differential count owing to both lack of cells and their fragmentation.

At MLD doses (i.e. 400r to 700r) although generally a similar picture was to be observed, the immature forms tended to appear in smears at a slightly later period, especially at the lower dose levels. However, in all goats they were prominent within a few days and continued thereafter; in non-survivors, the number of cells showing fragmentation of nuclei and vacuolization of cytoplasm increased as the overall number of cells decreased almost to vanishing point as death approached; whereas in survivors immature forms (typical, damaged and atypical) were prominently visible for 30 to 60 days after which they tended to decrease, at the same time as the overall numbers of granulocytes increased. Normal lobulated forms began to predominate in smears after 14 to 30 days. Nevertheless degenerating cells were observed in varying numbers in all survivors until the end of observations.

iii) Mononuclears (Fig 22)

a) Changes in the Mononuclear Count as a function of Survival, Time and Dose.

Table XXXVIII gives a summary of the mean cell counts and percentage changes in survivors and non-survivors.

Table XXXVIII

Mononuclears

Mean Cell Counts and Percentage Changes

Dose Range 400r to 700r

Period	Non-Survivors		Survivors	
	Cells/mm^3	Percentage of Control	Cells/mm^3	Percentage of Control
Pre-Irradiation Control	4735	-	4371	-
Post-Irradiation ½ hour	3068	- 35.21	2890	- 33.88
1 Day	247	- 94.78	585	- 86.62
2-7 Days	304	- 93.58	576	- 86.82
8-30 Days	346	- 92.69	701	- 83.96
31-60 Days	512	- 89.19	1221	- 72.07
61-210 Days	-		1718	- 60.70
7-12 Months	-			
13-18 Months	-			

At ½ hour after irradiation decreases of mononuclear cells were similar in both survivors (-34%) and in non-survivors (-35%). At one day, however, the depression in non-survivors (-95%) was slightly more profound than in survivors (-87%). Thereafter, this depression was maintained in non-survivors until death whereas in survivors it continued until about the 30th day after irradiation after which a very gradual recovery was observed. This recovery which paralleled that of the total leucocyte count was still not more than 40% of the pre-irradiation control value even after 7 months.

No dose/response interrelationship could be clearly observed.

Lymphocytes (Fig 25)

Minimum Values (Table XXXIX)

In non-survivors the maximum per cent decrease in lymphocytes was reached in approximately 6 days after irradiation and averaged over 97% below control values. In survivors, a similar depression (-94%) was

Table XXXIX

Lymphocytes

Goat No.	Dose	Control Mean Lymphocyte Count (cells/mm^3)	Minimum Lymphocyte Counts (cells/mm^3)	Days Post Irrad.	Survival Time (Days)	% Decrease: Minimum Recorded	% Decrease: Average for dose	% Decrease: Average for all goats	% Decrease: Average for dose range 400 - 700r
					Non-Survivors				
C229	2000	2838	NIL	3	8	100	100		-
C234	1000	6831	NIL	3	8	100	100		-
C175	700	5214	188	10	11	96.4			
F 40	700	3801	92	9	13	97.6	96.9		
F 27	700	2796	92	1	46	96.7			
F 14	650	5683	52	13	42	99.1			
F24	650	5232	187	2	14	96.4	97.7	97.6	97
C 315	600	5017	54	11	30	98.9	98.9		
C118	550	3400	135	2	18	96			
C200	550	4988	142	2	12	97.1	96.6		
F 20	550	4063	132	6	12	96.8			
					Survivors				
F 31	650	3782	125	2		96.7	96.7		
F 30	600	4160	151	14		96.4			
M 9	600	4188	104	22		97.5	96.9		
M 3	500	3152	312	28		90.1			
M 1	500	3118	281	1		91.0	90.4	94.2	94.2
C240	500	6743	616	3		90.1			
M 10	400	3811	58	30		98.5			
W316	400	4068	240	3		94.1	95.4		
W302	400	4007	262	1		93.5			

Mean time to reach minimum.

Non-survivors (all) - 5.6 days

(400-700r) 6.2 days.

Mean time to reach minimum.

Survivors - 11.6 days

reached in about 12 days after irradiation.

Monocytes (Fig 26)

The decrease of the monocytes closely paralleled that of the lymphocytes but their relative rate of recovery to near normal values between 60 and 100 days was quicker.

Discussion

The following general factors were taken into consideration when trying to interpret the leucocytic response in goats exposed to X radiation :

i) Under normal conditions the various formed elements in the circulating blood of animals are at a relatively constant level indicating a dynamic equilibrium between their production and use. Toxic influences, including irradiation, disturb this equilibrium (Cronkite & Brecher, 1955; Whitby & Britton, 1957).

ii) There is normally a balanced response from the haematopoietic tissue to demands made by these toxic disturbances. This is reflected in the leucocytic distribution within the circulating blood.

iii) Absolute numbers of cell types are more important than total leucocyte counts because of the differences in the proportions of granulocytes to mononuclears in individual animals.

iv) A leucocytosis can often occur in animals as a specific response to non-specific stimuli. Although usually regarded as resulting from bacterial infection, a neutrophilia can arise whenever cell necrosis occurs anywhere in the animals body. An early neutrophilia can therefore be interpreted as a sensitive indicator of recent tissue damage; in like manner the appearance of immature or degenerated cells in the blood circulation can also be a manifestation of injury.

The degree of the neutrophilia or cellular differentiation roughly reflects the extent of the tissue damage or possible bone marrow hyperplasia.

v) Similarily, a continuing neutropenia may be an indication of hypoplasia of the bone marrow. Moreover, where a secondary bacterial infection does not elicit a compensating neutrophilic response (as was so often observed in this study) it is strong evidence of bone marrow damage.

vi) The levels of circulating eosinophils is governed by many complex factors some of a homeopathic nature. In this study helminth parasitism was eliminated as far as possible; some types of metazoan infestation produce an eosinopenia that may continue until the animal's death while in the case of helminths lying loose in the bowel an eosinophilia of recovery is usual. Cell necrosis caused by radiation can produce histamine at local sites which, after entering the blood circulation, may stimulate the bone marrow to produce more eosinophils (Archer, 1960).

vii) The most important criteria of the severity of the leucocytic reaction are the intensity of the shift to the left (i.e. presence of immature forms) and the absence of eosinophils. Favourable signs are the reappearance of eosinophils and mature cells.

viii) Monocytes fluctuate in numbers and appearance; a monocytosis is an uncommon reaction in animals but is sometimes shown by those recovering from bacterial infections. In this study monocytes and lymphocytes were regarded as identical and classified as "mononuclear cells".

The findings of the leucocytic response in goats after exposure to MLD doses of X radiation in this study were in agreement generally with those reported for other species of large animals (Burro, Rust et al 1954; Cattle, Brown et al, 1961; Calves, Rosenfeld, 1958; Schultze et al, 1959; Sheep, Trum and Rust 1958; Goat, Swift et al, 1946; Cronkite 1950; Pig, Cronkite, 1950, Rust et al, 1954; Dog, Alpen et al, 1958).

One of the most sensitive indicators of radiation injury in animals is a lymphopenia which commences promptly after exposure and becomes maximal at about 24 to 36 hours (Trowell, 1952; Cronkite and Bracher, 1955). In this study the mononuclear cells were the most sensitive of the blood elements indicated by a very rapid and profound lymphopenia which was greatest at 24 hours and again some 6 days after irradiation in non-survivors and continuing in survivors to 30 days after irradiation. There was also observed an extreme sensitivity of the granulocytes which after a sharp increase in numbers on the first day following irradiation, practically disappeared from the blood circulation by death in non-survivors or by the 19th day in survivors. The resilience of recovery of the mononuclear cells as compared with the granulocytes was a feature of this study and was in general agreement with the findings reported by Rosenfeld (1958) and Brown et al (1961) in cattle after gamma irradiation. In the burro this is reversed (Rust et al, 1954), but probably reflects a species difference rather than a difference in response to irradiation. The observation by Swift et al (1946) in their study on goats that the decrease in lymphocytes was less on a percentage basis than polymorphonuclear leucocytes in both non-survivors and survivors could not be substantiated.

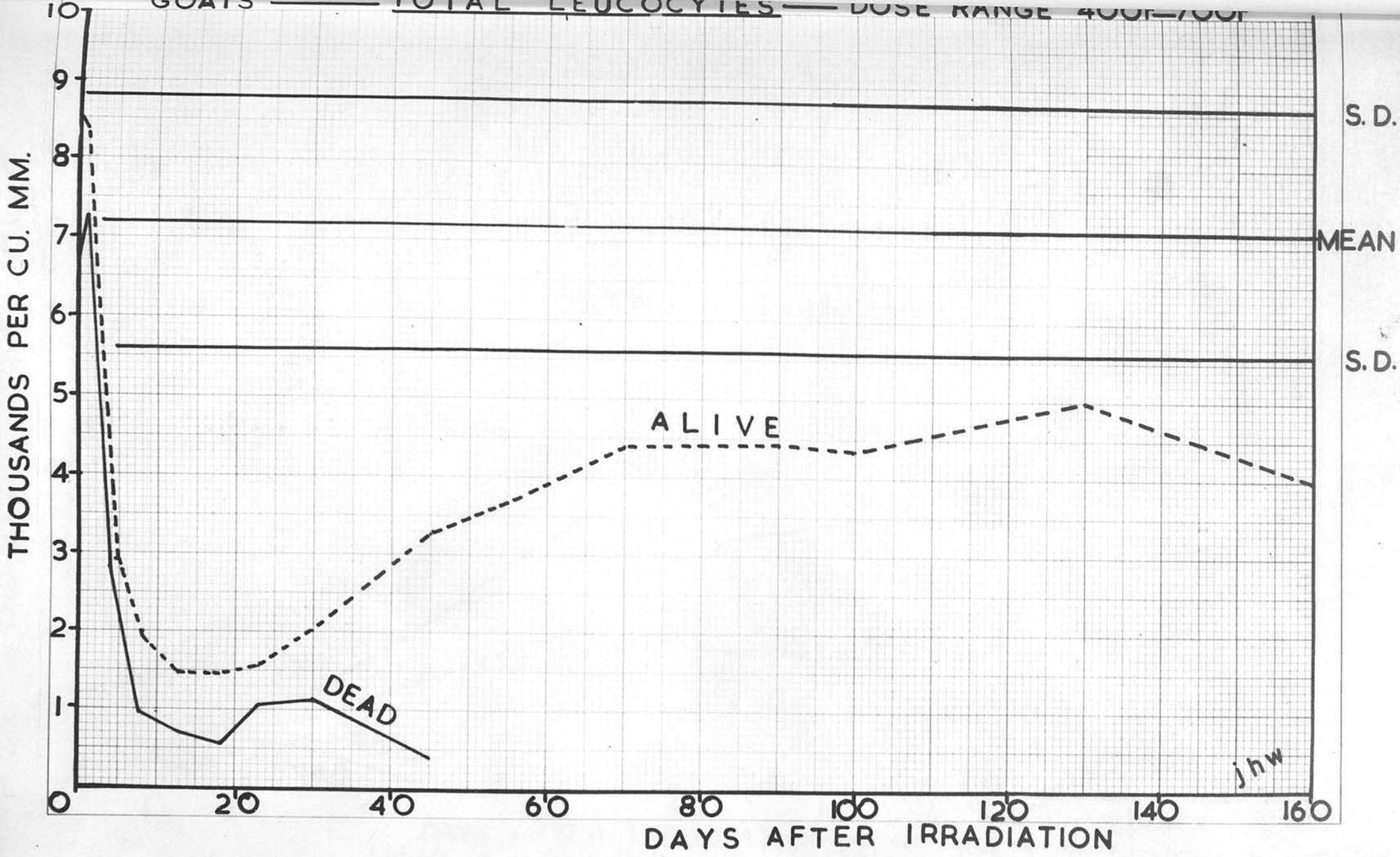

GOATS —— TOTAL LEUCOCYTES —— DOSE RANGE 400r–700r
THOUSANDS PER CU. MM.
10
9
8
7
6
5
4
3
2
1
0
S. D.
MEAN
S. D.
ALIVE
DEAD
jhw
2'0
4'0
6'0
8'0
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 21

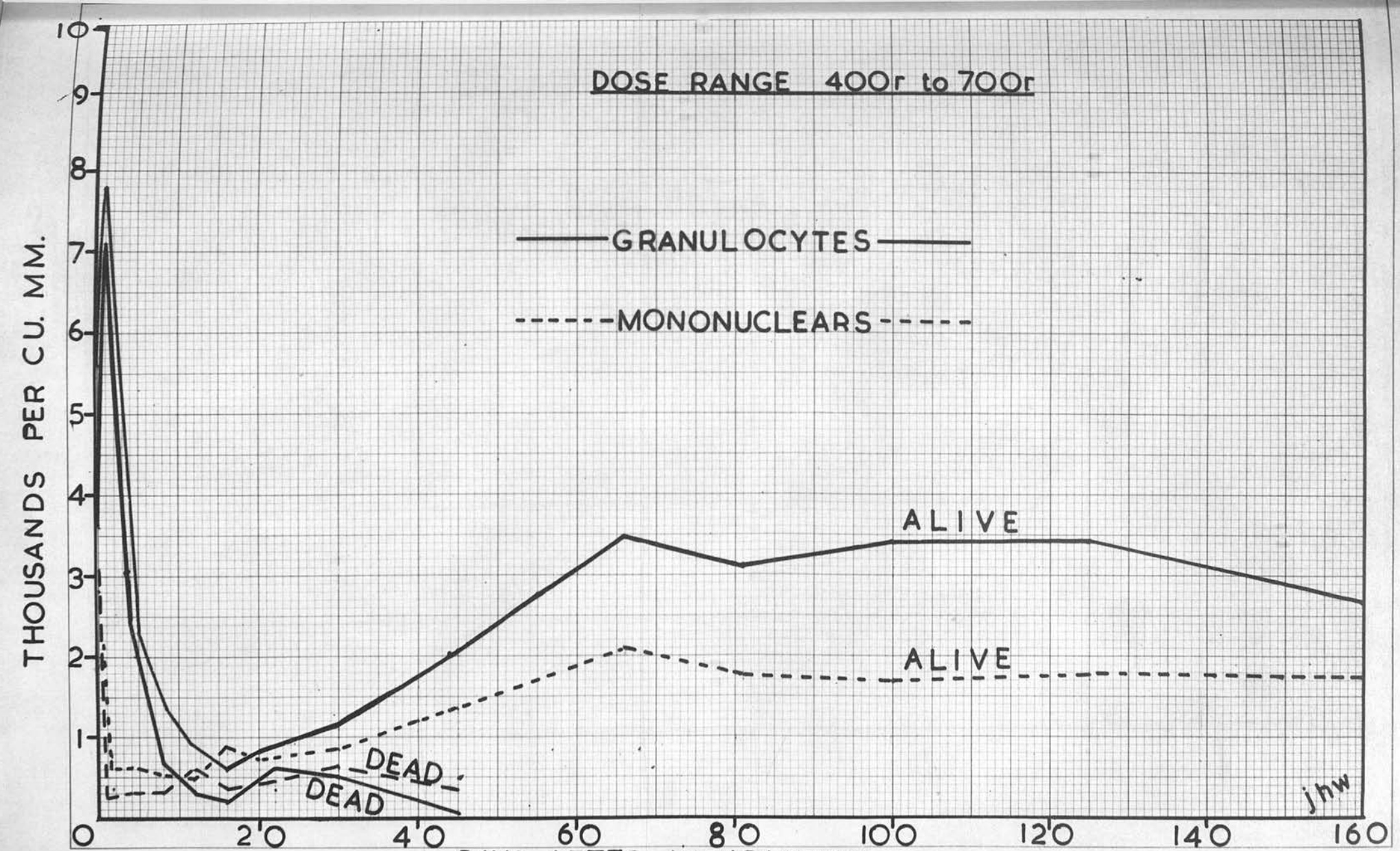


Fig. 22

DAYS AFTER IRRADIATION

Fig.24

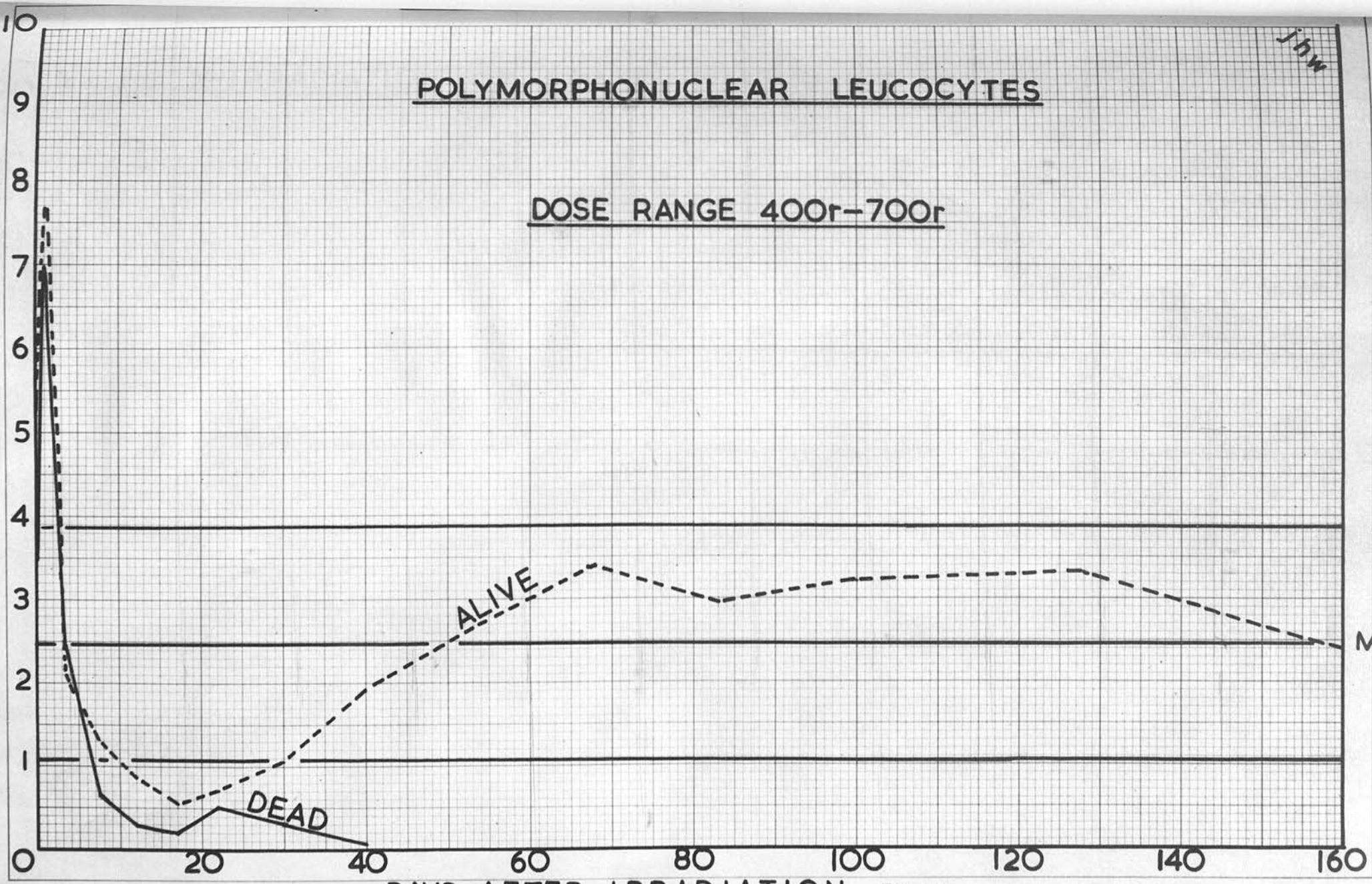


Fig.23

DAYS AFTER IRRADIATION

Fig.24

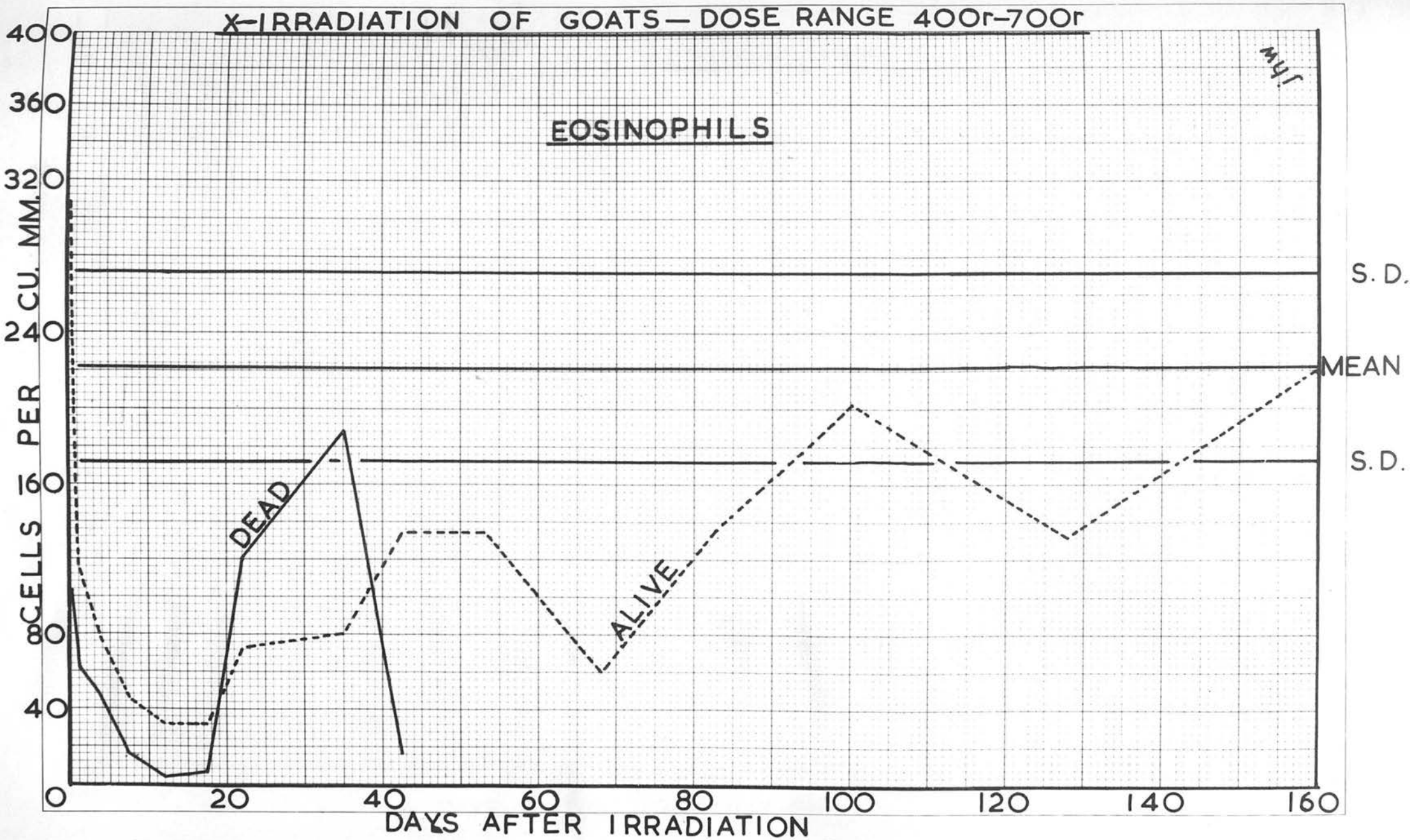

X-IRRADIATION OF GOATS — DOSE RANGE 400r–700r
EOSINOPHILS
M.U.
400
360
320
240
160
80
40
0
CELLS PER CU. MM.
S. D.
MEAN
S. D.
DEAD
ALIVE
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig.24

X IRRADIATION OF GOATS

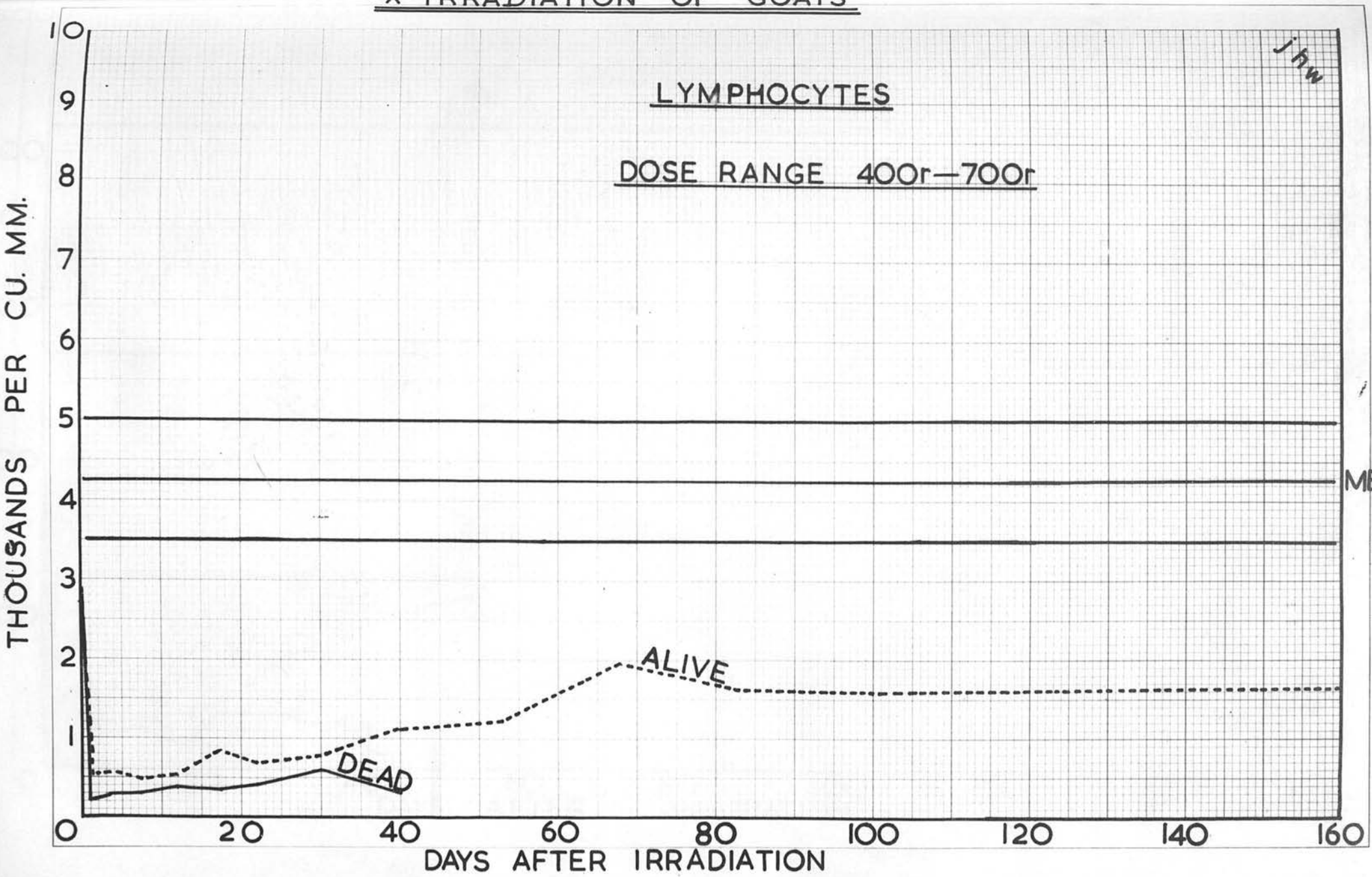


Fig. 25

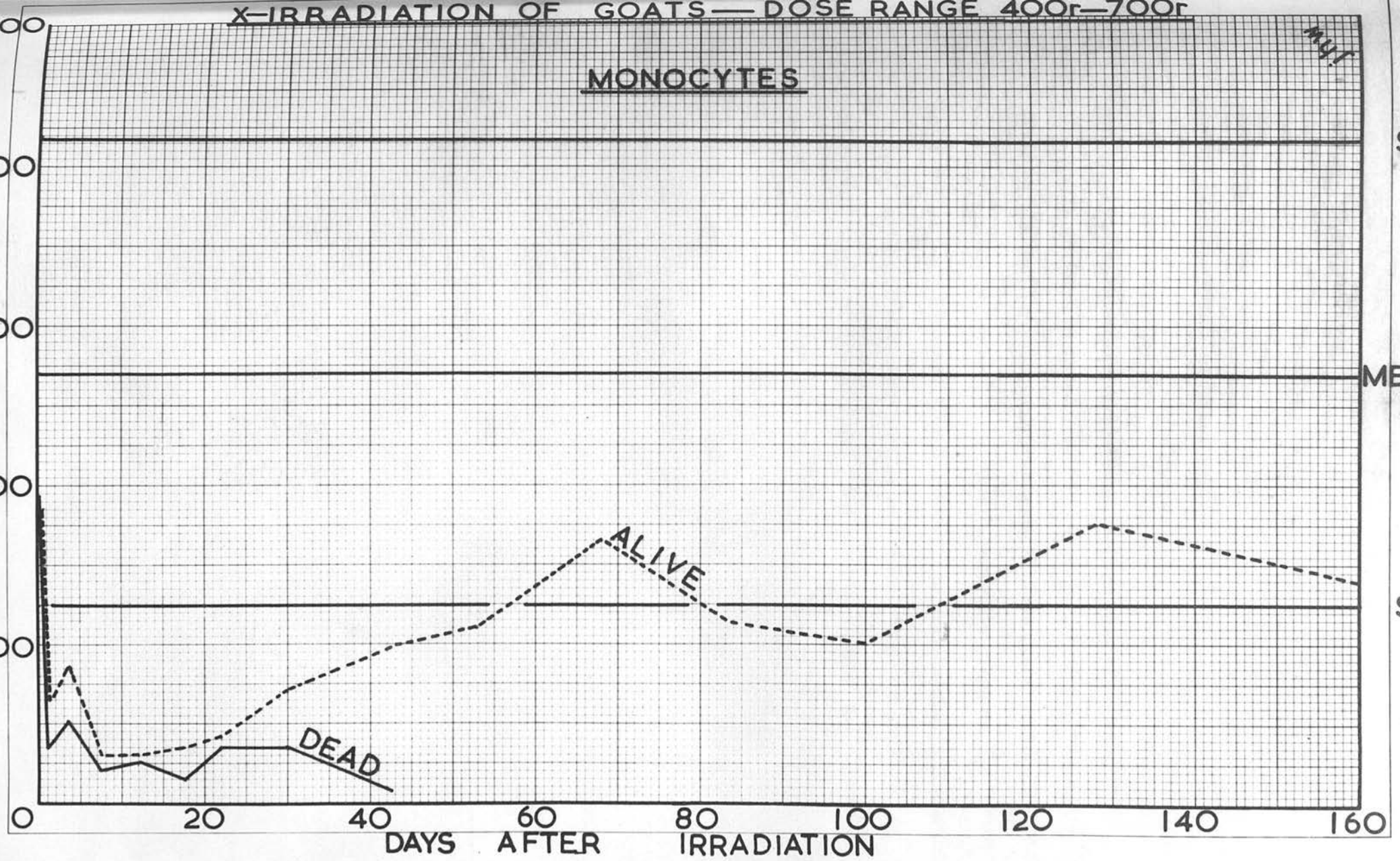

X-IRRADIATION OF GOATS — DOSE RANGE 400r—700r
MONOCYTES
S.D.
MEAN
S.D.
ALIVE
DEAD
CELLS PER CU.MM.
500
400
300
200
100
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 26

The Erythrocytic Picture

Changes in the red blood cells were measured by total cell counts, haemoglobin and haematocrit determinations together with fragility tests and estimation of sedimentation rates.

i) Total Red Blood Cells

Changes in the RBC counts as a function of Time, Survival and Dose.

Table XL gives a summary of the rbc counts and percentage changes in survivors and non-survivors. Figure 27 is a graphic representation of the general erythrocytic response; from this graph it can be seen that the effects were not dramatic in either survivors or non-survivors although a terminal oligocythaemia is evident in those goats which died as a result of irradiation. The maximal changes which occurred are presented in Tables XLI and XLII. At ½ hour after irradiation the mean counts had been depressed more (-12%) in non-survivors than in survivors (-3.6%). From the 1st to about the 30th day the average value in non-survivors was some 5% above normal reaching a maximum count (18.4% above control) between the 7th and 8th day; by contrast survivors during the same period showed counts some 5% below normal after which there was a progressive fall to 23% below control values between the 31st and 60th day post irradiation. Thereafter in survivors a gradual improvement set in but even 7 months after irradiation values were still slightly below the mean but well within the normal range. Minimum counts in survivors were reached between 41 and 42 days. Although there was a slight dose/response relationship apparent at ½ hour after irradiation i.e. the higher doses tending to depress counts more, none could be clearly discerned thereafter. There was evidence to suggest that at the highest doses considerable increases of rbcs counts occurred terminally which was not seen at the lower levels (see Figure 20).

Table XL

Red Blood Cells

Mean Cell Counts and Percentage Changes

Dose Range 400r to 700r

Period	NON-SURVIVORS		SURVIVORS	
	Millions of Cells per cu mm.	Percentage of Control	Millions of Cells per cu mm.	Percentage of Control
Pre-Irradiation Control	13.98	-	16.23	-
Post-Irradiation				
½ hr	12.28	- 12.16	15.64	- 3.64
1 Day	14.70	+ 5.15	16.27	+ 0.25
2-7 Days	14.70	+ 5.15	15.44	- 4.87
8-30 Days	14.77	+ 5.65	14.66	- 9.67
31-60 Days	11.67	- 16.52	12.51	- 22.92
61-210 Days	-		14.59	- 10.11

Table XLI

Effects of X-Irradiation on Goats

Total Erythrocytes (Maximum Values)

Goat No.	Dose (r)	Control RBC Count$_3$ (Cells/mm^3 x 10^6)	Maximum RBC Count$_3$ (Cells/mm^3 x 10^6)	Hrs/Days Post Irradiation when Max. recorded	Survival Time (Days)	% Increase: Maximum Recorded	% Increase: Mean for Dose	% Increase: Mean for all goats	% Increase: Mean for Dose Range 400r - 700r
				Non-Survivors					
C229	2000	15.44	17.29	8 days	8	12	12	-	-
C234	1000	18.80	25.80	7 days	8	37.2	37.2		-
C175	700	13.35	13.70	1 day	11	2.6			
F 40	700	12.21	13.44	7 days	13	10.1	5.5		
F 27	700	13.10	13.61	4 days	46	3.9			
F 14	650	18.50	21.50	22 days	42	16.2			
F 24	650	15.40	19.90	7 days	14	29.2	22.7	19.5	
C315	600	14.43	16.26	4 days	30	12.7	12.7		18.4
C118	550	13.40	16.56	7 days	18	23.6			
C200	550	14.08	18.72	9 days	12	33.0	30.3		
F 20	550	11.40	15.30	6 days	12	34.2			
				Survivors					
F 31	650	12.15	16.50	10 days		35.8	35.8		
F 30	600	13.17	15.28	4 days		16.0			
M 9	600	18.45	18.80	½ hr		1.9	9.0		
M 3	500	17.85	18.80	7 days		5.3			
M 1	500	16.68	19.50	3 days		16.9	9.1	14.0	14.0
C240	500	18.94	19.88	468 days		5.0			
M 10	400	14.15	14.51	134 days		2.5			
W316	400	18.57	22.21	8 days		19.6	15.0		
W302	400	16.10	19.80	118 days		23.0			

Mean time to reach maximum count :

Non-survivors : 7.5 days

Survivors : 84 days

Total Erythrocytes (Minimum Values)

Goat No.	Dose (r)	Control RBC count ($Cells/mm^3$ x 106)	Minimum RBC count ($Cells/mm^3$ x 106)	Hrs/Days Post Irradiation when min. recorded	Survival Time (Days)	% Decrease: Minimum Recorded	% Decrease: Mean for Dose	% Decrease: Mean for all goats	% Decrease: Mean for dose range 400r - 700r
				Non-Survivors					
C229	2000	15.44	12.51	½ hr	8	19.0	19.0	-	-
C234	1000	18.80	18.20	4 days	8	3.2	3.2		-
C175	700	13.35	10.70	2 days	11	20.0			
F 40	700	12.21	10.26	½ hr	13	16.0	35.4		
F 27	700	13.10	3.90	44 days	46	70.2			
F 14	650	18.50	15.33	27 days	42	17.0			
F 24	650	15.40	14.60	½ hr	14	5.0	11.0	18.8	20.5
C315	600	14.43	9.02	29 days	30	35.5	35.5		
C118	550	13.40	11.91	15 days	18	11.1			
C200	550	14.08	12.51	½ hr	12	11.2	6.9		
F 20	550	11.40	11.60	1 day	12	+ 1.7			
				Survivors					
F 31	650	12.15	8.70	6 days		28.4	28.4		
F 30	600	13.17	7.50	48 days		43.1	38.5		
M 9	600	18.45	12.20	2 days		33.9			
M 3	500	17.85	12.90	49 days		27.7			
M 1	500	16.68	10.50	126 days		37.0	34.8		
C240	500	18.94	11.43	56 days		39.7		34.1	34.1
M 10	400	14.15	8.80	30 days		37.8			
W316	400	18.57	14.70	42 days		20.8	32.5		
W302	400	16.10	9.84	15 days		38.9			

Mean Time to reach minimum.

Non-Survivors — All Goats - 11.1 days

Dose Range 400 to 700r - 13.1 days

Survivors — 41.6

ii) Haemoglobin

Changes in the Haemoglobin content as a function of Time, Survival and Dose.

Table XLIII presents a summary of the mean amounts of haemoglobin in the blood of goats exposed to X radiation at different periods after irradiation. The graph at Figure 28 shows the similarity between the effects of irradiation on the haemoglobin content and red blood cell counts. The maximal changes which occurred are presented in Tables XLIV and XLV. There was an overall reduction in Hb at ½ hour in both survivors and non-survivors; the reduction in those goats which died (-13%) was significantly greater than in those which survived (-3.7%). However, values at 1 day post irradiation were slightly above control and were similar in both groups. From the 1st to about the 30th day non-survivors showed values just above normal but these were drastically reduced prior to death; a distinct diphasic minimal depression occurred in non-survivors, one during the first week post irradiation and the other terminally. Survivors, on the other hand, tended to show gradual decreases in Hb content after the first day post irradiation over a period of some 60 days. They had not recovered the pre irradiation level even after 7 months. The maximum depression of Hb was very similar in both groups; the mean time to reach minimum values in survivors (25.3% below control) was 37 days after irradiation.
A slight dose/response correlation was evident at ½ hour post irradiation i.e. lower Hb values at higher doses, and this persisted for one day. There after no interrelationship could be discerned although at the highest doses a haemoconcentration occurred terminally (see Figure 20).

Table XLIII

Haemoglobin

Mean Values and Percentage Changes

Dose Range 400r to 700r

Period	NON-SURVIVORS		SURVIVORS	
	Gms/100ml	Percentage of Control	Gms/100ml	Percentage of Control
Pre-Irradiation Control	12.31	-	12.96	-
Post-Irradiation				
½ hr	10.72	- 12.92	12.48	- 3.70
1 Day	12.80	+ 3.98	13.14	+ 1.39
2-7 Days	12.83	+ 4.22	12.73	- 1.78
8-30 Days	12.58	+ 2.19	11.68	- 9.88
31-60 Days	9.70	- 21.20	10.84	- 16.36
61-210 Days	-	-	12.44	- 4.01

Haemoglobin
(Minimum Values)

Goat No.	Dose	Control Hb level (gm/100 ml)	Minimum Hb level recorded (gm/100 ml)	Hrs/days Post Irradiation taken min. recorded	Survival Time (Days)	% Decrease: Minimum Recorded	% Decrease: Mean for Dose	% Decrease: Mean for all goats	% Decrease: Mean for Dose Range 400r - 700r
			Non-Survivors		Death				
C229	2000	16.45	12.2	½ hr	8 days	25.8	25.8	-	-
C234	1000	16.70	16.3	4 days	8 days	2.4	2.4		
C175	700	13.20	11.7	3 days	11 days	11.4			
F 40	700	11.10	8.0	½ hr	13 days	28.0	38.1		
F 27	700	10.75	2.7	44 days	46 days	74.9			
F 14	650	13.45	12.1	40 days	42 days	10.1		20.77	
F 24	650	11.00	9.7	½ hr	14 days	11.8	10.95		28.5
C315	600	13.30	8.1	29 days	30 days	39.1	39.1		
C118	550	12.65	11.9	½ hr	18 days	5.9			
C200	550	14.13	12.5	1 day	12 days	11.5	12.5		
F 20	550	11.25	10.4	½ hr	12 days	7.6			
			Survivors		Time at end of observations				
F 31	650	10.2	8.9	37 days	157 days	12.8	12.8		
F 30	600	12.27	7.8	42 days	239 days	36.4			
M 9	600	12.1	8.6	22 days	164 days	28.9	32.65		
M 3	500	12.3	9.8	22 days	190 days	21.3		25.3	25.3
M 1	500	13.35	9.5	52 days	179 days	28.8	24.77		
C240	500	15.3	11.6	49 days	584 days	24.2			
M 10	400	10.6	8.6	43 days	172 days	18.9			
W316	400	15.95	12.1	42 days	213 days	24.1	25.0		
W302	400	14.55	9.9	25 days	225 days	32.0			

Mean Time to reach Minimum Values

Non-Survivors (all goats) - 11 days (400 - 700r) - 13 days

(There is a biphasic reduction in Non-Survivors)

Survivors - 37 days

Table XLV

Effects of X-irradiation on Goats

Haemoglobin
(Maximum Values)

Goat No.	Dose	Control Hb level (gm/100 ml)	Maximum Hb level recorded (gm/100 ml)	Hrs/Days Post Irrad. when Maximum recorded	Survival Time (Days)	% Increase: Maximum Recorded	% Increase: Mean for Dose	% Increase: Mean for all Goats	% Increase: Mean for Dose Range 400r - 700r
				Non-Survivors	Death				
C229	2000	16.45	16.7	8 days	8 days	1.5	1.5		-
C234	1000	16.70	22.0	7 "	8 "	31.7	31.7		-
C175	700	13.20	13.3	1;6 "	11 "	0.75			
F 40	700	11.10	11.8	9 "	13 "	6.3	5.3		
F 27	700	10.75	11.7	11 "	46 "	8.8			
F 14	650	13.45	16.4	20 "	42 "	21.9		15.6	
F 24	650	11.00	14.4	7 "	14 "	30.9	26.4		15.4
C315	600	13.30	14.7	1 "	30 "	10.5	10.5		
C118	550	12.65	16.4	1 "	18 "	29.6			
C200	550	14.13	15.0	11 "	12 "	6.2	19.8		
F 20	550	11.25	13.9	6 "	12 "	23.6			
				Survivors	End of observations				
F 31	650	10.20	12.7	94 "	175 days	24.5	24.5		
F 30	600	12.27	14.5	4 "	239 "	18.2			
M 9	600	12.10	13.0	114; 156 "	164 "	7.4	12.8		
M 3	500	12.30	13.9	12 "	190 "	13.1			
M 1	500	13.35	15.1	1 "	179 "	13.1	9.2	11.6	11.6
C240	500	15.30	15.5	296 "	584 "	1.3			
M 10	400	10.60	12.5	106 "	172 "	17.9			
W316	400	15.95	16.3	121; 142 "	213 "	2.2	8.9		
W302	400	14.55	15.5	118 "	225 "	6.5			

Mean Time to Reach Maximum Values

Non-Survivors 7.5 to 8 days

Survivors 96 to 103 days

iii) Packed Cell Volume

Changes in the PCV as a function of Time, Survival and Dose.

It can be seen from Table XLVI and the graph (Figure 29) that PCV values paralleled closely that ofthe rbc counts and Hb content. The maximal changes which occurred are given in Table XLVII. At ½ hour after irradiation the depression in non-survivors (-15.6% of control) was significantly greater than in survivors (-3%). At 1 day both groups were above control values but non-survivors (6%) were higher than survivors (2.5%). Thereafter non-survivors remained above normal values, although decreasing in extent gradually with time, up to 30 days after which there was a precipitous drop to 32% below controls. In survivors, however, PCV values were about normal for about 7 days after irradiation and thereafter tended to be slightly depressed. The maximum depression of PCV was similar in both survivors (-23.8%) and in non-survivors (-27.8%) but the rate of decrease was considerably more gradual in survivors. Maximum per cent increases were also similar in both groups being reached in non-survivors (14% above control) in 5 days and in survivors (11.6%) in 49 to 53 days.
There was a slight dose/response correlation at ½ hour (below control) and at 1 day (above control) but thereafter little or no inter-relationship could be discerned.

Table XLVI

Packed Cell Volume (PCV)

(Haematocrit)

Mean Values and Percentage Changes

(Dose Range 400r to 700r)

Period	NON-SURVIVORS		SURVIVORS	
	Mean Values (%)	Percentage of Control	Mean Values (%)	Percentage of Control
Pre-Irradiation Control	32.34		33.21	
Post-Irradiation				
½ hr	27.28	- 15.65	32.22	- 2.98
1 Day	34.28	+ 6.00	34.05	+ 2.53
2-7 Days	33.60	+ 3.90	33.23	+ 0.09
8-30 Days	32.64	+ 0.93	30.61	- 7.83
31-60 Days	21.96	- 32.10	28.52	- 14.12
61-210 Days	-		30.03	- 9.58

iv) The Erythrocytic Indices (MCHC, MCH and MCV)

Anaemia is generally classified by three criteria viz 1) the concentration of haemoglobin within the red blood cell; 2) the average size of the cell; and 3) the presence or absence of regenerative changes in the cellular elements in the blood.

The maximal changes which occurred in Hb concentration and cell size are presented in Tables XLVIII, XLIX and L, and graphs Figs 30, 31 and 32.

Table XLVII

Effect of X-Irradiation on Goats

Packed Cell Volume (PCV) Haematocrit

Dose (r)	Control Mean PCV (%)	Maximum PCV recorded (%)	Hrs/Days Post Irradiation when maximum recorded	Minimum PCV recorded (%)	Hrs/Days Post Irradiation when minimum recorded	Survival Time at death or at end of observations (days)	% Increase: Maximum recorded	% Increase: Mean for Dose	% Increase: Mean for all Goats	% Increase: Mean for Dose Range 400 to 700r	% Decrease: Minimum recorded	% Decrease: Mean for Dose	% Decrease: Mean for all Goats	% Decrease: Mean for Dose Range 400 to 700r
						Non-Survivors								
2000	38.75	47.00	8 days	29.50	½ hr	8 days	21.29	21.29	-	-	23.87	23.87		-
1000	41.50	59.50	7 days	41.00	4 days	8 days	43.37	43.37		-	1.20	1.20		-
700	35.00	36.00	1 day	32.00	9 days	11 days	2.86				8.57			
700	26.25	29.00	2 days	20.50	½ hr	13 days	10.48	6.33			21.90	33.43		
700	26.50	28.00	1,4 & 5 days	8.00	44 days	46 days	5.66		17.29		69.81		25.00	
650	37.50	45.00	20 days	16.14	41 days	42 days	20.00				56.96			
650	28.00	35.50	7 days	26.00	½ hr	14 days	26.79	23.40		13.95	7.14			27.77
600	38.25	38.00	1 day	22.00	29 days	30 days	- 0.65	- 0.65			42.48	42.48		
550	34.25	41.50	1 day	29.50	½ hr	18 days	21.17				13.87			
550	36.83	39.00	7 days	28.00	½ hr	12 days	5.89	20.13			23.98	14.37		
550	28.50	38.00	6 days	27.00	½ hr	12 days	33.33				5.26			
						Survivors								
650	27.00	30.00	2 & 10 days	21.00	59 days	157 days	11.11	11.11			22.22	22.22		
600	31.67	34.50	4 days	22.00	42 days	239 days	8.94	4.47			30.53			
600	34.00	34.00	1 day	24.00	24 days	164 days	0.0				29.41			
500	29.00	36.00	4 & 28 days	25.00	22 days	190 days	24.14		11.56	11.56	13.79		23.8	23.8
500	34.50	41.00	3 days	27.00	66, 126 & 154 days	179 days	18.84	17.40			21.74	21.50		
500	38.00	41.50	296 days	27.00	49 days	584 days	9.21				28.95			
400	28.50	34.00	8 days	23.00	25 & 43 days	172 days	19.30				19.30			
400	40.50	43.00	6 days	32.00	22 days	213 days	6.17	10.59			20.99	22.52		
400	35.75	38.00	118 days	26.00	15 days	225 days	6.29				27.27			

Mean Time to reach Maximum Value : Non-Survivors — All Goats ..... 5.5 to 6 days 400 - 700r 5 to 5.5.

Survivors ..... 49 to 53 days

Mean Time to reach Minimum Value : Non-Survivors ..... 11.5 days 400 - 700r - 13.7 days

Survivors ..... 36 to 48 days

Majority of Non-Survivors reached Minimum Values in ½ hr.

Table XLVIII

Effects of X-Irradiation on Goats

Erythrocytic Index MCHC (Mean Corpuscular Haemoglobin Concentration)

Goat No.	Dose (r)	Control Mean MCHC (%)	Maximum MCHC Recorded (%)	Hrs/Days Post Irradiation when maximum recorded	Minimum MCHC Recorded (%)	Hrs/Days Post Irradiation when minimum recorded	Survival Time at Death or at end of Observation (days)	% Increase: Maximum recorded	% Increase: Mean for Dose	% Increase: Mean for all Goats	% Increase: Mean for Dose Range 400 - 700r	% Decrease: Minimum Recorded	% Decrease: Mean for Dose	% Decrease: Mean for all Goats	% Decrease: Mean for Dose Range 400 - 700r
							Non-Survivors								
229	2000	41.00	47.20	2 days	35.50	8 days	8	15.12	15.12		-	13.41	13.41		-
234	1000	40.30	40.30	2 days	37.00	7 days	8	0.0	0.0		-	8.19	8.19		
275	700	37.75	38.00	6 days	35.40	3 days	11	0.66				6.23			
40	700	42.30	41.90	8 days	31.40	2 days	13	- 0.95	2.41			25.77	16.29		
27	700	40.55	43.60	22 days	33.70	44 days	46	7.52		10.36	10.98	16.89		9.20	
14	650	35.85	52.61	40 days	33.50	1 day	42	46.75				6.56	5.83		8.84
24	650	39.30	40.50	7 days	37.30	½ hr	14	3.05	24.9			5.09			
315	600	37.25	40.30	15 days	34.30	10 days	30	8.19	8.19			6.85	6.85		
118	550	36.90	42.40	7 days	37.60	2 days	18	14.91				- 1.90			
200	550	38.50	46.40	½ hr	36.80	1 day	12	20.52	11.19			4.42	4.07		
20	550	39.48	38.75	3 days	35.66	1 day	12	- 1.85				9.68			
							Survivors								
31	650	37.78	47.04	94 days	32.86	17 days	157	24.51	24.51			13.02	13.02		
30	600	38.73	46.40	14 and 130 days	32.96	48 days	239	19.80				14.90			
9	600	35.58	43.33	156 days	32.50	2 days	164	21.78	20.79			8.66	11.78		
3	500	42.49	46.33	12 days	33.89	28 days	190	9.04		16.94		20.24		14.22	
1	500	38.65	44.19	17 days	33.93	52 days	179	14.33	12.09		16.94	12.21	13.38		14.22
240	500	40.30	45.50	42 days	37.20	238 days	584	12.90				7.69			
10	400	37.19	48.08	106 days	30.00	8 days	172	29.28				19.33			
316	400	39.38	44.38	22 days	34.25	8 days	213	12.70	16.70			13.03	17.09		
302	400	40.69	44.00	167 days	33.00	25 days	225	8.13				18.90			

Mean Time to reach maximum values — Non-Survivors : All Goats ..... 10.2 days; Dose range 400 to 700r ... 12.0 days
Survivors : ..... 70 to 83 days

Mean Time to reach minimum values — Non-Survivors : All Goats ..... 7.2 days; Dose range 400 to 700r.... 7.1 days
Survivors : ..... 47 days

Table XLIX

Effects of X-Irradiation on Goats

Erythrocytic Index MCH (Mean Corpuscular Haemoglobin)

Goat	Dose	Control Mean MCH (μμg)	Maximum MCH Recorded (μμg)	Hrs/Days Post Irradiation when Maximum Recorded	Minimum MCH Recorded (μμg)	Hrs/Days Post Irradiation when Minimum Recorded	Survival Time at Death or at end of observations	% Increase: Maximum Recorded	% Increase: Mean for Dose	% Increase: Mean for all Goats	% Increase: Mean for Dose Range 400 - 700r	% Decrease: Minimum Recorded	% Decrease: Mean for Dose	% Decrease: Mean for all Goats	% Decrease: Mean for Dose Range 400 - 700r
							Non-Survivors								
0229	2000	10.25	10.10	7 days	9.65	1 & 8 days	8 days	- 1.46	- 1.46		-	5.85	5.85		-
0234	1000	8.90	9.30	1 day	8.50	7 days	8 days	4.49	4.49		-	4.49	4.49		-
0175	700	9.90	11.70	2 days	9.50	10 days	11 days	18.18				4.04			
F 40	700	9.10	9.20	9 days	7.20	2 days	13 days	1.10	20.25			20.88	13.59		
F 27	700	8.20	11.60	20 days	6.90	44 days	46 days	41.46				15.85			
F 14	650	7.28	10.11	27 days	7.09	40 days	42 days	38.87		13.15		2.61		10.73	
F 24	650	7.20	7.80	4 days	6.60	½ hr; 9 & 11 days	14 days	8.33	23.60		15.74	8.33			11.97
0315	600	9.22	11.30	8 days	8.10	4 & 22 days	30 days	22.56	22.56			12.15	12.15		
0118	550	9.40	10.50	3 & 17 days	8.90	14 days	18 days	11.70				5.32			
0200	550	10.10	10.40	½ hr	7.90	9 days	12 days	2.97	3.71			21.78	14.62		
F 20	550	9.85	9.50	3 days	8.20	10 days	12 days	- 3.55				16.75			
							Survivors								
F 31	650	8.40	11.61	6 days	6.73	10 days	157 days	38.21	38.21			19.88	19.88		
F 30	600	9.32	11.87	48 days	8.15	20 days	239 days	27.36	28.62			12.55	8.41		
M 9	600	6.56	8.52	2 days	6.28	½ hr	164 days	29.88				4.27			
M 3	500	6.90	9.31	28 days	6.15	½ hr	190 days	34.93				10.87			
M 1	500	8.03	10.76	126 days	7.20	14 days	179 days	34.00	36.56	30.61	30.61	10.34	9.13	11.73	11.73
0240	500	8.10	11.40	161 days	7.60	17 days	584 days	40.74				6.17			
M 10	400	7.49	10.69	58 days	7.65	134 days	172 days	42.72				+ 2.14			
W316	400	8.60	9.52	3 days	6.17	8 days	213 days	10.70	23.45			16.63	13.83		
W302	400	9.04	10.57	15 days	6.60	25 days	225 days	16.92				26.99			

Mean Time to reach Maximum values :

Non-Survivors: All Goats ..... 7.6 to 8.9 Days
Dose Range 400 to 700r ..... 8.4 to 10.0 Days

Survivors : ..... 49.7 Days

Mean Time to reach Minimum values :

Non Survivors: All Goats ..... 12.8 to 16.1 Days
Dose Range 400 to 700r ..... 14.8 to 18 Days

Survivors : ..... 25.3 Days

Table L

Effects of X-Irradiation on Goats

Erythrocytic Index MCV (Mean Corpuscular Volume)

Goat No.	Dose (r)	Control Mean MCV (cu.μ)	Maximum MCV recorded (cu.μ)	Hrs/Days Post Irradiation when Maximum recorded	Minimum MCV recorded (cu.μ)	Hrs/Days Post Irradiation when Minimum recorded	Survival Time at Death or End of Observations (days)	% Increase: Maximum	% Increase: Mean for Dose	% Increase: Mean for all Goats	% Increase: Mean for Dose range 400 - 700r	% Decrease: Minimum	% Decrease: Mean for Dose	% Decrease: Mean for all Goats	% Decrease: Mean for Dose range 400 - 700r
							Non-Survivors								
0229	2000	25.10	27.18	8 days	20.60	2 days	8	8.3	8.3			17.3	17.3		
0234	1000	22.00	23.60	1 day	22.40	4 days	8	7.3	7.3			+ 1.8	+ 1.8		
0175	700	26.20	31.77	2 days	26.19	½ hr	11	21.3				0.0			
F 40	700	21.45	23.00	2 days	20.00	1 hr	13	7.2	18.3			6.7	4.0		
F 27	700	20.18	25.53	18 days	19.12	12 days	46	26.5				5.3			
F 14	650	20.22	25.00	16 days	13.47	40 days	42	23.6	14	10.5	11.0	33.4	18.6	11.7	12.5
F 24	650	18.30	19.10	1 & 4 days	17.60	11 days	14	4.4				3.8			
0315	600	24.75	28.80	8 days	20.90	4 days	30	16.4	16.4			15.6	15.6		
0118	550	25.55	27.00	3 days	21.80	10 days	18	5.7				14.7			
0200	550	26.13	23.70	2 days	20.30	9 days	12	- 9.3	0.0			22.3	16.1		
F 20	550	25.00	25.90	1 day	22.20	½ hr & 10 days	12	3.6				11.2			
							Survivors								
F 31	650	22.24	28.74	6 days	18.18	10 days	157	29.2	29.2			18.3	18.3		
F 30	600	24.06	36.00	48 days	20.74	20 days	239	49.6	45.95			13.8	12.2		
M 9	600	18.43	26.23	2 days	16.49	½ hr	164	42.3				10.5			
M 3	500	16.25	27.48	28 days	16.64	126 days	190	69.1		35.3	35.3				
M 1	500	20.73	25.71	126 days	19.24	98 days	179	24.0	43.2			7.2	2.4	12.4	12.4
0240	500	20.00	27.30	238 days	19.50	15 days	584	36.5				2.5			
M 10	400	20.15	27.27	30 days	17.57	134 days.	172	35.3				12.9			
W316	400	21.84	24.71	3 days	16.58	22 days	213	13.1	22.5			24.1	20.5		
W302	400	22.21	26.42	15 days	16.77	34 days	225	19.0				24.5			

Mean Time to reach Maximum values : Non-Survivors — All Goats . . . . . 5.5 to 6 days; Dose Range 400r to 700r.... 6 to 6.3 days

Survivors . . . . . 55 days

Mean Time to reach Minimum Values : Non-Survivors — All Goats . . . . . 8.5 to 9.4 days; Dose Range 400r to 700r . . 9.7 to 10.8 days

Survivors . . . . . 51 days

v) Red Blood Cell Fragility

Figure 33 depicts the general trends in RBC fragility in survivors and non-survivors. The differences of fragility in irradiated goats as compared with normal goats was not significant; however and this is better illustrated in the graph, the mean fragility of the RBCs of non-survivors was greater than that of survivors. In both groups it was always above normal values and continued so until the end of observations.

vi) Erythrocyte Sedimentation Rate

The ESR in goats is extremely slow and irradiation did not have any consistent effects although in some goats there appeared to be a correlation (after prolonged periods of standing) between the ESR and the anaemic state of the animal. The stability of the blood suspension was different from the mean control in some irradiated goats. Table LI presents data on survivors and non-survivors over a period of 7 days. It will be seen that effects up to 3 days were similar in both groups but that thereafter the rate in non-survivors was significantly greater than in survivors.

Table LI

Erythrocyte Sedimentation Rate

(mm)

NON-SURVIVORS

	C229 2000	C234 1000	C175 700	F40 700	F27 700	F14 650	F24 650	C315 600	C118 550	C200 550	F20 550
1 hr	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
24 hrs	4	4	8	5	3	4	5	4	3	4	4
2 days	8	8	5	6	5	6	6	5	5	6	6
3 days	16	16	10	12	12	12	13	13	10	14	4
4 days	22	21	17	18	19	18	20	21	20	19	21
5 days	26	25	20	22	22	21	22	23	25	24	25
6 days	29	30	25	25	26	27	28	27	29	30	30
7 days	31	33	29	30	30	29	30	29	32	33	33

SURVIVORS

	Control (Mean)	400 W316	400 W302	400 MID	500 C240	500 MI	500 M3	600 F30	600 M9	650 F31
1 hr	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
24 hrs	4	3	3	2	5	3	3	5	4	5
2 days	7	5	5	6	9	5	5.5	9	8	10
3 days	10	6	7	11	13	6	8.5	15	11	16
4 days	14	8	10	16	17	9	11	17	13.5	21
5 days	17	10	13	20	20.5	11	13.5	20	16	26
6 days	20	11	14	24	23.5	13	16	23	19	30.5
7 days	21	12.5	16	27.5	26.5	15	19	25	22	33.0

DISCUSSION

In general the findings are in agreement with those reported for other animals. A progressive and considerable increase terminally in rbc count, haemoglobin content and haematocrit was noted in a few non-surviving goats which had received the higher doses; most of the other animals, both survivors and non-survivors, showed a gradual and irregular decrease but the rate of this depression was more gradual in survivors. The fact that a compensatory mechanism exists in healthy goats by which animals with a low rbc count have larger corpuscles than those with high counts (Holman, 1952) makes the diagnosis of anaemia in goats more difficult. It was considered that whenever a significant reduction in the red cell count in non-survivors was no longer apparent a probable cause was a simultaneous alteration in plasma volume had occurred to mask an underlying anaemia which could be greater than the rbc count indicated (Kahn et al, 1952). This is especially liable to occur in goats because of the extremely small size of the red blood cells and the fact that goat blood contains about 7 times as much trapped plasma as human blood (Klement et al, 1954; Wadsworth, 1957).

In normal goats and sheep, rbcs are destroyed within the body as they reach the end of their life span which ranges from 70 to 150 days (Tucker, EM, 1963). Certain toxic factors can cause the abnormal haemolysis of rbcs within the body e.g. by lead, phenothiazine, bracken poisoning, propylene glycol, some forms of allergy and so on. In this study it was observed that in normal adult goats rbcs showed some haemolysis in a mean saline concentration of 0.64% and were completely haemolysed at 0.4%: the mean corpuscular fragility (MCF) was found to be 0.5% NaCl. This phenomenon of partial haemolysis is explained by Hendry, 1947 as being the result of a proportion only of the rbcs haemolysing at any time whilst the remainder are unaffected. In non-survivors some haemolysis was shown at 0.72% NaCl whereas in survivors this occurred at 0.68%: again, complete haemolysis occurred in non-

survivors at 0.44% NaCl and in survivors at 0.42%.

The blood pigment released after haemolysis of rbcs is excreted by the liver or the kidney and often gives rise to a haemoglobinuria or to the presence of bilirubin in the urine. Both of these abnormalities were noted in irradiated goats; in addition, in histopathological examinations of post-mortem material haemosiderin was a common finding. It is probable that haemolysis was increased in irradiated goats by the presence of the haemolysing toxins of Clostridium welchii as a result of gastro-intestinal injury (Gordon et al 1940). The higher ESR in irradiated goats after 7 days poses the question whether abnormal fibrènogens had increased in amount due to the pyrexia (Smith, 1957) and that these had attracted heparinoid substances from the destruction of body mast cells (Thomas et al 1954). This would in part explain coagulation defects together with the terminal eosinophilia observed in some goats (Riley, 1959; Archer, 1954). The appearance of regenerative forms mainly normoblasts commonly observed in blood films of survivors and occasionally in non-survivors indicated erythropoietic activity.

The gradual reduction in erythrocytic elements in non-survivors after about the 14th day post-irradiation indicated a cessation of haematopoiesis which continued until death; this fall was progressive and more gradual in survivors up to the 60th day post irradiation after which an improvement was discernible.

In non-survivors, moreover, there was a sudden and massive decrease in the ~~formation~~ number of rbcs near death indicated by a dramatic fall in the PCV terminally. ~~confirming the cessation of erythropoiesis~~. At the same time there was a very low haemoglobin content (8.48 gm/ml) indicating a pathological anaemia which was not shown to such a degree in survivors. This anaemia was normochromic and normocytic. It was considered to be

caused mainly by post-haemorrhagic and dyshaematopoietic factors to which were added the destructive effects of a circulating bacterial haemolysin. The erythropoietic tissue in general showed an intial resistance to irradiation and this was followed by a progressive hypoplastic anaemia.

To sum up, in non-survivors there was terminal anaemia caused probably by a combination of haemorrhage, dyshaematopoiesis and abnormal intravascular haemolysis whereas in survivors an initial erythrocytosis was followed by a milder chronic hypoplastic anaemia.

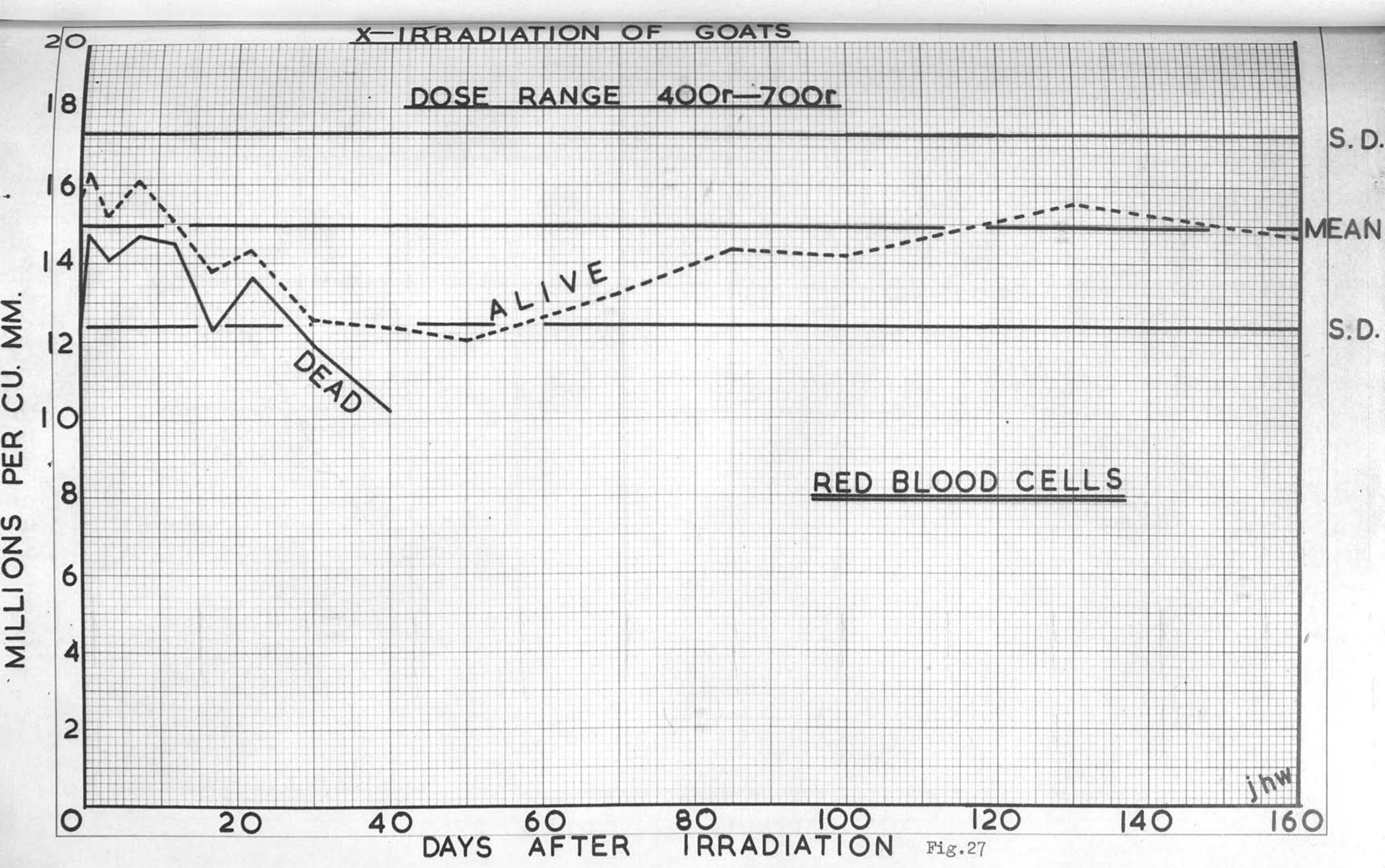

X—IRRADIATION OF GOATS
DOSE RANGE 400r—700r
S. D.
MEAN
S. D.
ALIVE
DEAD
RED BLOOD CELLS
jhw
MILLIONS PER CU. MM.
20
18
16
14
12
10
8
6
4
2
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig.27

Fig. 29

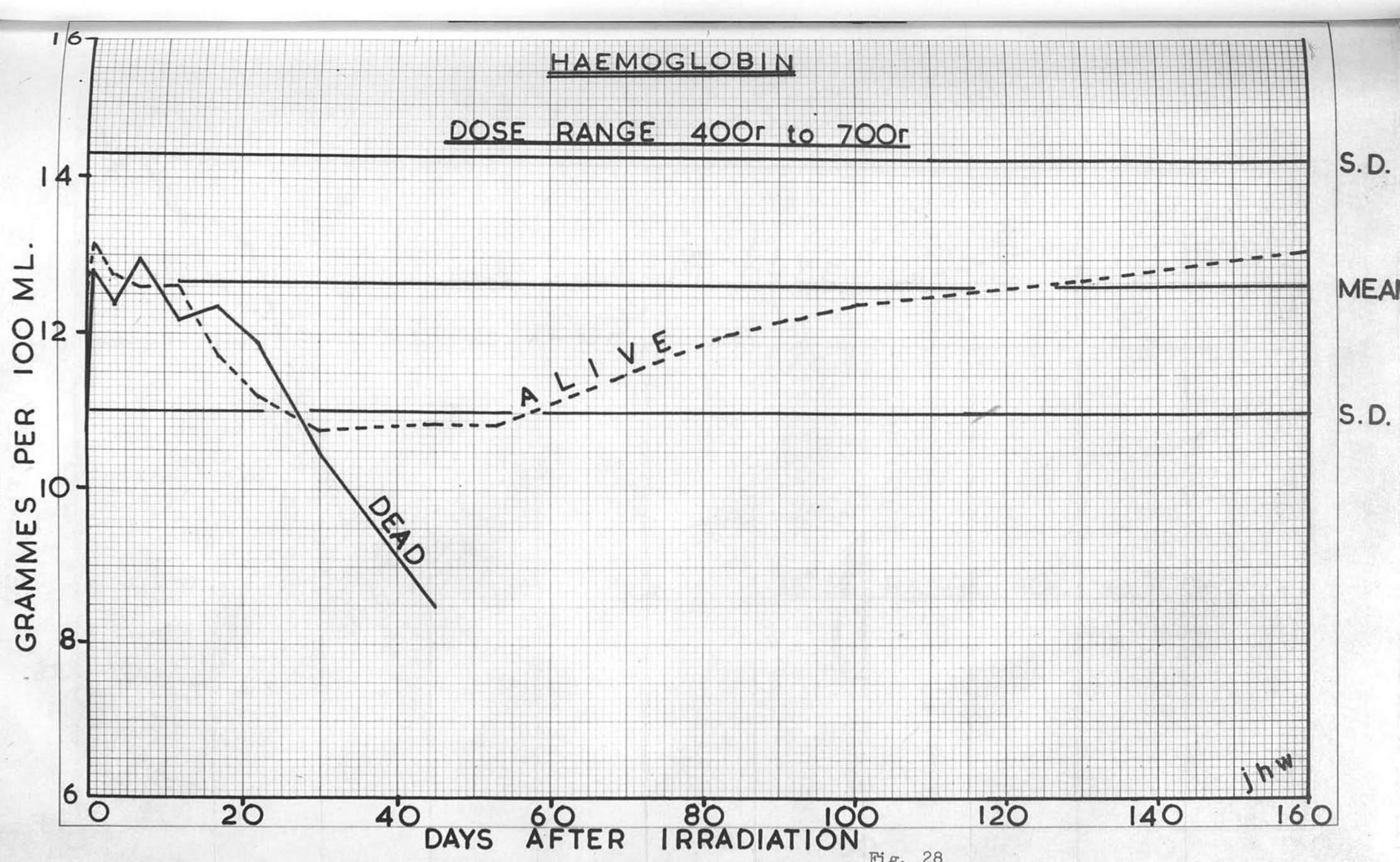

HAEMOGLOBIN
DOSE RANGE 400r to 700r
S.D.
MEAN
S.D.
ALIVE
DEAD
GRAMMES PER 100 ML.
16
14
12
10
8
6
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION
jhw

Fig. 28

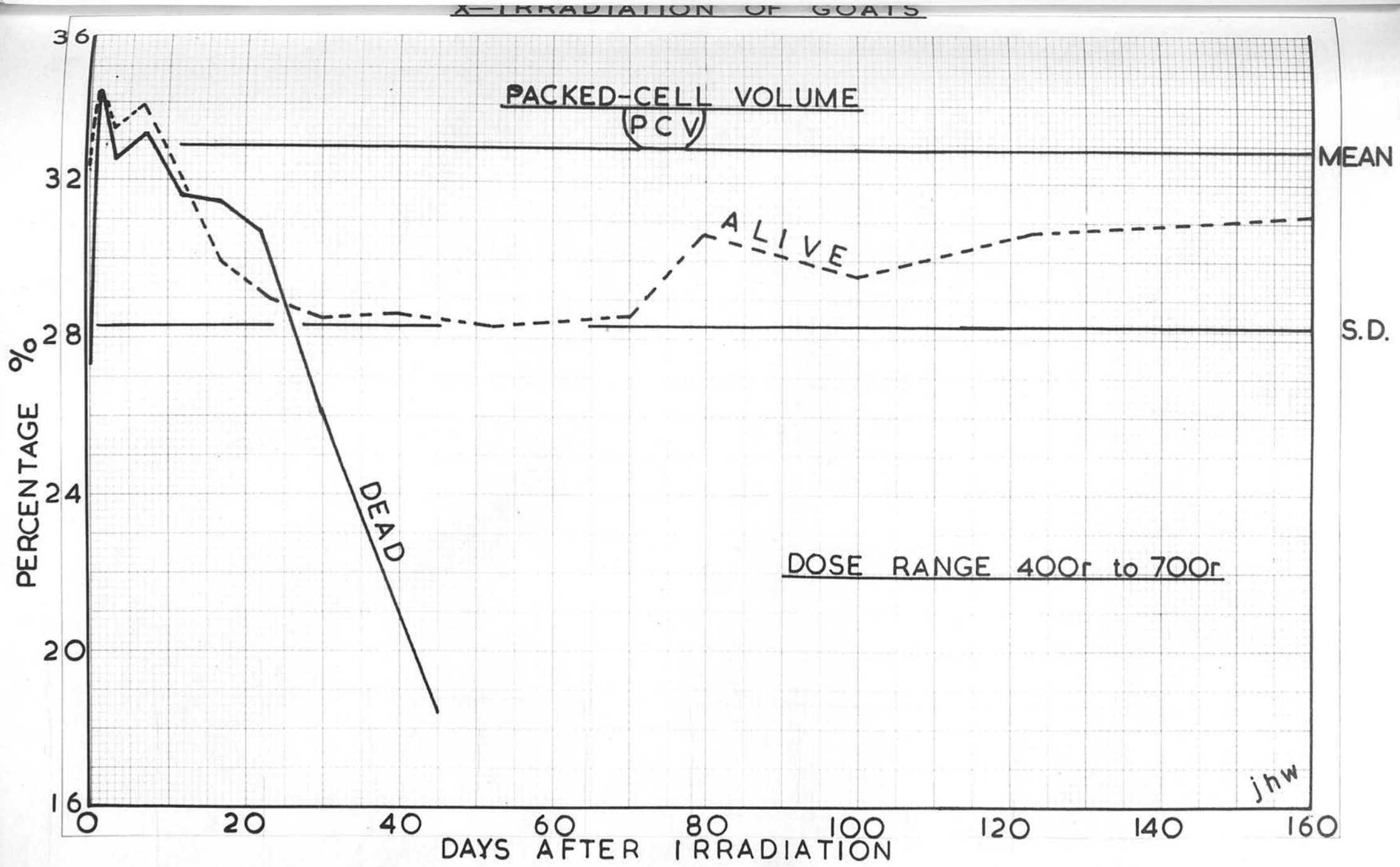

X—IRRADIATION OF GOATS
PACKED-CELL VOLUME
PCV
MEAN
S.D.
ALIVE
DEAD
DOSE RANGE 400r to 700r
jhw
PERCENTAGE %
DAYS AFTER IRRADIATION
36
32
28
24
20
16
0
20
40
60
80
100
120
140
160

Fig. 29

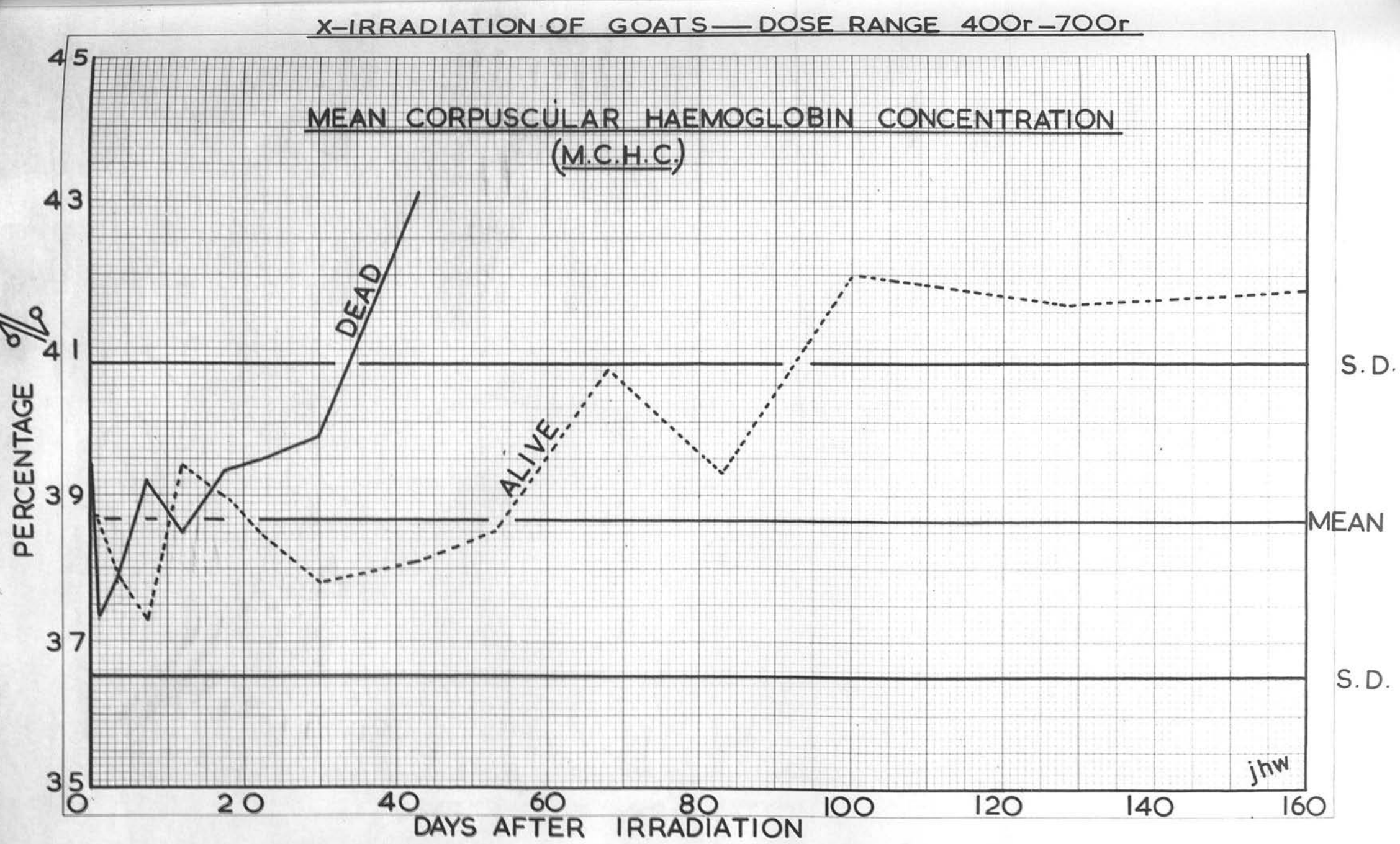

X-IRRADIATION OF GOATS — DOSE RANGE 400r – 700r
MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION
(M.C.H.C.)
45
43
41
39
37
35
%
PERCENTAGE
DEAD
ALIVE
S.D.
MEAN
S.D.
jhw
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 30

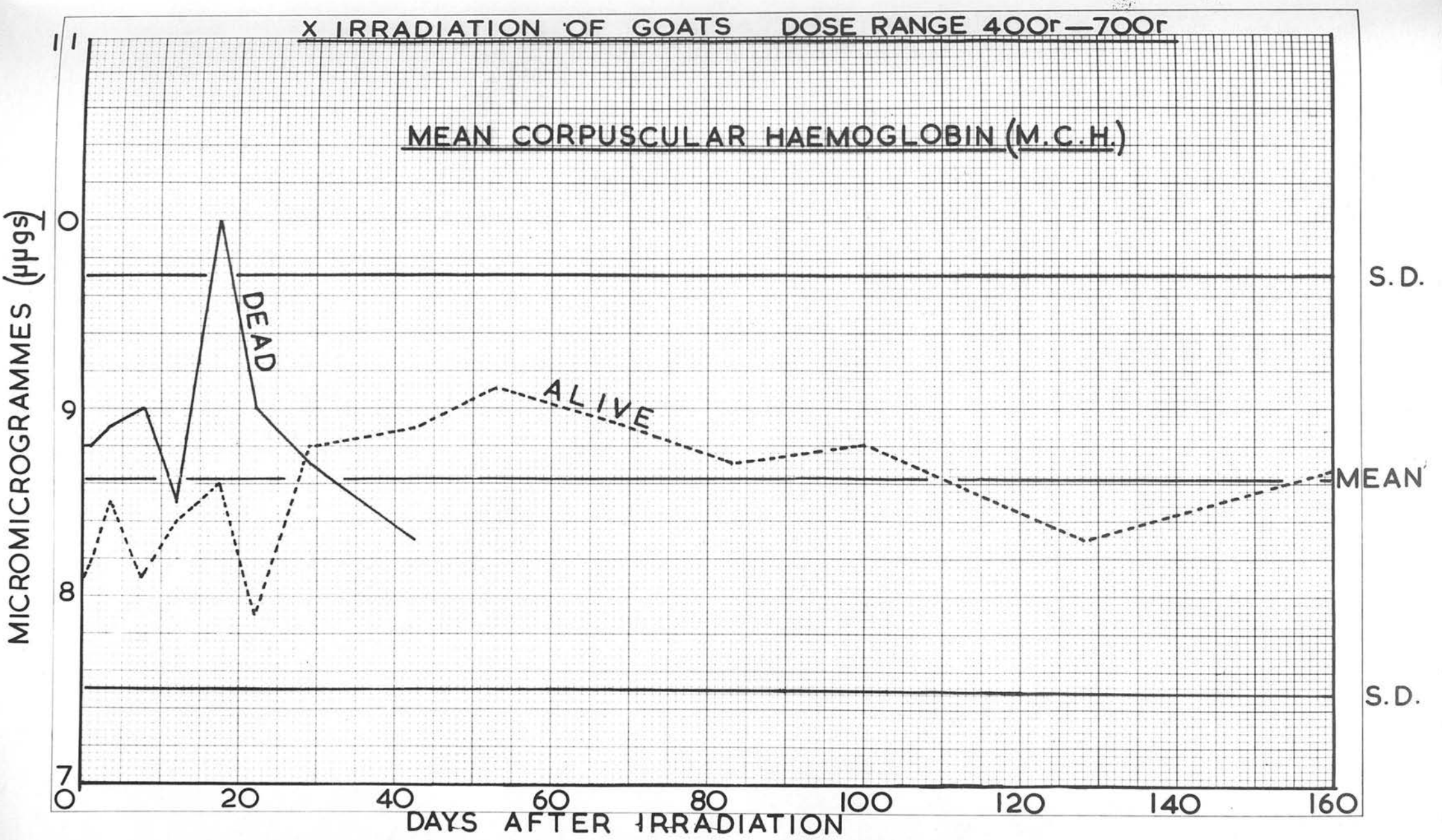

X IRRADIATION OF GOATS DOSE RANGE 400r—700r
MEAN CORPUSCULAR HAEMOGLOBIN (M.C.H.)
MICROMICROGRAMMES (μμgs)
11
10
9
8
7
DEAD
ALIVE
S.D.
MEAN
S.D.
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 31.

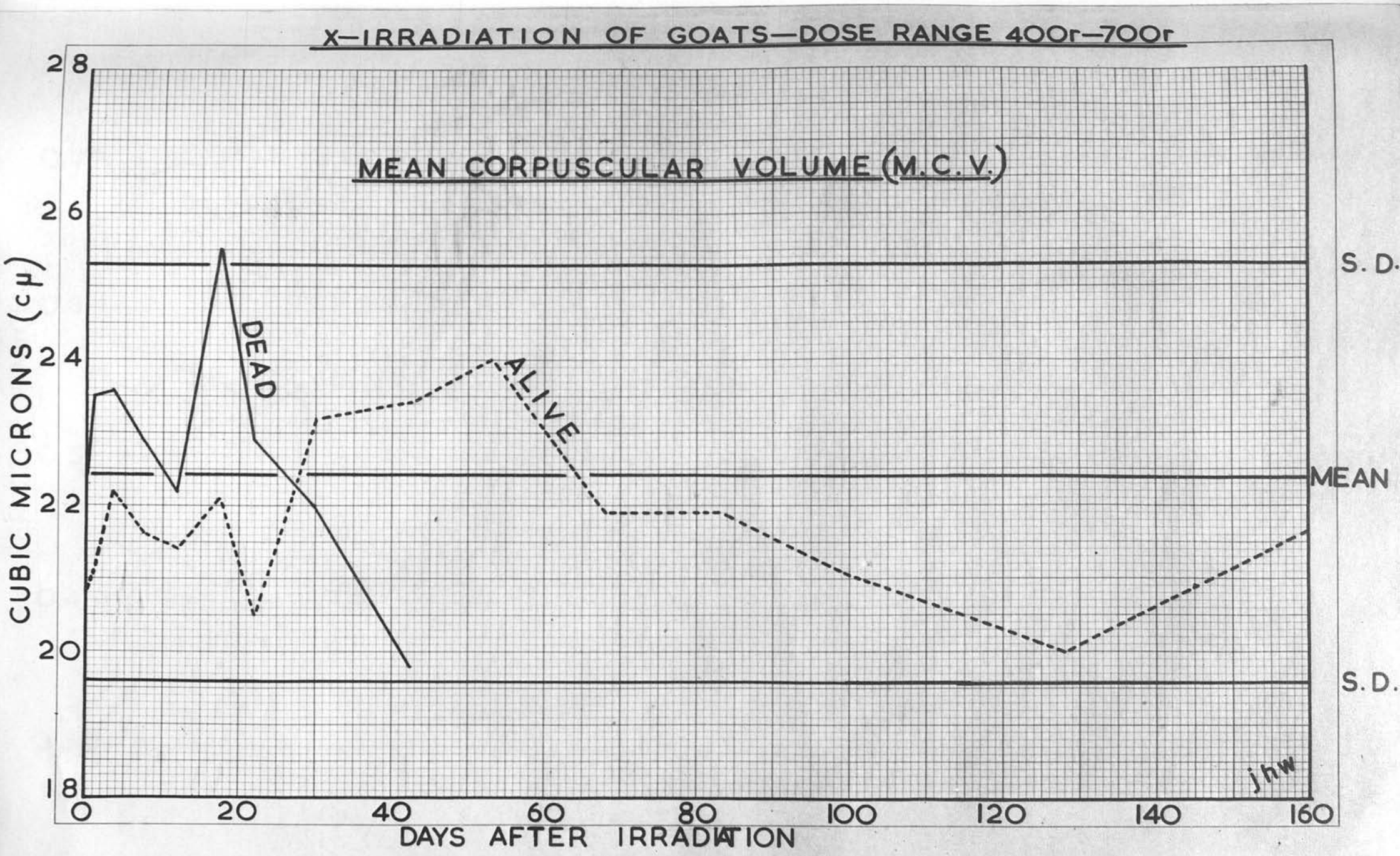

X-IRRADIATION OF GOATS—DOSE RANGE 400r—700r
MEAN CORPUSCULAR VOLUME (M.C.V.)
CUBIC MICRONS (cμ)
28
26
24
22
20
18
S.D.
MEAN
S.D.
DEAD
ALIVE
jhw
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 32

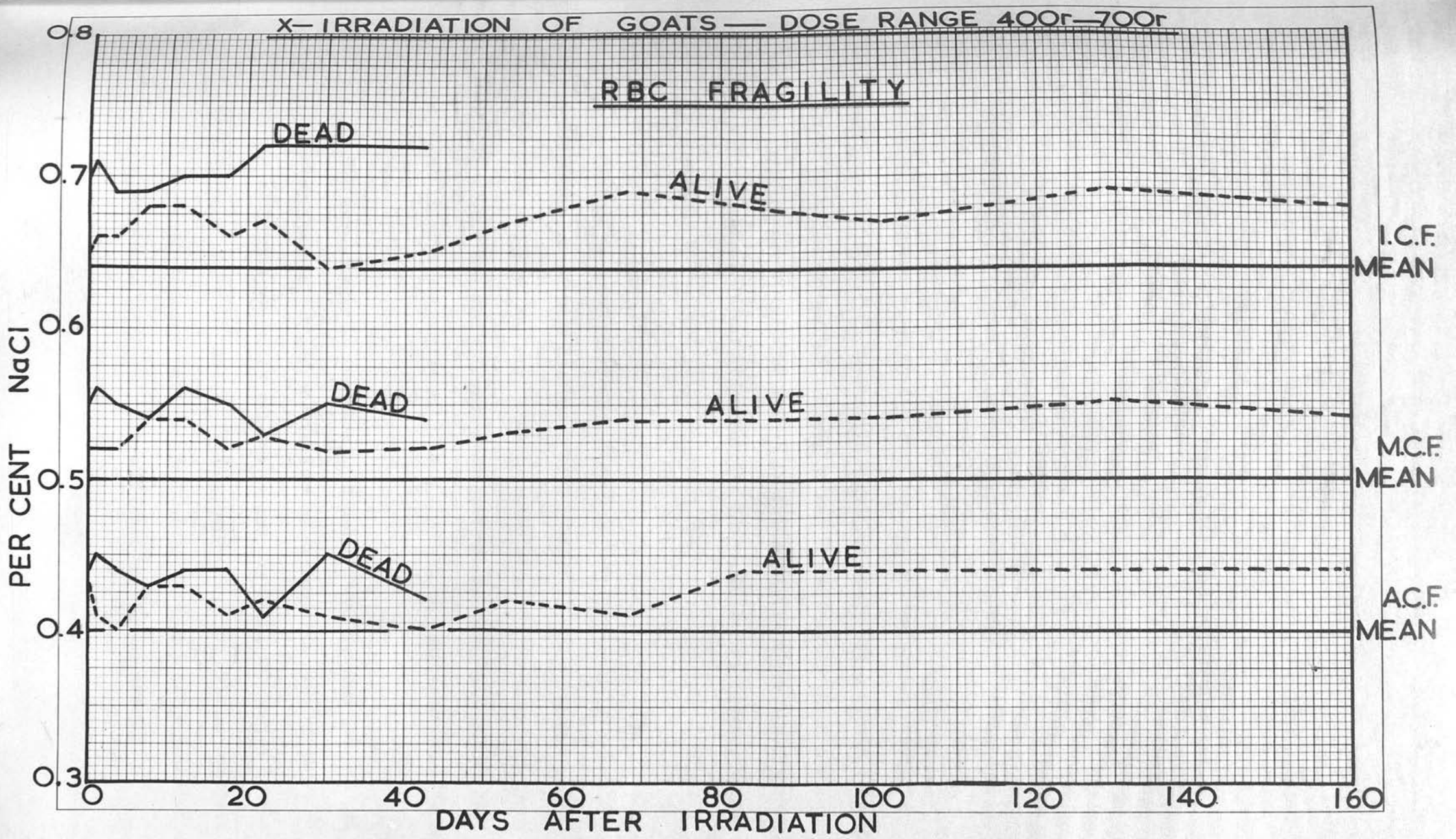

X— IRRADIATION OF GOATS — DOSE RANGE 400r—700r
RBC FRAGILITY
DEAD
ALIVE
DEAD
ALIVE
DEAD
ALIVE
I.C.F. MEAN
M.C.F. MEAN
A.C.F. MEAN
PER CENT NaCl
0.8
0.7
0.6
0.5
0.4
0.3
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig. 33

iii) The Thrombocytic Picture

Changes were measured by total platelet counts together with observations on coagulation time and clot retraction.

a) Platelets. Table LII presents the mean cell counts and percentage values, Figure 34 gives a graphical representation of the general trend and Table LIII the maximal changes which occurred.

At ½ hour post irradiation the count for the survivors had increased by a mean percentage value of +5.6% whereas non-survivors had decreased by -6.7%. At one day, however, the situation was reversed: non-survivors had increased by +6% over control values whilst survivors decreased by -12%. Thereafter in survivors there was a steady decrease to 57.2% below control at between 31 and 60 days after which a slow improvement occurred. The values in non-survivors after the first day paralleled those in survivors but the decreases became progressively larger right up to just prior to death when an average of -66% below control value was reached.

Maximum and Minimum Values (Table LIII)

The minimum percentage decreases were similar in non-survivors (-69%) and survivors (-73%); the mean time to reach minimal values in survivors was 46 days. Maximum per cent increases in non-survivors (+28%) was reached in an average time of 3 to 4 days and in survivors (+18.7%) in 99 days.

The count began to decrease in non-survivors between the 6th and 9th day after irradiation and declined at an approximate rate of about 30,000 to 40,000 per cubic millimetre per day until the lowest levels were reached prior to death. In survivors after the initial increase the count began to decrease between the 10th and 14th day after irradiation at an approximate rate of 20,000 per cubic millimetre per day.

Table LII

Platelets

Mean Cell Counts and Percentage Changes

Dose Range 400-700r

Period	NON-SURVIVORS		SURVIVORS	
	Thousands per cu. mm.	Percentage of Control	Thousands per cu. mm.	Percentage of Control
Pre-Irradiation Control	328	-	390	-
Post-Irradiation				
½ hour	306	- 6.71	412	+ 5.64
1 Day	348	+ 6.10	342	- 12.31
2-7 Days	298	- 9.15	296	- 24.10
8-30 Days	176	- 46.34	200	- 48.72
31-60 Days	140	- 57.32	167	- 57.18
Count just prior to Death	112	- 65.85	-	-
61-210 Days	-	-	230	- 41.03

Table LIII

Effects of X-Irradiation on Goats

Platelets

	Dose (r)	Control Mean Count (Cells/mm^3 x 10^3)	Maximum Count (Cells/mm^3 x 10^3)	Hrs/Days Post Irr. when max. recorded	Min Count (Cells/mm^3 x 10^3)	Hrs/Days Post Irr. when min recorded	Survival Time at Death or at end of observation (days)	% Increase: Maximum recorded	% Increase: Mean for dose	% Increase: Mean for all Goats	% Increase: Mean for Dose Range 400-700r	% Decrease: Minimum recorded	% Decrease: Mean for Dose	% Decrease: Mean for all Goats	% Decrease: Mean for Dose Range 400-700r
							Non-Survivors								
9	2000	375	380	½ hr	40	8 days	8	1.33	1.33		-	89.33	89.33		-
4	1000	277	275	3½ hrs	102.5	7 days	8	- 0.72	- 0.77		-	63.00	63.00		-
5	700	227	260	10 days	135	6 days	11	14.54				40.53			
0	700	547	485	2 days	168	10 days	13	-11.33	19.20			69.29	61.75		
7	700	285	440	1 day	70	44 days	46	54.39				75.44			
4	650	204	260	9 days	55	41 days	42	27.45	28.43	23.21		73.04	77.22	70.20	68.87
4	650	510	660	1 day	100	11 days	14	29.41				80.39			
5	600	417	510	1 day	9	18 days	30	22.30	22.30		28.30	97.84	97.84		
8	550	340	620	3 days	4	18 days	18	82.35				98.82			
0	550	262	268	2 days	120	9 days	12	2.29	39.33			54.20	61.11		
0	550	165	220	2 days	115	6 days	12	33.33				30.30			
							Survivors								
1	650	228	325	6 days	100	52 days	157 days	42.54	42.54			56.14	56.14		
0	600	459	590	6 days	42	18 days	239 days	28.54	36.38			90.85	68.99		
9	600	208	300	142 days	110	66 days	164 days	44.23				47.12			
3	500	625	735	½ hr	115	15 days	190 days	17.60				81.60			
1	500	370	555	½ hr	120	17 days	179 days	50.00	11.90	18.71	18.71	67.57	82.65	73.43	73.43
0	500	536	365	402 days	6.5	20 days	584 days	-31.90				98.79			
0	400	265	280	148 days	85	58 days	172 days	5.66				67.92			
6	400	470	485	183 days	90	77 days	213 days	3.19	5.81			80.85	72.92		
2	400	350	380	1 day	105	90 days	225 days	8.57				70.00			

Mean Time to reach minimum

Non-Survivors	All Goats	.....	16.2 days
	Dose Range 400 to 700r	..	18.1 days
Survivors		.....	45.9 days

Mean Time to reach maximum

Non Survivors	All Goats	.....	2.8 days
	Dose Range 400 to 700r	..	3.4 days
Survivors		.....	98.7 days

Improvement became evident after 60 days and normality restored about 7 months after irradiation. The response was not a function of dose. The depression just prior to death in non-survivors was almost identical at all dose levels.

b) Coagulation Time and Clot Retraction (Figs 35 and 36)

In non-survivors the coagulation time increased from a normal control average of 4.7 minutes to 22.4 minutes at the precise time of the greatest reduction in the platelet count. Although there was a concomitant impairment of clot retraction this did not obtain consistently thereafter. In fact, near death although clot retraction was nil, platelet counts and coagulation times were often good. In survivors, values were generally higher.

Discussion

By and large the general thrombocytic responses were considered to be similar to those seen in other animals (Cronkite, 1950; Rosenthal et al, 1950). Some goats had vast internal haemorrhages at post mortem (Photo 43) but firm blood clots were usually found in the visceral organs. In these cases it was assumed that the cause of the haemorrhages was a rupture of a vessel wall brought about probably by local vascular ulcerations which were themselves probably the result of extreme granulocytopenia. Histological evidence often confirmed this later. In one or two cases an apparent paradox was observed, namely blood taken shortly before death showing very prolonged clotting times or even being incoagulable whereas firm clots were visible in the visceral organs at autopsy.

A failure in the clotting mechanism was almost invariably prognostic of a fatal outcome in irradiated goats but minor defects often were not so. There was no constancy in the prolongation of coagulation time or clot retraction in individual goats but reduction in the numbers of platelets did occur in all goats which died.

The correlation of thrombocytopenia, post-irradiation haemorrhage prolonged coagulation time and clot retraction has been reviewed by Cronkite and Brecher (1952). Woods et al (1953) and Cronkite (1953) corrected temporarily the haemorrhagic effects of irradiation in animals by platelet transfusions. Cronkite et al (1952)had previously reported that the addition of platelets in vitro had corrected coagulation defects.

Prolongation of the clotting time undoubtedly implied a deficiency of one or more of the factors necessary for blood coagulation (Whitby and Britton (1957). Liver injury was considered to be at least one of these sources. According to Quick (1950) clot retraction is dependent on

i) the number of platelets ii) the nature of the surface surrounding the clot iii) the concentration of thrombin and iv) the red cell volume.

Proper clot retraction according to Whitby and Britton (1957) requires considerable numbers of platelets. If insufficient platelets are present the clot is soft friable and does not contract. In this study a defective clot retraction was certainly noted but this could not be correlated with the number of platelets observed terminally. A similar finding has been reported by Cronkite (1950) and Cohn (1952).

The possible causes of the haemorrhages and the subsequent anaemia following irradiation of animals have been discussed by many workers. Anaemia is a common finding following exposure of animals to MLD doses of whole body radiation and is responsible for some of the general clinical signs observed e.g. asthenia and body weight loss. This anaemia is caused firstly by local gross haemorrhages and secondly by a failure of the erythropoietic tissues: there is however a third reason due to a generalised haemorrhagic tendency brought about by more obscure causes of which a thrombocytopenia is considered by most

workers to be a very important, if not the most important, factor. (Cronkite and Brecher, 1954). Laccasgne and Lavedan (1922) were the first to observe a relationship between thrombocytopenia caused by irradiation and tissue bleeding. This parallelism between the post irradiation haemorrhagic tendency and the decrease in circulating platelets has been confirmed by many workers (Cronkite and Brecher 1952) and the concept of hyperheparinaemia put forward by Allen et al, 1948, and Jacobson et al, 1948 as a major cause of haemorrhage has been adequately refuted by Jackson et al (1952) and has been abandoned. It is now universally recognised that thrombocytopenia is the major cause of the haemorrhagic tendency causing a diversion of red blood cells into the lymph spaces and body cavities. Another probable reason for the observed anaemia of irradiation is the increased fragility, and destruction of red blood cells brought about by some haemolytic factor produced either as a direct result of irradiation or as a secondary effect of bacterial intoxication. In this study it is suggested that such a factor played a part and was derived from the toxins of Clostridium welchii which were absorbed into the circulation due to the denudation and damage to the gut mucosa as a result of irradiation.

Possibly of equal importance was the formation of particulate complexes within the sinusoidal circulation (Andersen, 1959). Haemorrhage and necrotising degenerative changes in many visceral organs were common findings in non-surviving irradiated goats and PAS-positive globules were observed in the heart, liver, kidney and other organs. Such globules possibly may have acted as emboli inducing the observed endothelial damage and in the presence of the coagulation defects enumerated may be considered as a predisposing cause of the haemorrhages.

There was no apparent inter relationship between the thrombocytic picture, dose, type of death or clinical evidence of haemorrhage. There was however a correlation between the gross haemorrhage seen at post mortem and in histological sections with the platelet count and the coagulation time. Clot retraction although correlated somewhat in degree was not so in time of appearance. (Table XLVI).

Conclusion

The alteration of platelet counts, coagulation times and clot retraction in the blood of goats after MLD doses of X radiation show an impairment of the blood clotting mechanism. Clot retraction disturbances were noticeable within 24 hours, reduction in the number of platelets and prolongation of coagulation times within 7 days. Normality was reached in 7 months in survivors.

Table LIV

The Correlation of the Thrombocytic and the Haemorrhagic Pictures in Goats dying after exposure to X Radiation

Goat No.	Dose (r)	Survival Time (days)	Evidence of Haemorrhage			Platelet Count (Thousands per cu. mm)			Coagulation Time (Minutes)			Clot Retraction (Percentage)		
			Clinical	Macroscopic	Microscopic	Control	Minimum	Days Post-Irrad.	Control	Maximum	Days Post-Irrad.	Control	Minimum	Days Post-Irrad.
C229	2000	8	+	+	+	375	40	8	4½	UC	7	50	Nil	3
C234	1000	8	+	+	+	277	102	7	5½	21	4	50	Nil	7
C175	700	11	-	+	+	260	135	6	3½	10	10	50	Nil	9
F 40	700	13	-	+	+	547	168	10	5½	25	7	50	Nil	6
F 27	700	46	-	+	+	285	70	44	3½	30	44	50	Nil	½ hr
F 14	650	42	+	+	+	204	55	41	6	15	40	50	Nil	1
F 24	650	14	-	+	+	510	100	11	4½	11½	11	50	10	11
C315	600	30	-	+	+	417	9	18	5	26½	22	50	Nil	½ hr
C118	550	18	+	+	+	340	4	18	5½	UC	16&18	50	Nil	2.18
C200	550	12	-	+	+	262	120	9	5	8	11	50	Nil	2.11
F 20	550	12	+	+	+	165	115	6	3½	8½	10	50	Nil	10

UC = Uncoagulated

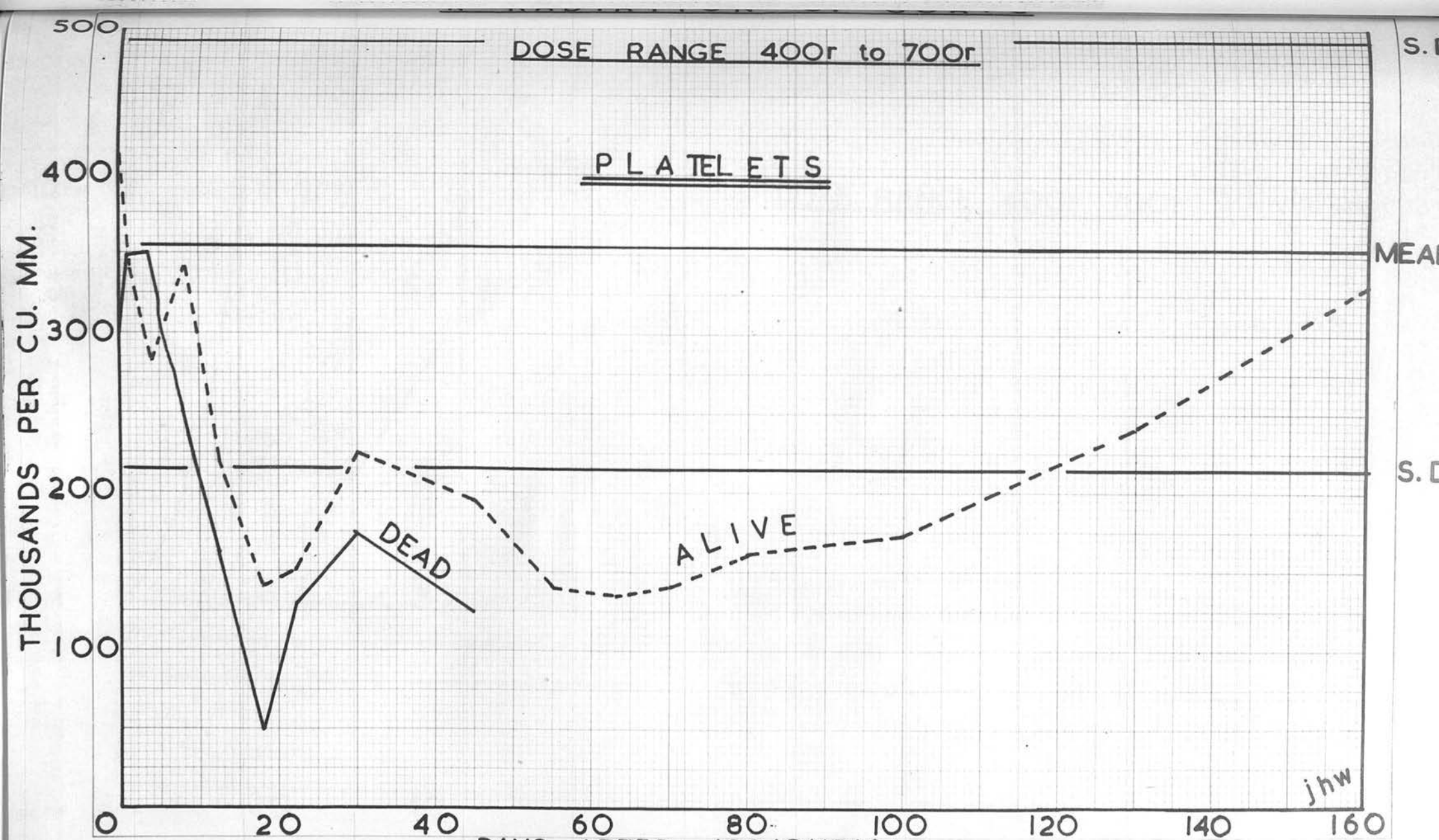

DOSE RANGE 400r to 700r
PLATELETS
THOUSANDS PER CU. MM.
500
400
300
200
100
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION
DEAD
ALIVE
jhw
MEA
S.D

Fig.34

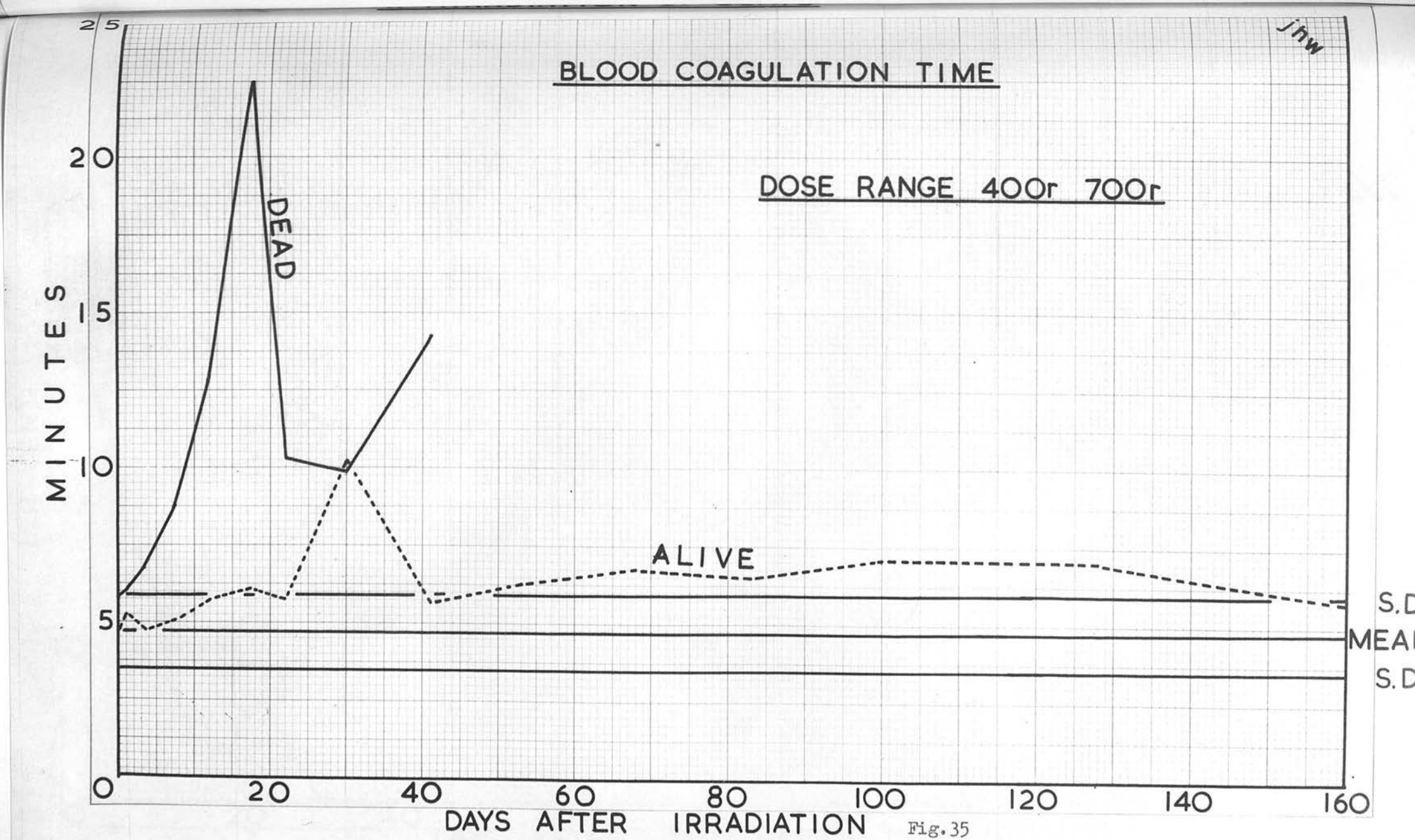

BLOOD COAGULATION TIME
DOSE RANGE 400r 700r
jhw
DEAD
ALIVE
MINUTES
25
20
15
10
5
0
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION
S.D
MEA
S.D

Fig. 35

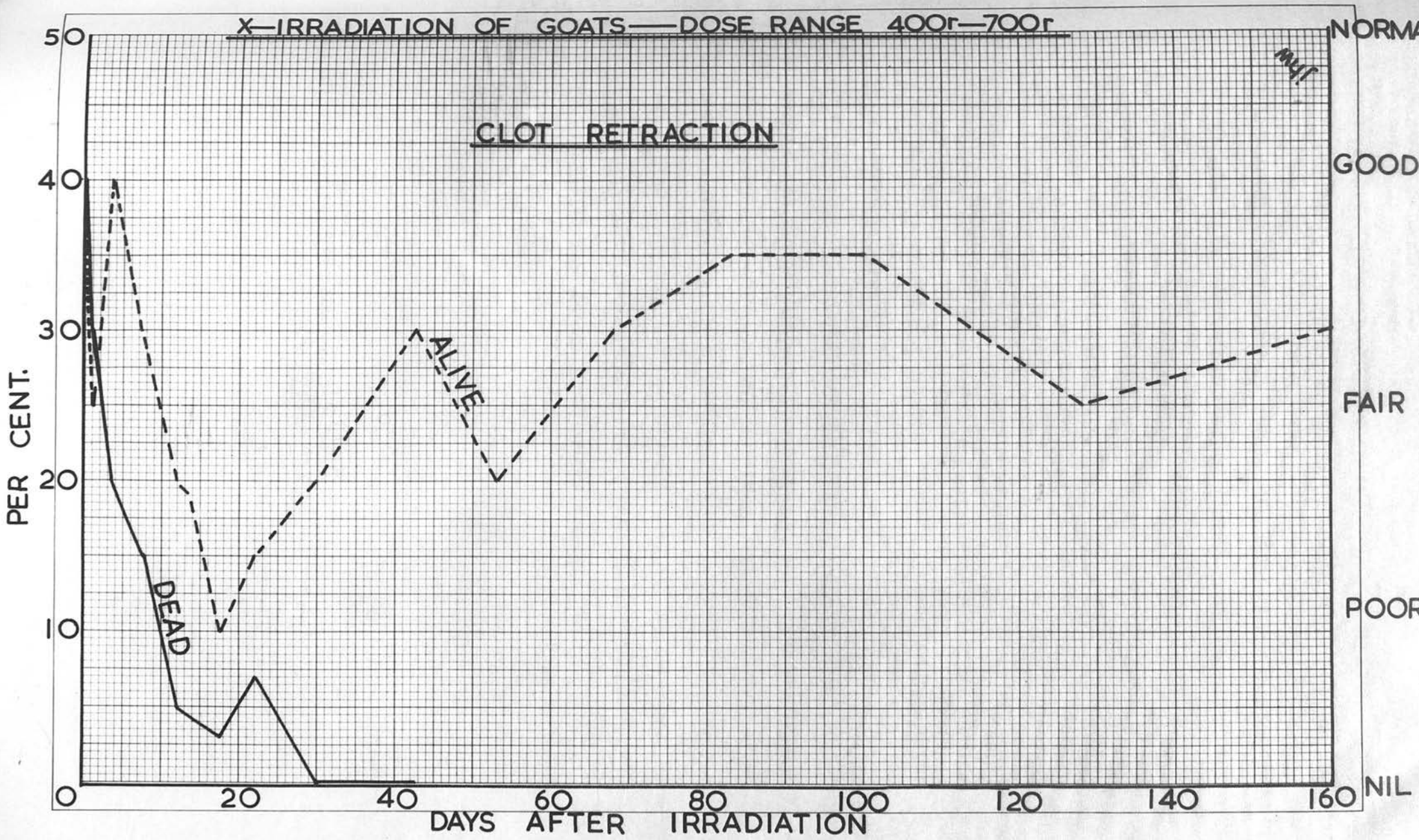

X—IRRADIATION OF GOATS——DOSE RANGE 400r—700r
CLOT RETRACTION
NORMA
GOOD
FAIR
POOR
NIL
PER CENT.
50
40
30
20
10
0
DEAD
ALIVE
20
40
60
80
100
120
140
160
DAYS AFTER IRRADIATION

Fig.36

2. Effects on the Bone Marrow

The general morphology of the bone marrow cells of the goat was found to be similar to that of human beings (Whitby & Britton, 1957) and also to that of other species of animals especially sheep, (Grunsell, 1955). This has been briefly described in a preliminary study (Wilkins, 1962) and is attached in the Appendix V(ii) Bone marrow examinations were made on 18 normal adult goats. The distribution of these 18 goats by breed, sex, age and weight is given in Table LV. Twenty two aspirated sternal bone marrow samples from irradiated goats were also examined and the distribution of these by dose and time after irradiation is given in Table LVI.

The mean values, with their standard deviations, of the cellular composition of the aspirated bone marrow of the normal and irradiated goats over four arbitrary time periods ($\frac{1}{2}$ hour, 2 to 7 days, 80 to 250 days and just prior to death) are given in Table LVII. This table also gives the myeloid/erythroid ratio and the percentage of marrow cells seen to be in mitosis.

Detailed results are given in the Appendix VI.

Table LV

Sternal Bone Marrow Biopsy

Before Irradiation

Distribution of 18 Normal Goats by Breed, Sex, Age and Weight

Breed	Sex	Age (Yrs)	Weight (lbs)
BS ... 10	Female ... 10	3 yrs ... 1	Over 225 lbs 1
BA ... 4	Male ... 4	4 to 5 yrs ...13	180 to 225 lbs .. 2
BT ... 3	Castrate.. 4	5 to 6 yrs ... 2	130 to 180 lbs .. 8
AN ... 1		Over 6 yrs ... 2	90 to 130 lbs .. 6

Table LVI

Sternal Bone Marrow Biopsy

After Irradiation

Distribution of 22 Examinations by Dose and Time

Period Post Irradiation	Dose (r)								
	2000	1000	700	650	600	550	500	400	TOTAL
½ hr.	1	-	2	-	2	1	-	1	7
2 - 7 days	1	1	-	1	1	-	2	1	7
80-250 Days	-	-	-	1	1	-	3	-	5
½ hr. or less before death	-	-	1	-	1	1	-	-	3

Bone Marrow Biopsy (Sternal Aspirate) Examinations

Table LVII

Mean Percentage Differential Marrow Cell Counts

Element	Pre-Irradiation		Post Irradiation							
	Normal Control		½ hour		2 to 7 days		80 to 250 days		Just before death	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Stem Cells	0.57	0.52	1.39	0.71	2.01	2.52	1.24	0.75	0.07	0.06
Myeloblasts	1.12	0.37	1.01	0.53	1.37	0.94	1.42	0.57	0.13	0.15
Promyelocytes	0.52	0.22	0.90	0.52	0.64	0.43	0.25	0.23	0.07	0.06
Myelocytes N.	1.16	0.39	1.50	1.10	3.09	2.75	1.82	0.82	0.23	0.06
" E.	0.54	0.31	0.44	0.31	0.17	0.23	0.64	0.79	0.07	0.06
" B.	0.05	0.08	0.03	-	0.01	-	0.00	-	0.00	-
Metamyelos N.	13.68	4.74	16.50	4.77	15.63	4.67	10.14	6.32	0.57	0.49
" E.	1.22	0.76	1.04	0.71	0.50	0.61	1.04	1.11	0.07	0.06
" B.	0.03	-	0.03	-	0.00	-	0.01	-	0.00	-
Polymorphs N.	8.15	3.54	6.07	4.69	5.44	2.27	11.08	3.16	0.80	0.75
" E.	0.40	0.33	0.31	0.25	0.37	0.23	1.42	1.52	0.07	0.06
" B.	0.01	-	0.01	-	0.01	-	0.06	-	0.00	-
Total Myeloid	26.89	5.04	28.14	7.28	27.24	5.20	27.42	8.75	2.00	1.37
Pronormoblasts	2.09	1.00	2.00	0.66	1.20	1.12	2.44	1.18	0.00	-
Normoblasts E.	4.22	2.28	2.43	0.80	2.57	2.22	5.16	1.10	0.23	0.21
" I.	13.40	7.95	7.56	5.77	8.94	4.51	16.42	2.90	1.73	0.85
" L.	43.13	10.80	43.57	14.54	27.39	5.60	31.72	6.80	53.53	4.25
Total Erythroid	62.83	6.37	55.56	11.55	40.10	9.41	55.74	5.78	55.50	3.38
Lymphocytes	2.60	1.60	6.09	6.07	9.07	3.90	2.40	0.35	6.23	2.20
Monocytes	1.49	1.46	1.41	0.63	1.70	1.28	1.20	1.05	0.47	0.63
Plasma Cells	0.35	0.31	1.47	1.07	1.71	0.71	1.62	0.78	28.43	3.10
Mast Cells	0.07	-	0.10	0.13	0.01	-	0.02	-	0.00	-
Megakaryocys	0.63	0.75	0.27	0.19	0.39	0.66	1.22	0.76	0.00	-
Necrobiotic	0.66	0.62	1.03	1.08	3.97	3.35	0.90	0.74	2.57	1.39
Smear Cells	4.00	1.93	4.14	2.76	13.64	6.93	7.42	4.46	5.90	3.05
Mitotic Cells	0.47	0.40	0.27	0.20	0.10	0.19	0.54	0.17	0.13	0.06
M/E Ratio	1/2.40		1/2.32		1/1.48		1/2.24		1/25	

Analysis of Data

The total myeloid and erythroid components of the findings were subjected to an analysis of variance. In the normal goats sex, age and weight and in irradiated goats dose and time after irradiation were considered as the most probable sources of variation. The data obtained from the goats just before death were not used in the analysis.

The results are set out in the accompanying Tables LVIII, LIX and LX.

Table LVIII

Bone Marrow of Normal Goats

Table of Analysis of Variance

1. Test for Difference between Sexes

Myeloid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Between Sexes (Corrected)	6	68.53	11.42	1	N.S.
Residual	9	340.62	37.85		
Total	15	409.15			

Erythroid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Between Sexes (Corrected)	6	5.58	0.93	1	N.S.
Residual	9	554.99	61.66		
Total	15	560.57			

Hence there was no statistical evidence to show a difference between the sexes, when corrected for differences between weights and ages. It followed that a multiple regression of myeloid and erythroid, elements on age and weight was permissible, differences in sex being not significant.

D.F. = Degree of Freedom (n-1); S.S. = Sum of Squares; M.S. = Mean Significant (S^2); V.R. = Variance Ratio; N.S. = Not Significant.

Table LIX

Bone Marrow of Normal Goats

Table of Analysis of Variance

Tests for Dependence on Age and Weight

Myeloid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Age	1	20.663	20.663	1	N.S.
Extra due to Weight	1	2.365	2.365	1	N.S.
Total due to regression	2	23.028			
Residual	15	409.151	27.277		
Total	17	432.179			

Erythroid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Age	1	24.496	24.496	1	N.S.
Extra due to Weight	1	104.577	104.577	2.8	N.S.
Total due to regression	2	129.073			
Residual	15	560.568	37.371		
Total	17	689.641			

Hence there was no statistical evidence to suggest a correlation between either the myeloid or erythroid elements with age or weight.

D.F. = Degree of Freedom (n-1); S.S. = Sum of Squares; M.S. = Mean Significant (S^2); V.R. = Variance Ratio; N.S. = Not Significant.

Table LX

Bone Marrow of Irradiated Goats

Table of the Analysis of Variance

Test for Dependance on Dose and Time Post Irradiation

(The data obtained from goats just before death was NOT included in the analysis)

Myeloid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Time	1	35.536	35.536	1	N.S.
Extra due to Dose	1	51.357	51.357	1.17	N.S.
Total due to regression	2	86.893			
Residual	16	702.479	43.905		
Total	18	789.373			

Erythroid

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Time	1	235.909	235.909	2.04	N.S.
Extra due to Dose	1	446.011	446.011	3.86	N.S.
Total due to regression	2	681.920			
Residual	16	1850.298	115.644		
Total	18	2532.218			

Hence the data did not show any statistical evidence of a correlation between either myeloid or erythroid elements with dose or time post-irradiation.

D.F. = Degree of Freedom (n-1); S.S. = Sum of Squares; M.S. = Mean Significant (S^2); V.R. = Variation Ratio; N.S. = Not Significant.

Discussion

(Photos 27 to 38)

The use of sternal puncture for bone marrow aspiration possesses well known drawbacks, for example, there is always some dilution with sinusoidal blood even in the best specimens. Many modifications of techniques have been employed by different workers to overcome the inaccuracies inherent in sternal bone marrow aspiration examinations: for example, Gordon (1941) advocated centrifugation of the samples to concentrate the cells and Davidson (1941) and Davidson et al, 1943, adopted the very satisfactory method of making smears and marrow spreads from solid flecks of marrow selected from the bloody aspirate. This latter method has been employed with comparatively satisfactory results by many veterinary workers (Grunsell, 1951; Wilde, 1961). Davidson's techniques suitably modified was used throughout this study.

Consideration was given in this work to the possibility of making total and differential nuclear cell counts but, after a few rather unsatisfactory attempts and in view of the very variable results obtained by other workers, the idea was abandoned. Reich and Kolb (1942) stated that quantitative determinations on aspirated marrow are inaccurate and Osgood and Seaman (1944) came to a similar conclusion after reviewing the matter fully. They quoted many workers on human marrow and demonstrated the enormous variation in the total numbers of nucleated marrow cells found. Veterinary workers in the same field have reported similar findings in examinations of the marrow cells of various animal species, for example, in horses by Archer 1954; in cattle by Wilde, 1963; in cats by Sawitsky and Meyer 1947 and in dogs by Rekers and Coulter, 1948.

It was fully realised that, because the cells of the marrow and their relative proportions tend to alter simultaneously after undergoing the deleterious effects of ionizing radiations, the data collected from the random examinations of aspirated sternal marrow of irradiated goats would have only a limited value. However although there is no universally uniform method of bone marrow aspiration nor for that matter of marrow cell identification and nomenclature yet, when all techniques are standardised as far as possible for both normal and irradiated animals, it is reasonable to assume that the examinations do give, at the least, indications of the major trends in bone marrow physiology. It was with these limited objectives in view that the data obtained were subjected to study and analysis.

The aspirate, which was never more than 1 ml. in quantity, consisted of much sinusoidal blood in which "flecks" of marrow were easily discernible. Also present were variable amounts of glistening fat globules. Direct smears from the aspirate and squashed fleck smears were made.

Direct Smears usually showed few nucleated cells mixed with a predominance of blood and more mature cells, which made examinations more tedious. (Photo 28).

Squashed Fleck Smears usually showed many marrow cells only slightly mixed with sinusoidal blood. Megakaryocytes were often seen but this depended on the amount of reticulum to be seen in the fleck. (Photos 29 and 30).

Fixed Histological Sections of marrow flecks were not satisfactory for the marrow cells were almost impossible to identify but the actual infra structure of the marrow was more easily appreciated. (Photo 31).

The immediate squashed fleck smear was the most satisfactory and was used in every case, although marrow-blood smears and sections were also examined as required.

According to Bloom and Bloom (1954) the effects of irradiation on the number of cells is manifested by

i) cessation of mitosis thus causing a reduction because the source of supply is cut off

ii) the cells dying off at subsequent mitoses and thus reducing the overall numbers

iii) the cells undergoing degeneration and thereby reducing the overall numbers.

At dose levels in excess of approximately 1000r the probability of the survival of the marrow cells is extremely remote: this was certainly the case in goats receiving 700r and over, where a marked hypoplasia or aplasia of the marrow was obvious. A confusing issue clearly pointed out by Hulse (1957) is that the normal progress of the maturing erythroblast involves the gradual degeneration of its nucleus in exactly the same way as any cell affected by radiation. It was therefore difficult to determine whether in any particular case the appearance of degenerative changes was due to the effects of irradiation or was a natural process of ageing.

The depressive effect of irradiation on the marrow was manifested in the circulating blood by a general picture which varied from a frank anaemia to one in which there was only a lowered leucocytic count. On the other hand, evidence of the regeneration of marrow cells was detected by the finding of young leucocytes and normoblasts.

Marked destruction of marrow cells was usually seen in early specimens examined soon after irradiation but some regeneration was seen in those taken between 2 and 7 days whereas a large measure of recovery was observed in later aspirates. The erythrocytic precursor marrow cells

appeared to diminish most rapidly between 2 and 7 days followed belatedly by the leucocytic precursors. Reactive erythroblastic and leucoblastic processes were often very evident in the specimens taken from survivors. Megakaryocytes had largely disappeared by the 7th day post irradiation, especially in non-survivors. Monocytes and lymphocytes increased proportionately up to the 7th day after which they decreased. Mast cells after an initial increase in numbers immediately after irradiation later decreased severely. Necrobiotic cells increased up to the 7th day, after which in survivors they decreased to normal values whereas in non-survivors they did not. Mitosis was very rare between 2 to 7 days in all goats; thereafter it was not seen in non-survivors but in survivors was enhanced. Sometimes large numbers of plasma cells were seen especially near death demonstrating a terminal reactive plasmocytosis; another observation at this period was the present in a few goats of an abnormally large number of eosinophils.

From the myeloid/erythroid ratio estimated at the four arbitrary time periods viz ½ hour, 2 to 7 days, 80 to 250 days and just prior to death, it was concluded that there was little or no effect on the bone marrow discernible at ½ hour but that between the 2nd and 7th day after irradiation a distinct suppression of erythroblastic activity occurred. Thereafter in survivors normality was achieved between 80th and 250th day whereas in non-survivors the leucoblastic activity was reduced to practically nothing.

In goats which died early, aplasia was generally observed whereas in those dying later regenerative reparative foci were seen resulting in an immature hypoplastic bone marrow. The marrow was cell-poor with prominent sinusoids, wasted fat cells and usually a gelatinous matrix as a background.

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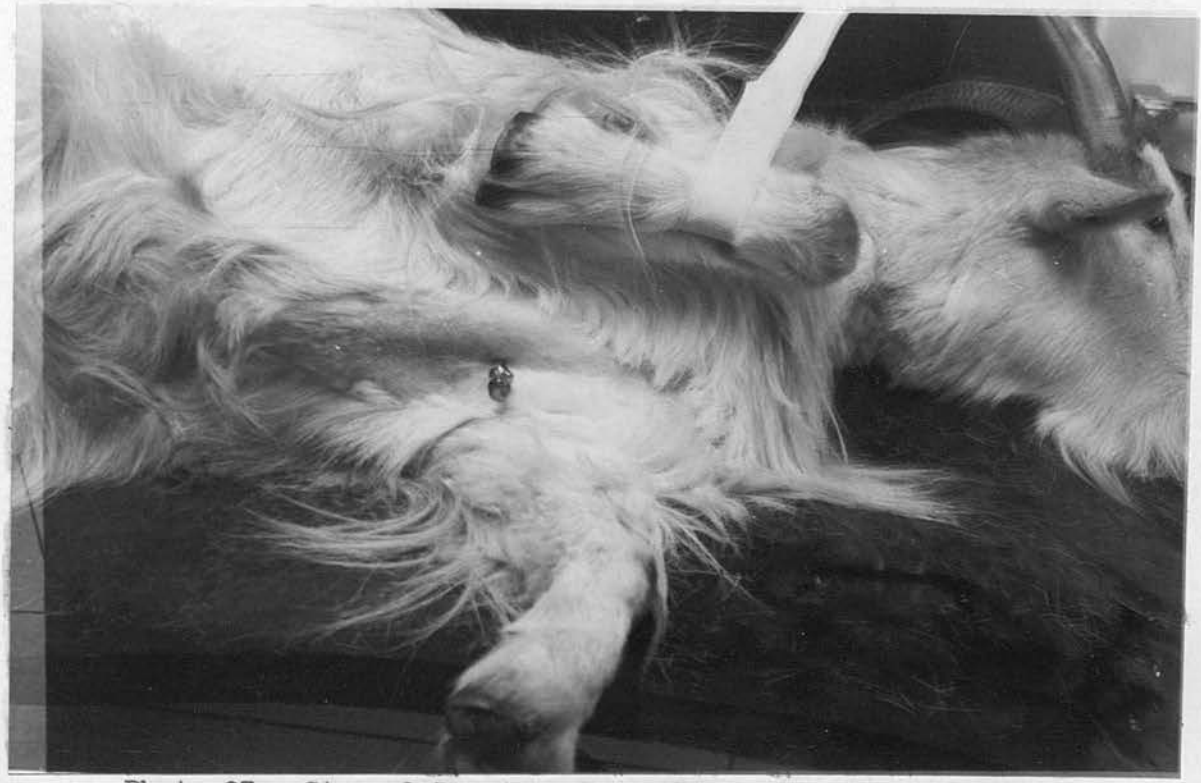

Photo 27. Sternal Puncture Bone Marrow Biopsy.

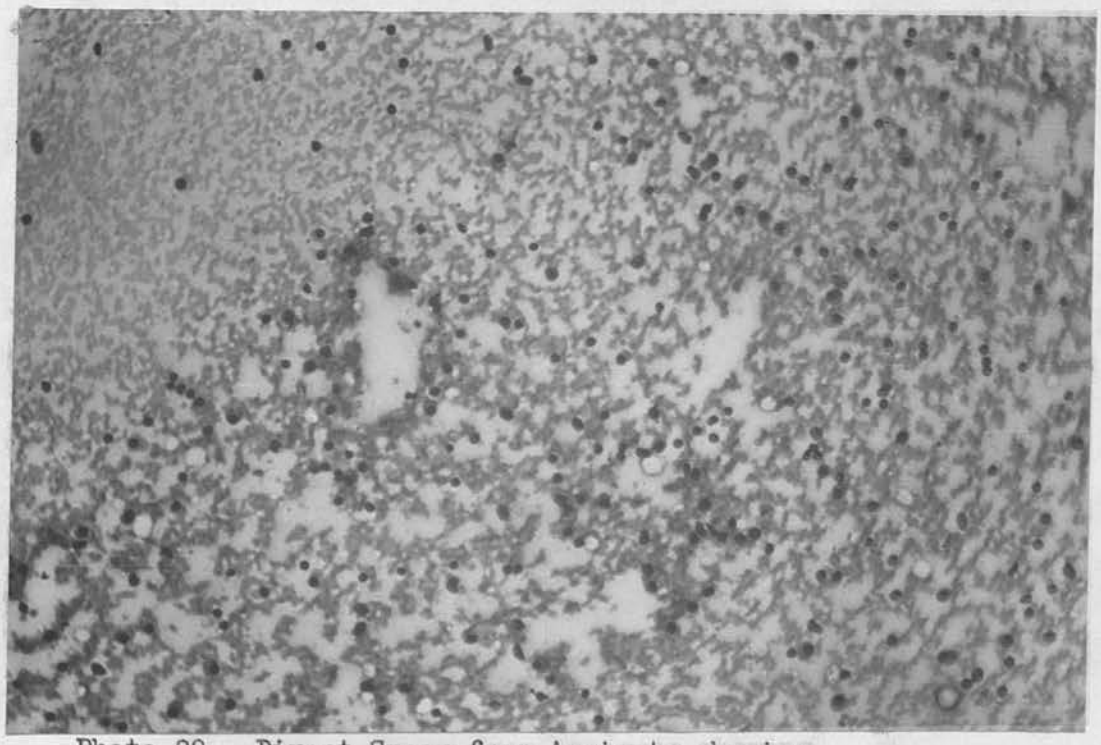

Photo 28. Direct Smear from Aspirate showing few nucleated cells and a predominance of sinusoidal blood. (x 200)

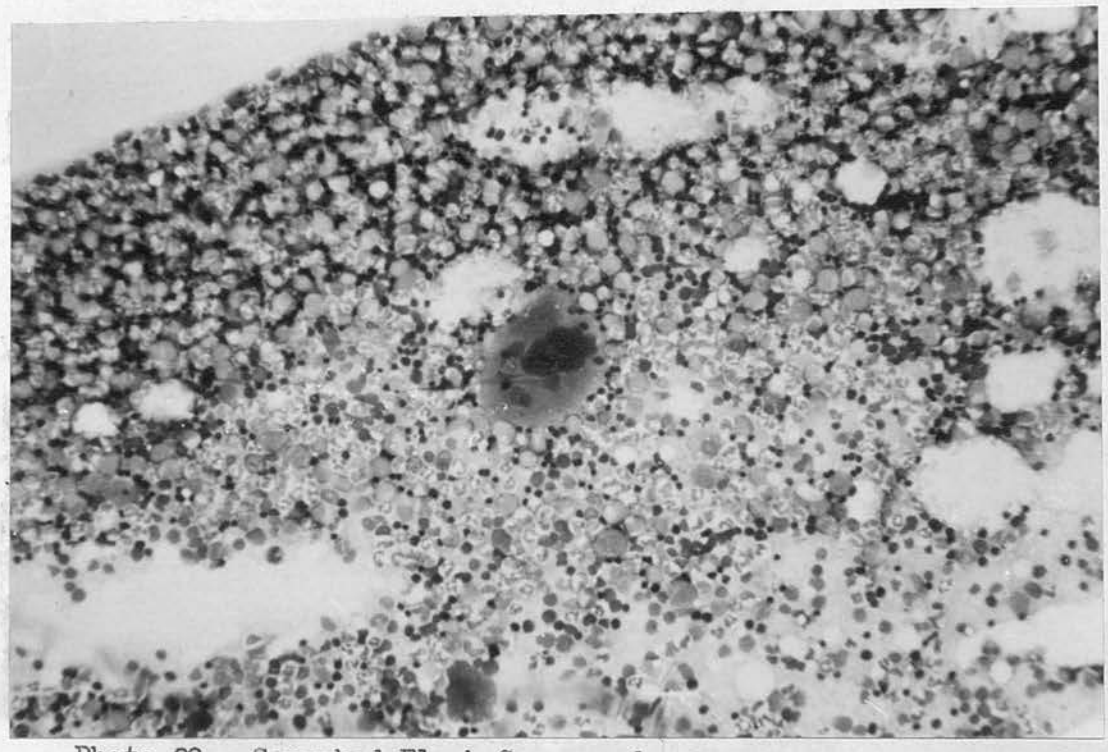

Photo 29. Squashed Fleck Smear made soon after irradiation (x 300)

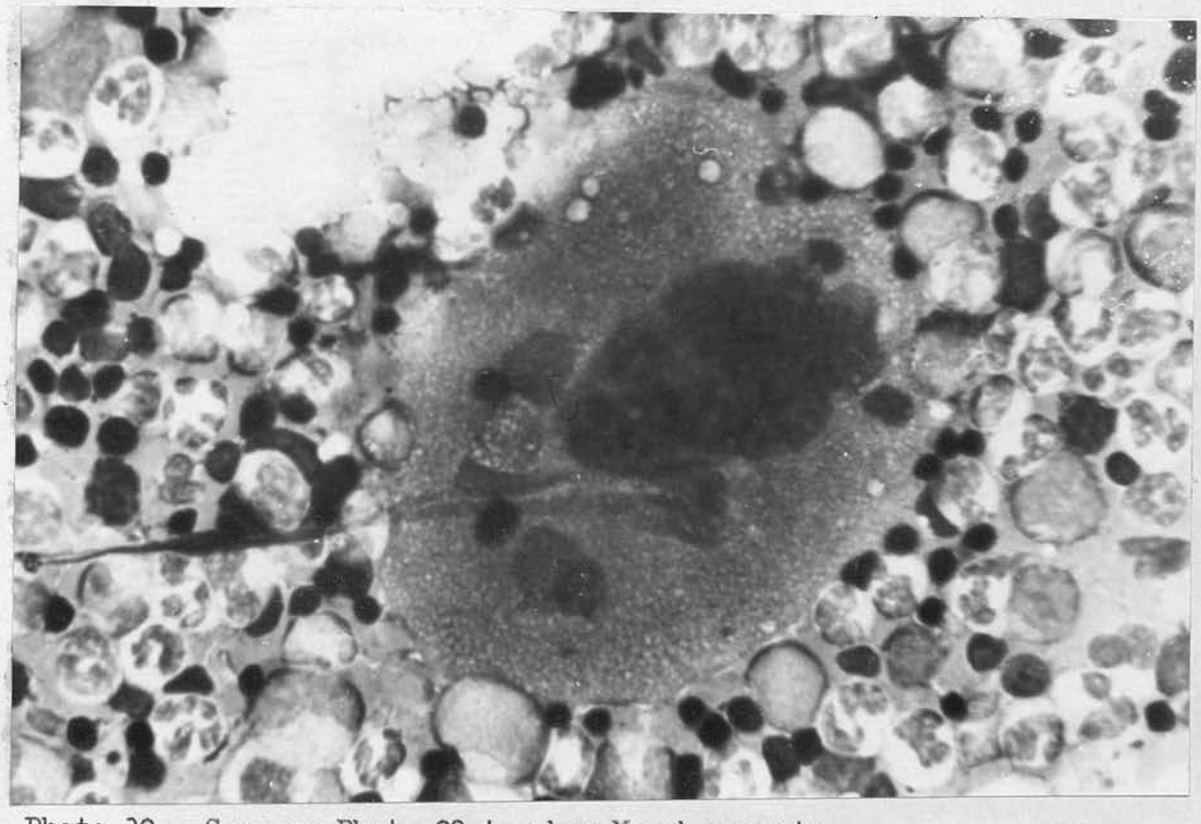

Photo 30. Same as Photo 29 to show Megakaryocyte and Normoblastic reactive process (x 1000)

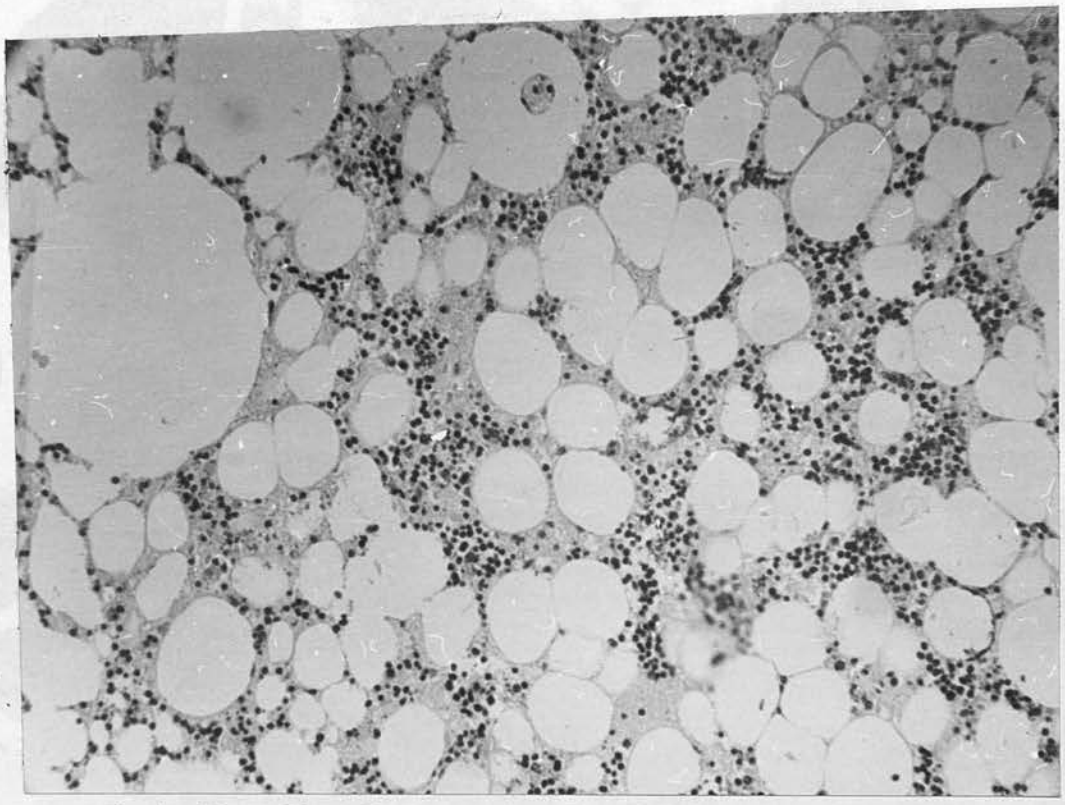

Photo 31. Histological Section of Marrow Fleck showing much fat and clumps of surviving marrow cells. (x 200)

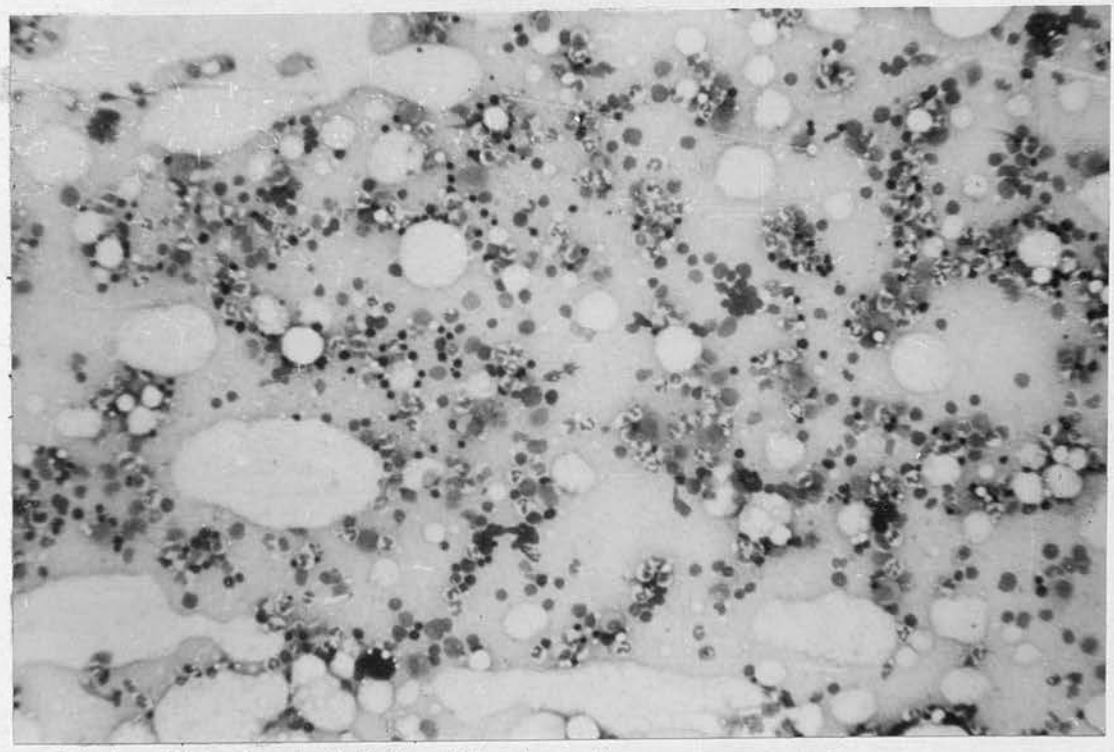

Photo 32. Wasted fat cells in a homogeneous gelatinous matrix with a leucoblastic reactive process (x 350)

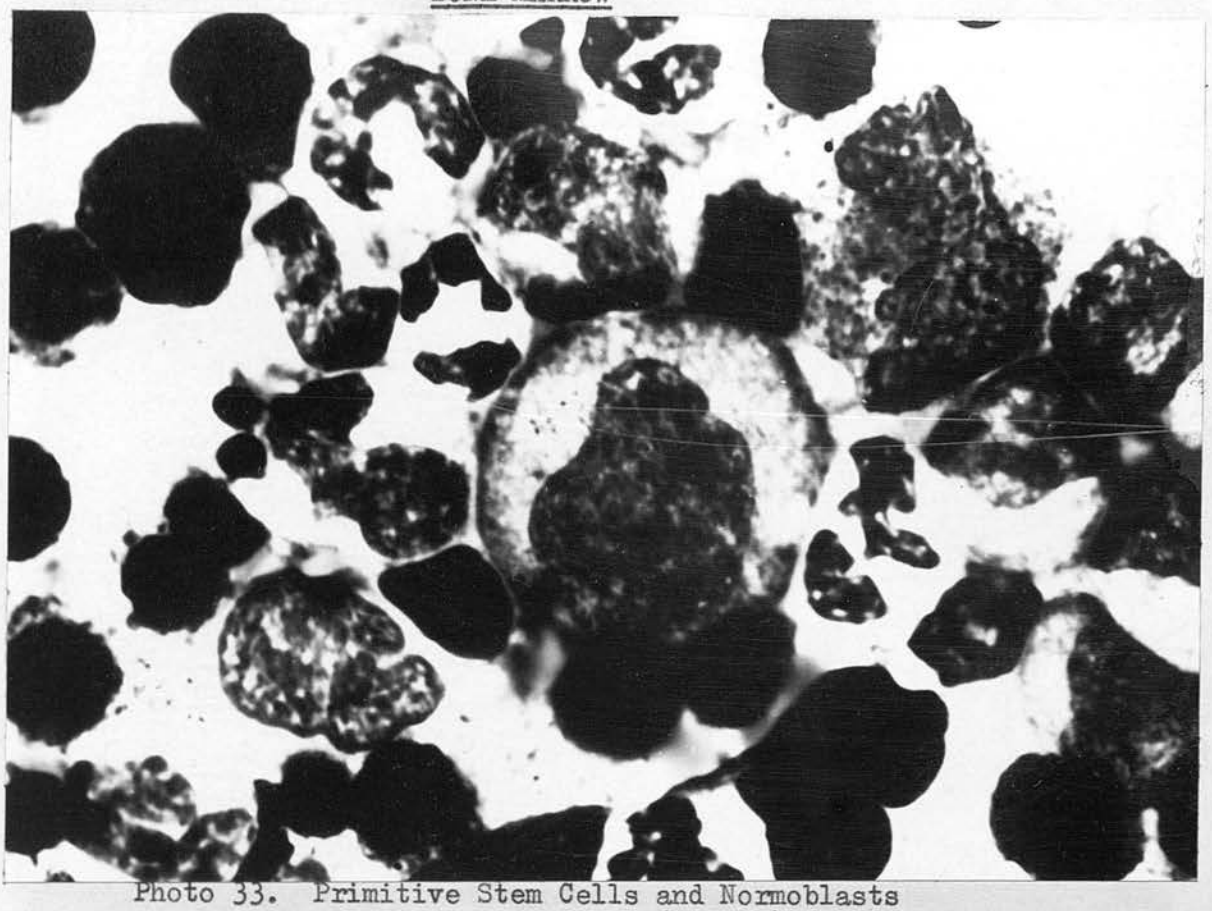

Photo 33. Primitive Stem Cells and Normoblasts in erythroblastic reaction (x 1200)

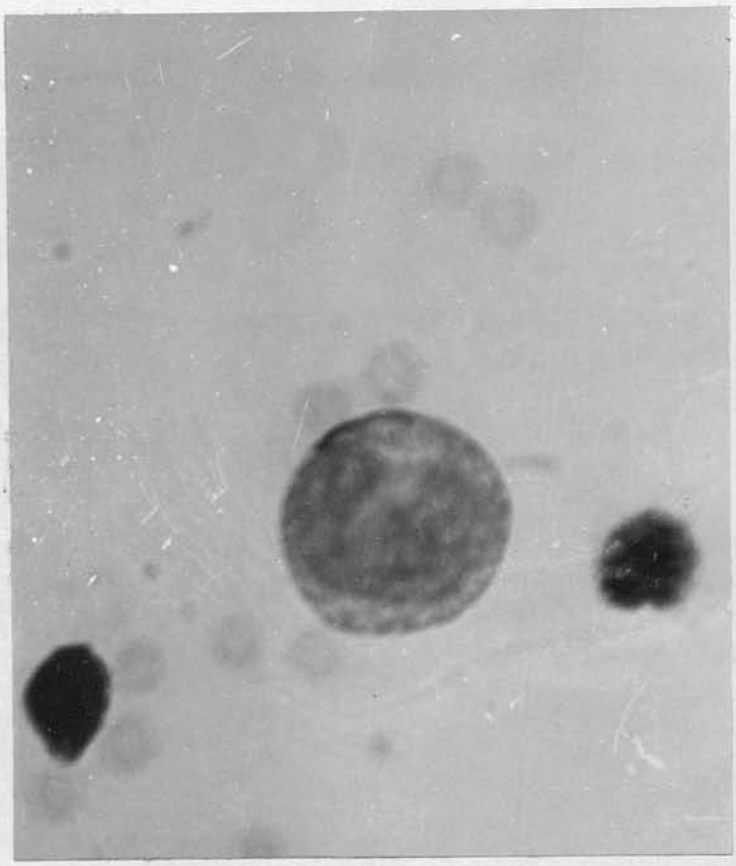

Photo 34. A Myeloblast and two Late Normoblasts (x 1000)

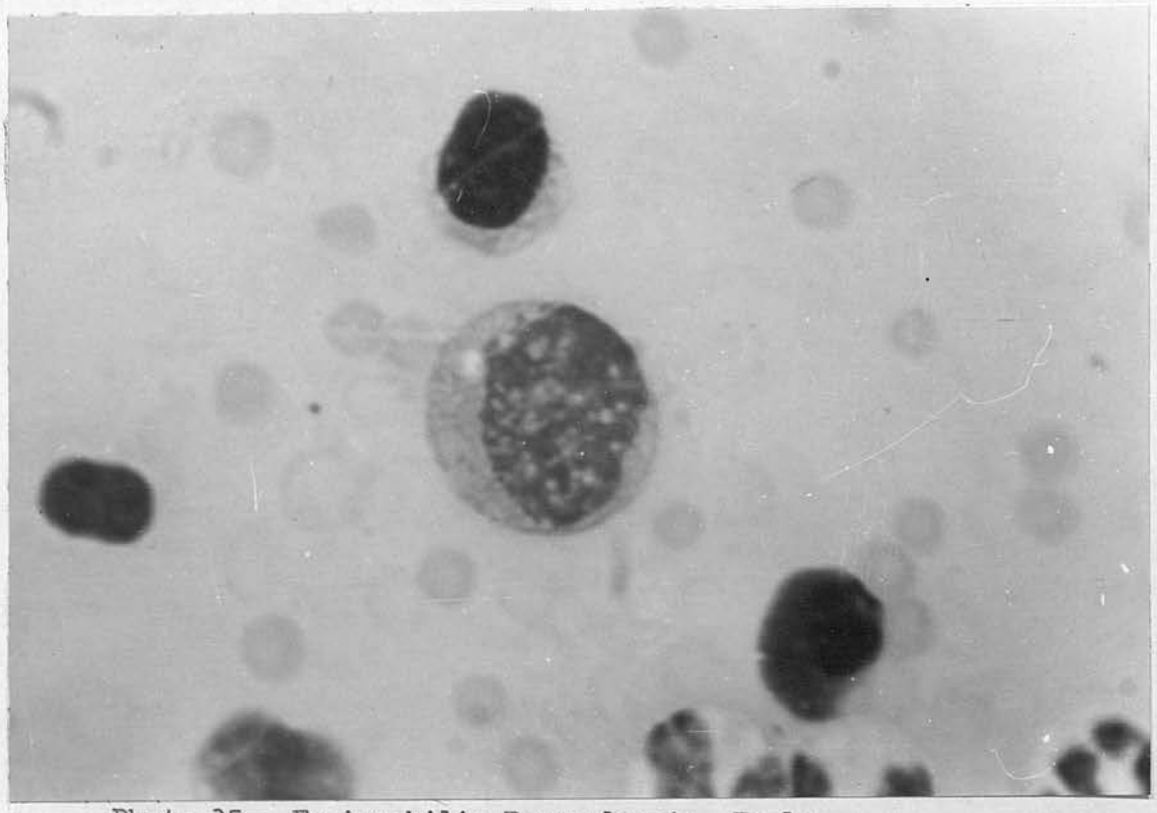

Photo 35. Eosinophilic Promyelocyte, Early Metamyelocyte and Normoblasts (x 1000)

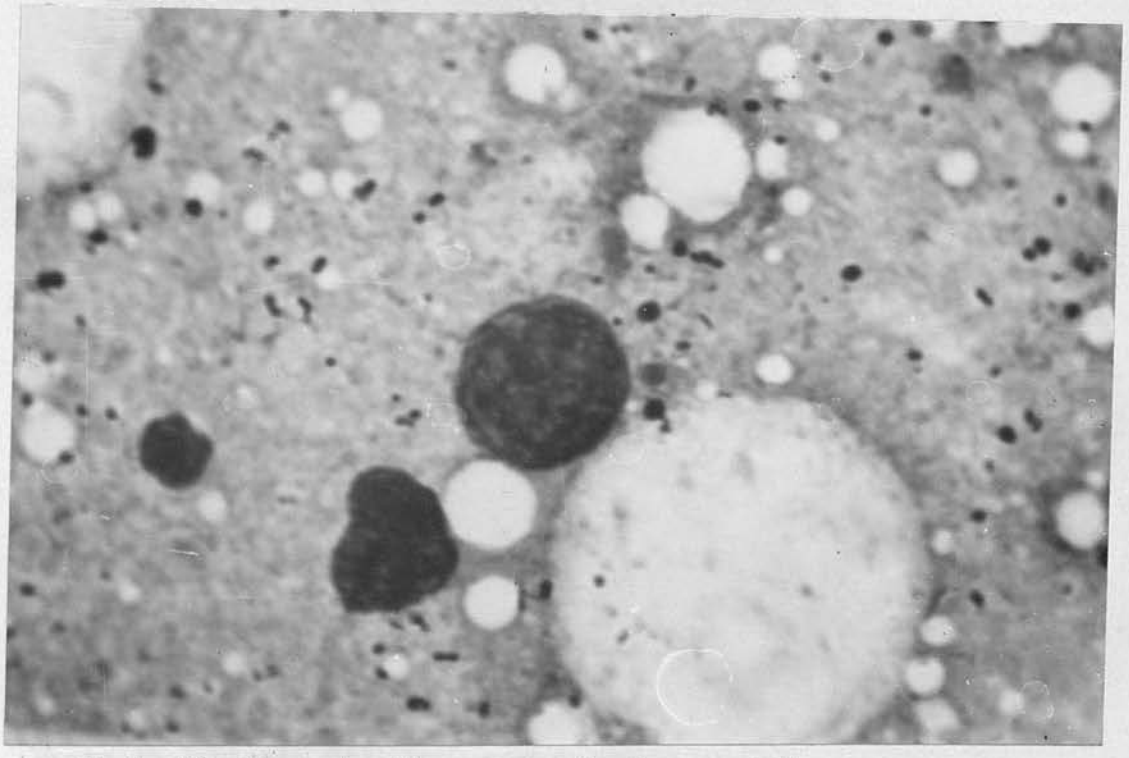

Photo 36. Hypoplastic marrow showing wasted fat cells in a gelatinous matrix with a few normoblasts (x 1000)

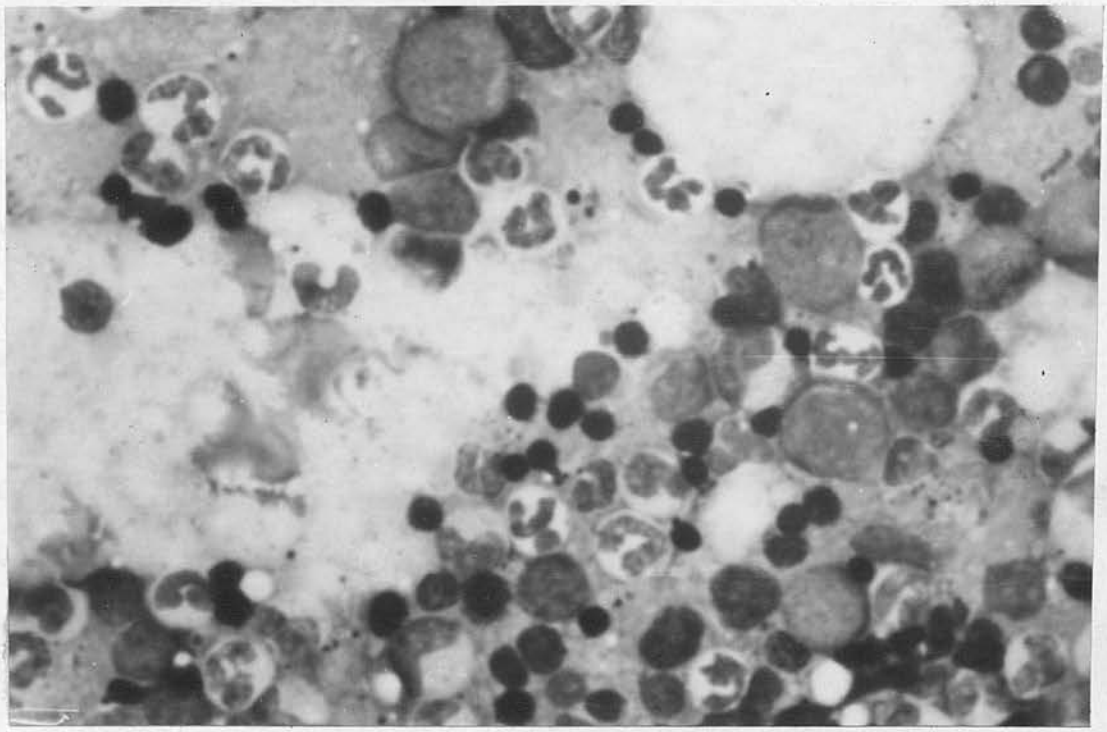

Photo 37. A leucoblastic reactive process showing leucocytes in various stages of development (x 500)

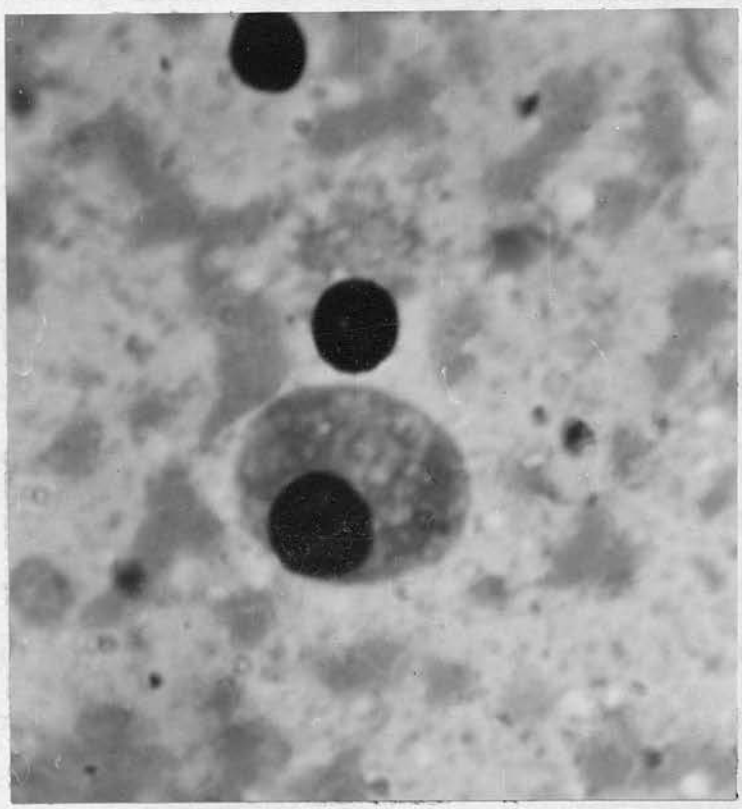

Photo 38. Plasma Cell and Late Normoblasts (x 1000)

3. Pathology

a) Morbid Anatomy (Photos 39 to 46)

The major post mortem findings of the eleven goats which died after exposure to X irradiation, are given in Table LXI. Table LXII lists the post mortem findings in goats (also numbering eleven) which died from other diseases or were destroyed during the same time period. The observations have been tabulated under four main headings, namely, haemorrhage, oedema, ulceration and miscellaneous.

In the majority of irradiated animals frank haemorrhages, ecchymoses and petechiae occurred in the body cavities, alimentary tract, lungs, trachea, heart and lymphatic tissues; oedema was also widely discernible; and ulcerations were observed in many parts of the intestinal mucosa. Of the miscellaneous findings, cystic ovaries were seen in four out of seven female goats irradiated; seven goats showed gelatinous bone marrow; three focal necrotising lesions in the liver; fatty livers were seen in five; enlarged gall bladders in four; and three goats had enlarged flabby hearts. White calcareous material near the pancreas and gall bladder were observed in three goats.

The post mortem lesions in these irradiated animals were in marked contrast to the almost negative findings in the goats which died from natural causes or which were destroyed. An interesting observation was the similarity of the main findings in the three goats diagnosed as having died from enterotoxaemia with those of the irradiated animals. The extent and degree of the lesions in the latter however were enhanced.

Survivors of the acute radiation syndrome which were autopsied some 18 to 30 months after exposure were normal as seen on examination. Death in irradiated goats were considered to be of two main kinds, namely:

i) Cardiac, with which were associated haemorrhage and haematopoietic failure

ii) Toxic, in which the predominant signs appeared to be a toxaemia.

According to this classification there were 3 toxic and 8 cardiac deaths amongst the irradiated goats.

Summary of Regional Post Mortem Findings in Irradiated Goats which died.

i) Organ Weights

The percentage organ to body weights of the main vital organs of the irradiated and unirradiated groups are given in Table LXIII. The average percentage heart weights in both groups were very similar. The weights of the lungs, spleen, and kidneys were greater in the irradiated goats but that of the liver was less.

ii) Musculo-Skeletal System, Subcutaneous Tissue and Skin.

Two goats showed intramuscular haemorrhages but no intra-articular lesions were seen. A few animals showed multiple petechial, ecchymotic and subepidermal haemorrhages especially of the orbital region of the head; an oedematous swelling of the facial region was characteristic of these cases. Loss of hair was seen in two goats and one goat suffered from an extensive pustular dermatitis.

iii) Central Nervous System

The CNS showed no anatomical changes except in the case of one goat with a slight haemorrhage into the spinal cord and one other with a superficial cerebral haemorrhage.

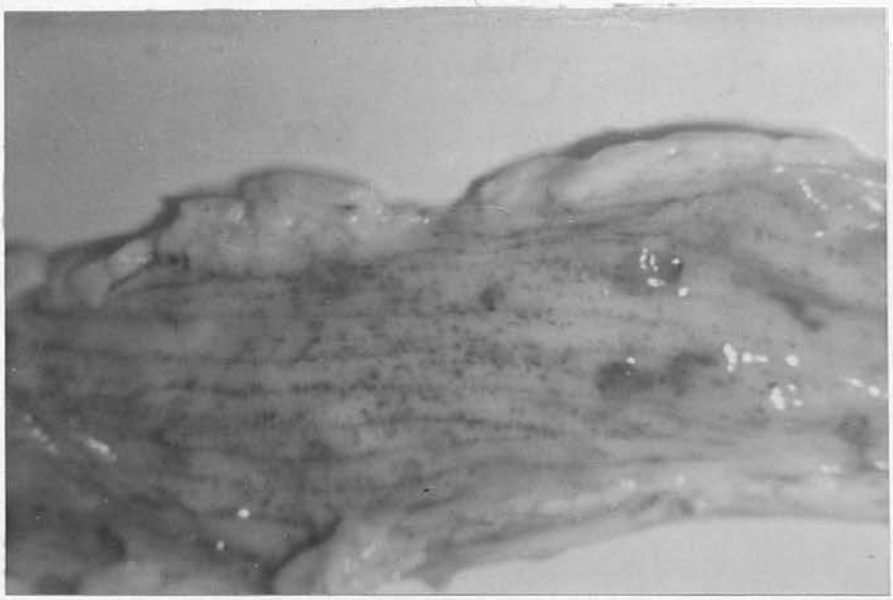

Photo 39. Haemorrhage and ulceration in the mucosa of the large intestine ("Zebra Markings"). Goat exposed to 700r survival time 11 days.

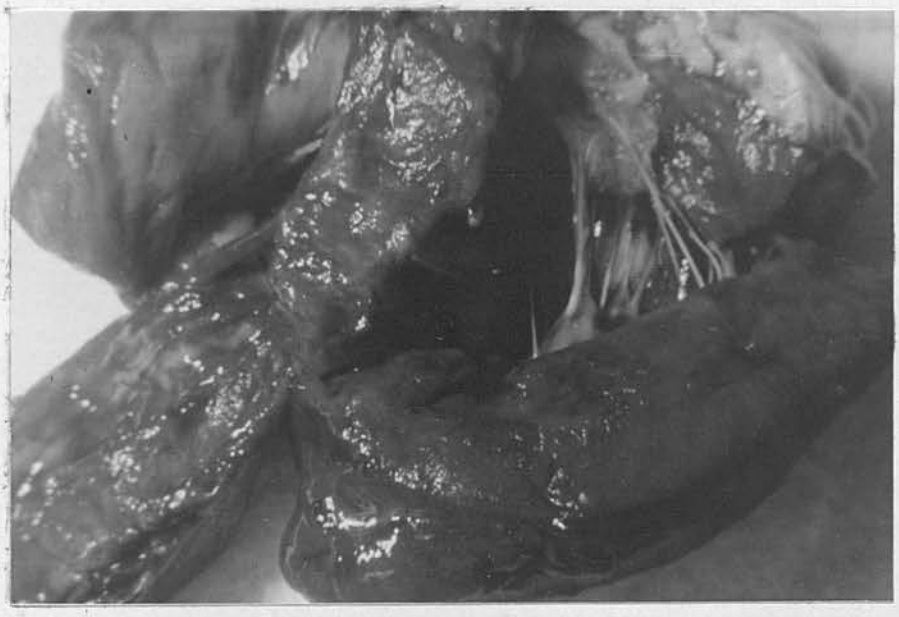

Photo 40. Longitudinal section of wall of left ventricle of heart showing epicardial myocardial and endorcardial haemorrhage. Goats exposed to 650r. Survival time 42 days.

iv) Cardio-Vascular System

Heart

Nearly all goats examined manifested subendocardial petechiae, ecchymoses or haemorrhages. Fibrosanguineous pericarditis was seen in five, sub-epicardial haemorrhages in five and myocardial haemorrhages in four goats. The heart valves were affected in all of these cases. One goat showed a strikingly atrophied and haemorrhagic organ best described as a "strawberry heart" (Photos 40 and 41) in which all cardiac tissue was involved. The mean percentage heart weight to body weight (0.544) did not differ from the normal (0.54).

v) Haemopoietic System

a) Lymph Nodes

The majority of lymph nodes were enlarged, haemorrhagic and oedematous. They were soft and mushy when cut. Some goats, especially those at the highest doses, also manifested a few shrunken lymph nodes.

b) Spleen

A common finding was subcapsular haemorrhages in the spleens. Spleens were generally larger than those found in normal goats: the mean percentage spleen to body weight was 0.270% in irradiated goats and 0.193% in normals.

c) Bone Marrow

Seven goats showed a gelatinous bone marrow in the long bones and five manifested a very fatty marrow both in the sternum and long bones. The bone marrow could be described as hypoplastic judging from the colour and fat deposition (Photo 42).

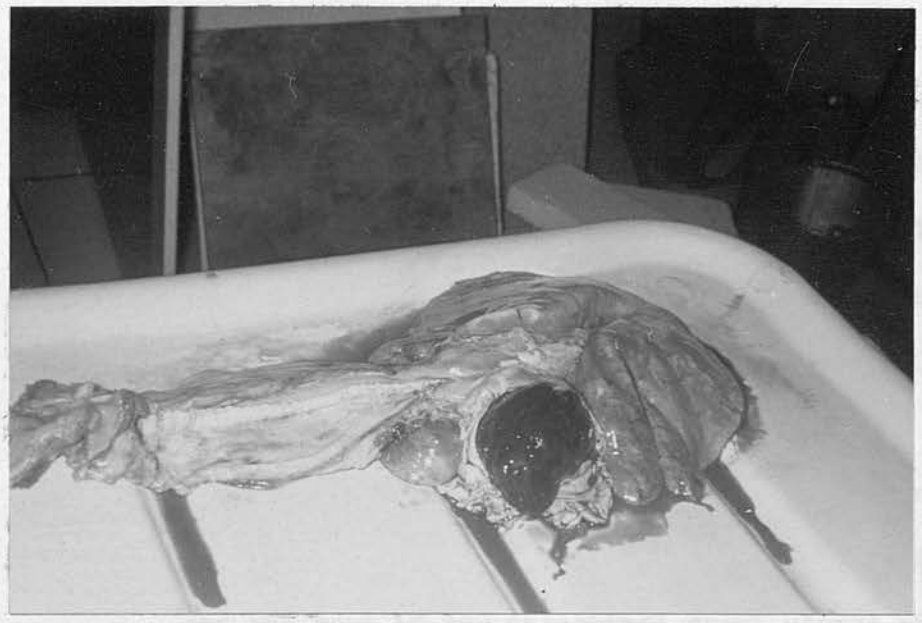

Photo 41. Cardiac Haemorrhage ("Strawberry Heart")

Photo 42. Sub-pleural Haemorrhage in Sternal area.
Hypoplastic Bone Marrow

vi) Alimentary System

a) Gastro-Intestinal Tract.

Haemorrhages and oedema were often seen in the pharynx and oral cavity but stomatitis or ulceration of this region was not observed. The oesophagus and the rumen (first stomach) were normal in all cases. The reticulum (second stomach) and the omasum (third stomach) were normal in the majority of cases but a few showed ecchymotic haemorrhages in serosa and mucosa. The glandular fourth stomach (abomasum) and the rest of the intestinal tract showed widespread petechial, ecchymotic or gross haemorrhages generally located beneath the mucosa and the serosa (Photo 44). Haemorrhages were also seen within the muscle bundles. Large haemorrhages were often observed in the omentum and mesentery and sometimes filled the abdominal cavity (photo 43).

The small intestine and colon, especially the caecum, were frequently filled with a purple semi-liquid, which was partially decomposed blood. The distal or floating colon and the rectum often showed a characteristic appearance owing to massive submucosal haemorrhages and ulcerations occurring in the mucosal folds; this has been described as "zebra markings". (Photo 39).

Photo 43. Abdominal Haemorrhage

Photo 44. Haemorrhages and inflammation in the Abomasum and Trachea

b) Liver

The liver and gall bladder on the whole were lighter in weight in the irradiated than in the normal goats. The mean percentage organ to body weight was 1.86% in irradiated goats and 2.35% in the normals. Five goats showed fatty livers and four had grossly enlarged and haemorrhagic gall bladders. Focal haemorrhagic areas were visible on the liver surface in four animals; and focal necrotising areas were observed in three goats. (Photo 45).

c) Pancreas.

The pancreas was usually quite normal except for occasional peri-pancreatic haemorrhage. Three goats showed white "calcareous spots" in or near the organ and two had haemorrhages actually within the substance.

vii) Respiratory System

The macroscopic lesions in the naso-pharynx consisted of a few petechiae or ecchymoses beneath the mucosa in some goats. Oedema of the epliglottis and larynx generally was noticeable in over half the goats. The larynx, trachea and the bronchi showed submucosal petechiae and ecchymoses and were often filled with bloody froth and mucus. Sub-pleural petechiae and ecchymoses were prominent in all lungs examined and the pulmonary parenchyma was invariably oedematous and showed massive haemorrhages in one or both lobes. (Photo 46). The bronchial lymph nodes were usually large and haemorrhagic. The mean percentage organ to body weight of the lungs of irradiated goats (2%) was greater than that of normal goats (1.76%).

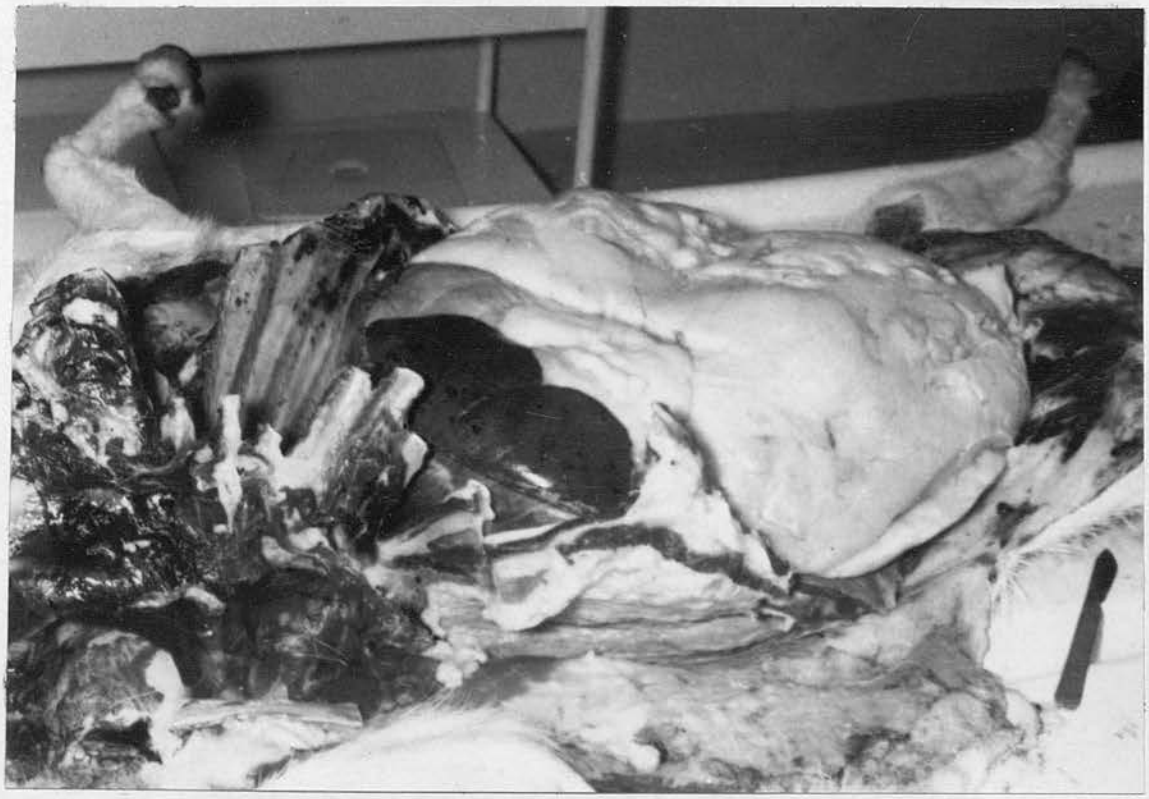

Photo 45. Focal Subcapsular Haemorrhages in Liver

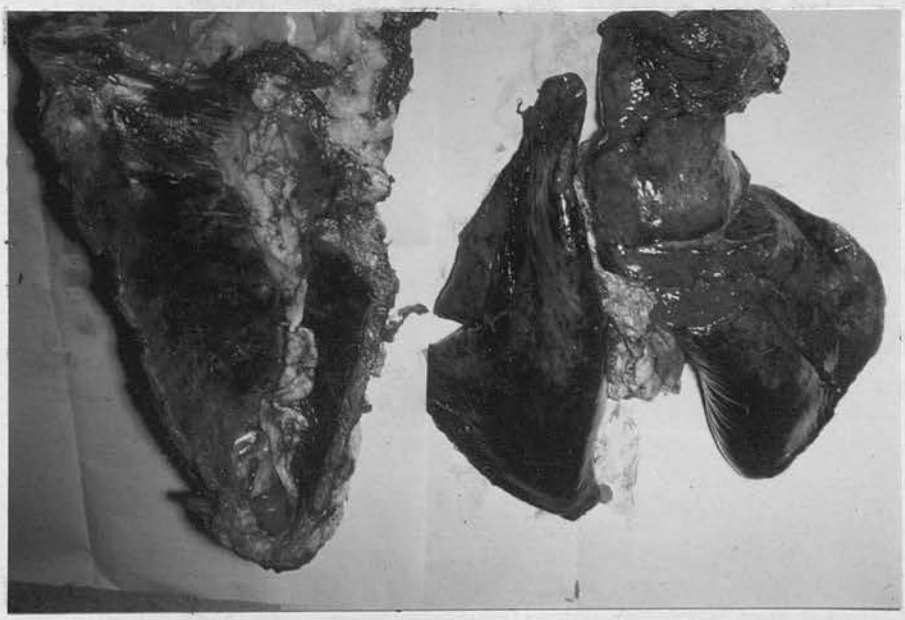

Photo 46. Haemorrhages in Lungs and Sternal Sub-pleural haemorrhages

Sub-pleural haemorrhages of the thoracic cavity were often massive in extent and occurred in all but one goat whereas sub-peritoneal haemorrhages in the abdominal cavity were observed in only 3 goats and were discrete and petechial in nature. (Photo 42 and 46).

viii) Genito-Urinary System

The mean percentage organ to body weight of the kidneys was slightly larger in irradiated goats (0.456%) than in normal goats (0.341%). The kidneys on macroscopic examination usually appeared to be normal; four however showed petechial or eccymotic haemorrhages on section. In one goat the capsule was thickened and adherent to a shrunken kidney. Extra-renal haemorrhage was seen on three occasions.

The bladder often showed submucosal and subserosal haemorrhages. Peri-vesical ecchymoses were also occasionally seen. The ureters were always normal in appearance.

The ovaries showed varying degrees of changes. Superficial petechiae or ecchymoses were sometimes observed. Frank haemorrhages were seen in two goats. Four out of seven irradiated females showed cystic ovaries whereas two out of six normal goats had cystic ovaries on autopsy.

ix) Endocrine System

The adrenals were usually normal or slightly enlarged in appearance although in two goats gross haemorrhages were observed. The weight of the adrenals in normal goats was between 4 and 5 gms whereas in the irradiated animals they were between 7 and 8 gms.

Sub-pleural haemorrhages of the thoracic cavity were often massive in extent and occurred in all but one goat whereas sub-peritoneal haemorrhages in the abdominal cavity were observed in only 3 goats and were discrete and petechial in nature. (Photo 42 and 46).

viii) Genito-Urinary System

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ix) Endocrine System

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The thyroids weighed between 8 and 10 gms in irradiated goats and were small elongated and lozenge-shaped; in normal goats they were slightly larger 10 to 12 gms but quite similar in appearance. No abnormalities could be detected.

Table LII

EFFECTS OF X-IRRADIATION OF GOATS

Major Findings at Post-Mortem of Irradiated Goats

Goat No.	C229	C234	C175	F40	F27	F14	F24	C315	C118	C200	F20	No. of Goats
Dose (r)	2000	1000	700	700	700	650	650	600	550	550	550	in which found
Survival Time	8	8	11	13	46	42	14	30	18	12	12	
HAEMORRHAGE												
Skin	-	-	-	-	-	-	-	-	-	-	+	1
Subcut. Tissues	-	-	-	-	-	-	-	-	+	+	+	3
Intramuscular	-	-	-	-	-	-	-	-	+	+	-	2
Brain	-	-	-	+	-	-	-	-	-	-	-	1
Spinal Cord	+	-	-	-	-	-	-	-	-	-	-	1
Eye	-	-	-	-	-	-	+	-	+	-	-	2
Orbital Cavity	-	-	-	-	-	-	-	+	-	-	+	2
Pharynx	+	+	++	++	-	++	-	-	++	-	++	7
Larynx	+	+	++	++	-	++	-	-	++	-	++	7
Trachea	+	+	++	++	-	++	+++	++	++	++	++	10
Tonsils	-	-	-	+	-	-	-	+++	-	-	++	3
Thoracic Lymph Nodes	+	++	+++	+++	+	+++	+	+	+++	+	+++	11
Pleural Cavity	-	-	-	+	+	+	-	++	-	-	-	4
Parietal Pleura	+	+	+	+	-	+	+	+	+++	++	++	10
Lungs	+++	+	++	+++	+	++	+++	+	+++	++	++	11
Heart												
1) Pericardium	-	+	-	-	++	-	-	-	+	+	++	5
2) Epicardium	+	-	-	-	++	-	+	-	-	+	+	5
3) Myocardium	+	-	-	-	+	-	+	-	-	-	+	4
4) Endocardium	-	+	-	+	+++	+	+++	++	+	+	+++	9
Parietal-Peritoneum	+	-	-	-	-	-	+	-	+++	-	-	3
Visceral Peritoneum	+	-	+	+	-	-	-	+	+	-	+	6
Rumen	-	-	-	-	-	-	-	-	-	-	-	0
Reticulum	-	+	-	-	-	-	-	+	-	-	-	2
Omasum	-	-	-	+	+	-	+	+	-	-	-	4
Abomasum	++++	++++	+++	+	++	+	++	+	+	+	+	11
Duodenum	+	+	++	++	+	+	+	++	+	+	+	11
Small Intestine	+	+	+	++	+	+	+	++	++	+	+	11
Large Intestine	++++	++	++++	++	+++	+++	+++	+	+++	++	+	11
Rectum	++	-	++	-	++	-	-	-	-	-	-	3
Spleen	-	-	-	-	+	-	+	-	-	+	+	4
Liver	-	-	-	+	+	-	-	+	+	-	-	4
Pancreas	-	-	-	-	-	-	-	-	-	+	+	2
Mesenteric Lymph Nodes	+	+	-	-	-	+	+	+	+	-	+	7
Kidneys	-	-	-	-	-	+	-	+	+	-	+	4
Bladder	-	-	-	-	-	-	-	+	-	-	+	2
Adrenals	-	-	-	-	-	+	-	-	-	-	+	2
Ovaries	-	-	-	-	+	-	-	-	-	-	+	2
OEDEMA												
Pharynx	+	+	-	-	-	+	-	-	+	-	+	5
Larynx	+	+	-	-	-	+	-	-	+	-	+	5
Trachea	+	+	-	-	-	-	-	-	-	+	-	3
Tonsils	-	-	-	+	-	-	-	+	-	-	+	3
Lymph Nodes	-	-	+	+	+	+	+	+	-	+	+	8
Pleural Cavity	-	-	-	+	+	+	-	+	-	-	-	4
Lungs	+	+	+	+	+	+	+	+	+	+	+	11
Pericardial Sac	-	+	-	-	-	-	-	-	+	-	+	3
Peritoneal Cavity	-	-	-	-	-	-	-	-	++	-	-	1
Ovaries (cystic)	-	-	+	-	+	-	+	-	-	-	+	4
ULCERATION												
Abomasum	+	+	+	-	+	-	-	-	+	-	+	6
Duodenum	+	+	+	+	-	-	+	+	-	-	-	6
Small Intestine	+	+	-	+	-	-	+	+	-	-	-	5
Caecum	+	+	+	+	+	+	+	+	+	+	-	10
Colon	+	+	+	+	+	-	+	-	+	-	-	7
Rectum	+	-	-	-	-	-	+	-	-	-	-	2
MISCELLANEOUS												
Liver Necrosis	+	-	-	-	-	-	+	-	-	-	+	3
Fatty Liver	-	-	-	-	+	+	+	+	+	-	-	5
Enlarged Gall Bladder	-	+	-	+	+	-	-	-	+	-	-	4
White "Calcareous" Spots in or near Pancreas	+	-	-	+	-	-	-	-	+	-	-	3
Enlarged Flabby Heart	-	-	-	+	-	-	-	+	-	-	+	3
Atrophy of Heart	-	-	-	-	-	+	-	-	-	-	-	1
Cysts in Ovary	-	-	+	-	+	-	+	-	-	-	+	4
Fatty Bone Marrow	-	-	-	-	+	+	+	+	+	-	-	5
Gelatinous Bone Marrow	+	+	+	-	-	-	+	-	+	+	+	7

Table LXII

Major Findings at Post-Mortem of Goats dying from natural causes/euthanasia

Goat No. Cause of Death	M7 Bladder worm in Cranial Cavity	C136 Euthan-asia	F4 Avian T.B.	107C Entero-toxaemia	F103 Euthan-asia	F304 Broken Neck	F253 Entero-toxaemia	M8 Entero-toxaemia	F29 Euthan-asia	F303 Euthan-asia	F37 Euthan-asia	No. of Goats in which found
HAEMORRHAGE												
Skin	-	-	-	+	-	-	-	-	-	-	-	1
Subcut Tissue	-	-	-	-	-	-	-	-	-	-	-	0
Intramus "	-	-	-	-	-	-	+	-	-	-	-	1
Brain	-	-	-	-	+	-	-	-	-	-	-	1
Spinal Cord	-	-	-	-	-	+	-	-	-	-	-	1
Eye	-	-	-	-	-	-	-	-	-	-	-	0
Orbital Cavity	-	-	-	-	-	-	-	-	-	-	-	0
Pharynx	-	-	-	+	-	-	-	-	-	-	-	1
Larynx	-	-	-	+	-	-	-	-	-	-	-	1
Trachea	-	-	-	++	-	-	-	-	-	-	-	1
Tonsils	-	-	-	-	-	-	-	-	-	-	-	0
Thoracic Lymph Node	-	-	-	-	-	-	+	-	-	-	-	1
Pleural Cavity	-	-	-	-	-	-	-	-	-	-	-	0
Parietal Pleura	-	-	-	+	-	-	+	+	+	-	-	4
Visceral "	-	-	-	-	-	-	+	-	-	-	-	1
Lungs	+	+	-	+	-	+	+	-	-	-	-	5
Heart												
1) Pericardium	-	-	-	-	-	-	-	-	-	-	-	0
2) Epicardium	-	-	-	-	-	-	+	-	-	-	-	1
3) Myocardium	-	-	-	-	-	-	-	+	-	-	-	1
4) Endocardium	++	-	-	-	-	-	++	+	-	+	-	4
Parietal Peritonium	-	+	-	-	-	-	-	-	-	-	-	1
Visceral "	-	-	-	+	-	-	-	-	-	-	-	1
Rumen	-	-	-	-	-	-	-	+	-	-	-	1
Reticulum	-	-	-	-	-	-	-	+	-	-	-	1
Omasum	-	-	-	-	-	-	-	+	-	-	-	1
Abomasum	+	-	-	+	-	-	+	++	-	-	-	2
Duodenum	+	-	-	+	-	+	+	+	-	-	-	5
Small Intestine	-	-	-	+	-	+	+	+	-	-	-	4
Large Intestine	-	-	-	+	-	+	++	+	-	-	-	4
Rectum	-	-	-	-	-	-	-	+	-	-	-	1
Spleen	-	-	-	-	-	-	-	-	-	-	-	0
Liver	-	-	-	-	-	-	+	-	-	-	-	1
Pancreas	-	-	-	-	-	-	+	-	-	-	-	1
Mesenteric Lymph Glands	-	-	+	-	-	-	-	-	-	-	-	1
Kidney	-	+	-	-	-	-	-	-	-	-	-	1
Bladder	-	++	-	-	-	-	-	-	-	-	-	0
Adrenals	-	-	-	-	-	-	-	-	-	-	-	1
Ovaries	-	-	-	+	-	-	-	-	-	-	-	0
Testes	-	-	-	-	-	-	-	-	-	-	-	
OEDEMA												2
Pharynx	-	-	-	-	-	-	+	+	-	-	-	2
Larynx	-	-	-	-	-	-	+	+	-	-	-	1
Trachea	-	-	-	+	-	-	-	-	-	-	-	0
Tonsils	-	-	-	-	-	-	-	-	-	-	-	3
Lymph Nodes	-	-	+	-	-	-	+	+	-	-	-	0
Pleural Cavity	-	-	-	-	-	-	-	-	-	-	-	5
Lungs	+	+	-	+	-	+	-	-	-	+	-	0
Pericardial Sac	-	-	-	-	-	-	-	-	-	-	-	1
Peritoneal Cavity	-	+	-	-	-	-	-	-	-	-	-	2
Ovaries	-	-	+	+	-	-	-	-	-	-	-	1
Kidney	-	+	-	-	-	-	-	-	-	-	-	
ULCERATION												0
Abomasum	-	-	-	-	-	-	-	-	-	-	-	0
Duodenum	-	-	-	-	-	-	-	-	-	-	-	0
Small Intestine	-	-	-	-	-	-	-	-	-	-	-	0
Caecum	-	-	-	-	-	-	-	-	-	-	-	0
Colon	-	-	-	-	-	-	-	-	-	-	-	0
Rectum	-	-	-	-	-	-	-	-	-	-	-	
MISCELLANEOUS												0
Liver Necrosis	-	-	-	-	-	-	-	-	-	-	-	2
Fatty Liver	+	-	-	+++	-	-	-	-	-	-	-	1
Enlarged Gall Bladder	-	+	-	-	-	-	-	-	-	-	-	
White "calcareous" spots in or near Pancreas	-	-	-	-	-	-	-	-	-	-	-	0
Enlarged Flabby Heart	-	-	-	-	-	-	-	-	-	-	-	0
Atrophy of Heart	-	-	-	-	-	-	-	-	-	-	-	0
Cysts in Ovary	-	-	+	+	-	-	-	-	-	-	-	2
Fatty Bone Marrow	-	-	-	+	-	-	-	-	-	-	-	1
Gelatinous Bone Marrow	-	-	-	-	-	-	-	-	-	-	-	0

Weight of Organs and Percentage of Organ to Body Weights in Goats

Unirradiated Goats

Goat No.	Total Body Wt.	Heart		Lungs		Liver & Gall Bladder		Spleen		Kidneys	
	(Kg.)	Wt. in gm	%	Wt. in gm	%	Wt. in gm	%	Wt. in gm	%	Wt. in gm	%
F 103	59.1	425	0.72	1500	2.54	1500	2.54	100	0.169	150	0.254
F 304	20.1	82	0.41	325	1.62	675	3.37	34	0.170	103	0.514
F 253	50.0	350	0.70	1200	2.40	1500	3.00	129	0.258	293	0.586
C 107	75.4	260	0.34	700	0.93	1100	1.46	99	0.131	162	0.215
F 4	55.9	300	0.54	1000	1.79	1600	2.86	250	0.447	200	0.358
C 136	81.8	250	0.31	950	1.16	1500	1.83	75	0.092	200	0.244
M 8	73.6	375	0.51	1250	1.70	1600	2.17	110	0.149	300	0.407
M 7	64.5	400	0.54	1300	1.74	1450	1.95	100	0.134	220	0.295
F 37	64.5	400	0.62	1120	1.74	1475	2.29	150	0.232	200	0.310
F 29	68.2	400	0.59	1200	1.76	1500	2.20	100	0.147	160	0.235
F 303	45.5	300	0.66	900	1.98	1000	2.20	90	0.198	150	0.330
Mean for 11 Goats	60.8	322	0.54	1040	1.76	1355	2.35	112	0.193	194	0.341

Weight of Organs and Percentage of Organ to Body Weights in Goats

Irradiated Goats .

Goat No.	Dose (r)	Total Body Wt.	Heart		Lungs		Liver & Gall Bladder		Spleen		Kidneys	
		(Kg.)	Wt. in gm	%	Wt in gm	%	Wt. in gm	%	Wt. in gm	%	Wt. in gm	%
C229	2000	86.4	350	0.405	750	0.87	1300	1.51	110	0.127	209	0.242
C234	1000	65.0	270	0.415	1000	1.54	1250	1.92	79	0.122	145	0.223
C175	700	50.5	260	0.515	1125	2.23	750	1.49	110	0.218	140	0.277
F 40	700	48.2	250	0.519	1500	3.11	1250	2.59	150	0.311	550	1.142
F 27	700	47.3	400	0.846	1250	2.64	1250	2.64	200	0.423	160	0.338
F 14	650	37.7	175	0.464	700	1.86	1000	2.65	100	0.265	140	0.371
F 24	650	35.0	175	0.500	800	2.29	675	1.93	73	0.209	137	0.391
C315	600	58.2	250	0.430	1000	1.72	700	1.20	120	0.206	200	0.344
C118	550	106.8	450	0.421	1400	1.31	1500	1.40	250	0.234	225	0.211
C200	550	48.6	350	0.720	800	1.64	650	1.34	200	0.411	300	0.617
F 20	550	56.8	425	0.748	1600	2.82	1000	1.76	250	0.440	490	0.862
Mean for 11 Goats		58.2	305	0.544	1084	2.00	1030	1.86	149	0.270	245	0.456

b) Histopathology (Photos 47 to 66)

The major histopathological findings in the organs and tissues of the eleven goats which died after exposure to X radiation are given in the Tables LXIV and LXV.

Briefly it may be said that the following were observed in ALL goats viz:

i) Congestion, oedema and haemorrhage in the lungs

ii) Cellular degeneration in the kidneys

iii) Petechiae in the adrenal gland

iv) Normal mitotic activity in the epithelium of all fore-stomachs

v) Petechial and ecchymotic haemorrhages into the connective tissue of the abomasum, small and large intestines

vi) Thrombosis of the vessels of the small intestine and liver

A MAJORITY of the goats demonstrated the above lesions together with

i) Endocardial and myocardial petechial haemorrhages

ii) Degeneration of the cells of the lymph nodes with oedema and haemorrhage

iii) Atalectasia, emphysema and consolidation in the lungs

iv) Hypoplasia of the bone marrow

v) Hyperaemia and atrophy of the renal glomeruli

vi) Presence of large numbers of plasma cells together with cellular degeneration in the spleen and much haemosiderin

vii) Atrophic ovarian follicles

viii) Fatty livers with focal necrosis and thrombosis with oedematous gall bladders

ix) Profound epithelial changes in the gastro-intestinal tract together with oedema and haemorrhage into serosa, connective tissue and musculature, and also vascular thrombosis and intimal endothelial proliferation.

Table LXIV

+

Summary of the major histopathological findings observed in eleven goats dying after exposure to X radiation

Body System	Organ/Tissue		Lesion	Total Numbers
CNS	Brain (Cerebrum)		Haemorrhage	2
	Spinal cord		"	2
Cardio-vascular	Heart		Haemorrhage	
			Pericardial	1
			Epicardial	5
			Myocardial	7
			Endocardial	10
	Blood vessels		Endothelial swelling	6
			Thrombosis	10
Haemopoietic	Lymph nodes		Haemorrhage	10
			Oedema	7
			Cell degeneration	10
			Cell regeneration	5
	Spleen		Haemosiderosis	7
			Many plasma cells	8
			Cell degeneration	7
	Bone Marrow		Aplasia	3
			Hypoplasia	8
Alimentary	Gastro-intestinal Tract		see Table LXV	-
	Liver		Haemorrhages	11
			Necrosis	9
			Fatty	10
	Gall Bladder		Haemorrhages	3
			Oedema	6
			Calcareous spots	2
	Pancreas		Haemorrhages	7
			Acinous cell degeneration	3
			Calcareous spots	2
Respiratory	Trachea/bronchi		Haemorrhages	10
			Metaplasia in epithelial cells	8
	Lungs		Haemorrhages	11
			Atalectasis	10
			Emphysema	10
			Consolidation	7
			Vascular hyalinization	9
			Thrombosis	9
Genito-urinary	Kidney	Glomerulus	Haemorrhages	8
			Swelling	4
			Atrophy	6
			Necrosis	4
		Tubules	Haemorrhages	9
			Degeneration	11
		Interstitial	Thrombosis	7
	Bladder		Haemorrhages	7
			Oedema	5
			Epithelial desquamation	3
	Ovary		Atrophic follicles	7
			Cystic	4
			Haemorrhages	4
			Primitive ova	6
Endocrine	Adrenal		Haemorrhages	11
			Degeneration	4
	Thymus		Atrophy	11
	Thyroid		Functional Stimulation	1

A FEW goats also showed the following :-

i) Pericardial and epicardial effusions and haemorrhages

ii) Haemorrhage into the wall of the gall bladder

iii) Aplasia of the bone marrow

iv) Perivascular haemorrhage in the CNS

Summary of Regional Histopathology

i) Central Nervous System

The CNS was usually normal. Two goats showed massive haemorrhages into the brain substance and two others into the spinal cord.

ii) Cardio-Vascular System

a) Heart

The general picture was one of peri-vascular bleeding with some hyalinization of the connective tissue. Endocardial haemorrhage was the most common finding and occurred in all but one goat. Myocardial bleeding was seen in seven animals and epicardial haemorrhage in five. There was no degeneration of muscle cells nor thrombosis. However in some cases "caterpillar" cells and intravascular globules that were PAS-positive were seen. (Photo 49). Occasionally globules were noted in the myoplasm especially in the right side of the heart.

b) Blood Vessels

A few animals showed telangiectasis of cutaneous blood vessels. Effects on large vessels were nil but in arterioles and venules and in the capillary beds of the major organs profound effects in the endothelium were discernible. These effects consisted mainly of a swelling of the endothelium

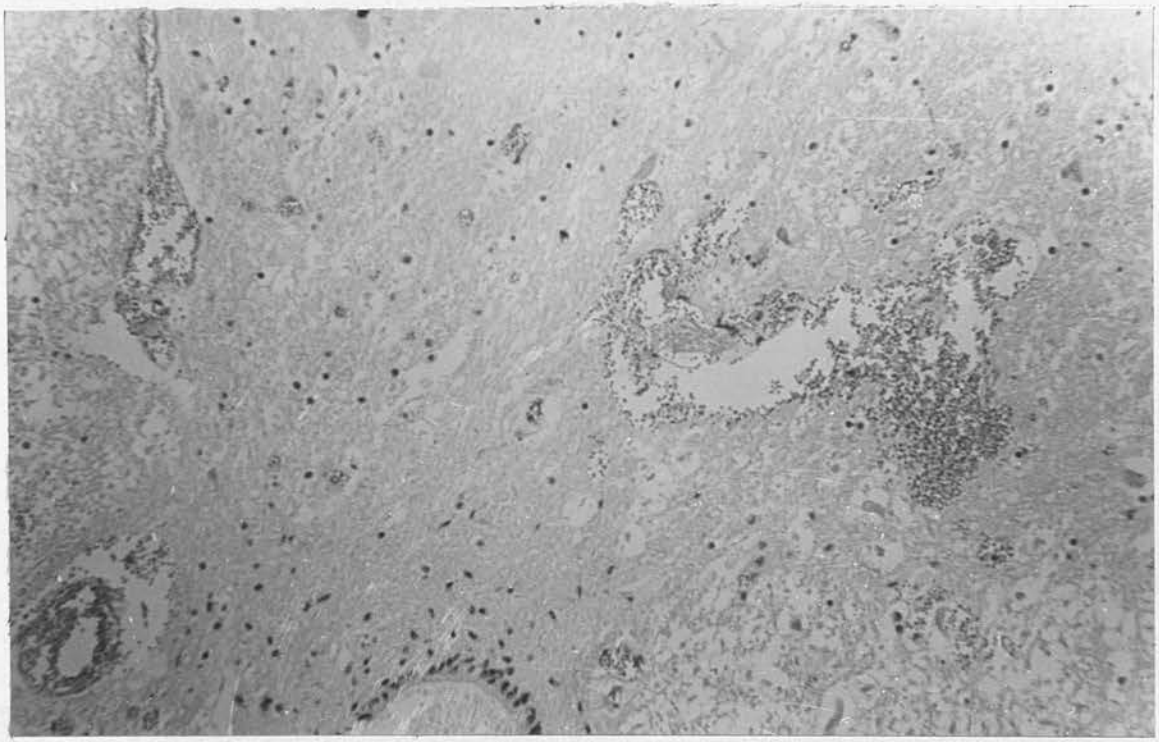


Photo 47. Cerebrum showing perivascular haemorrhage. H and E x 180

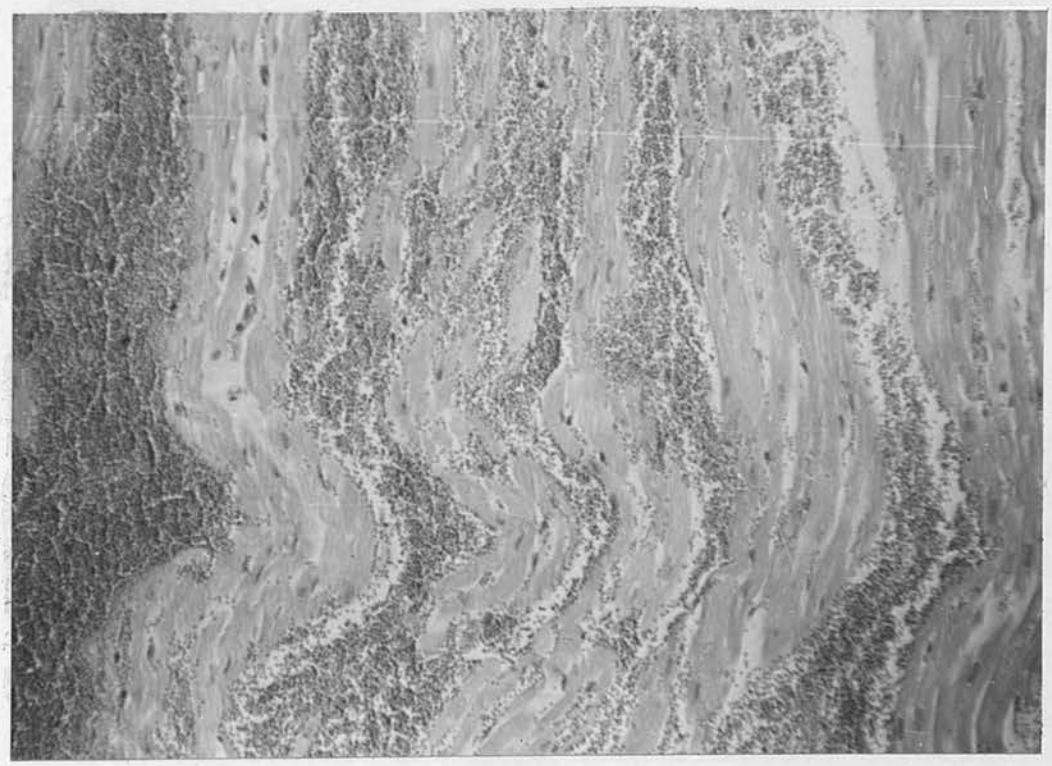


Photo 48. Myocardial haemorrhage H and E x 180

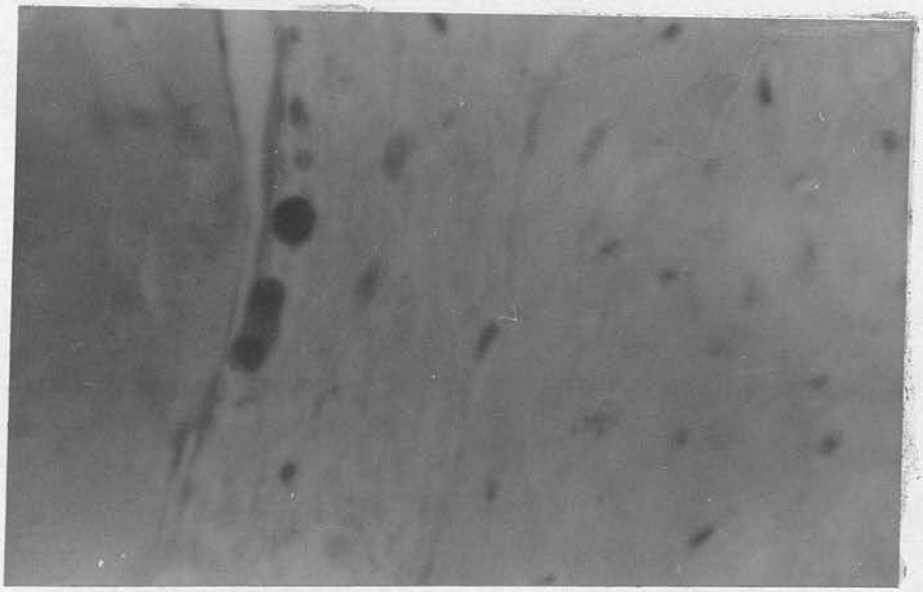


Photo 49. Globules in myocardium adjacent to A-V node. P.A.S. x 1000

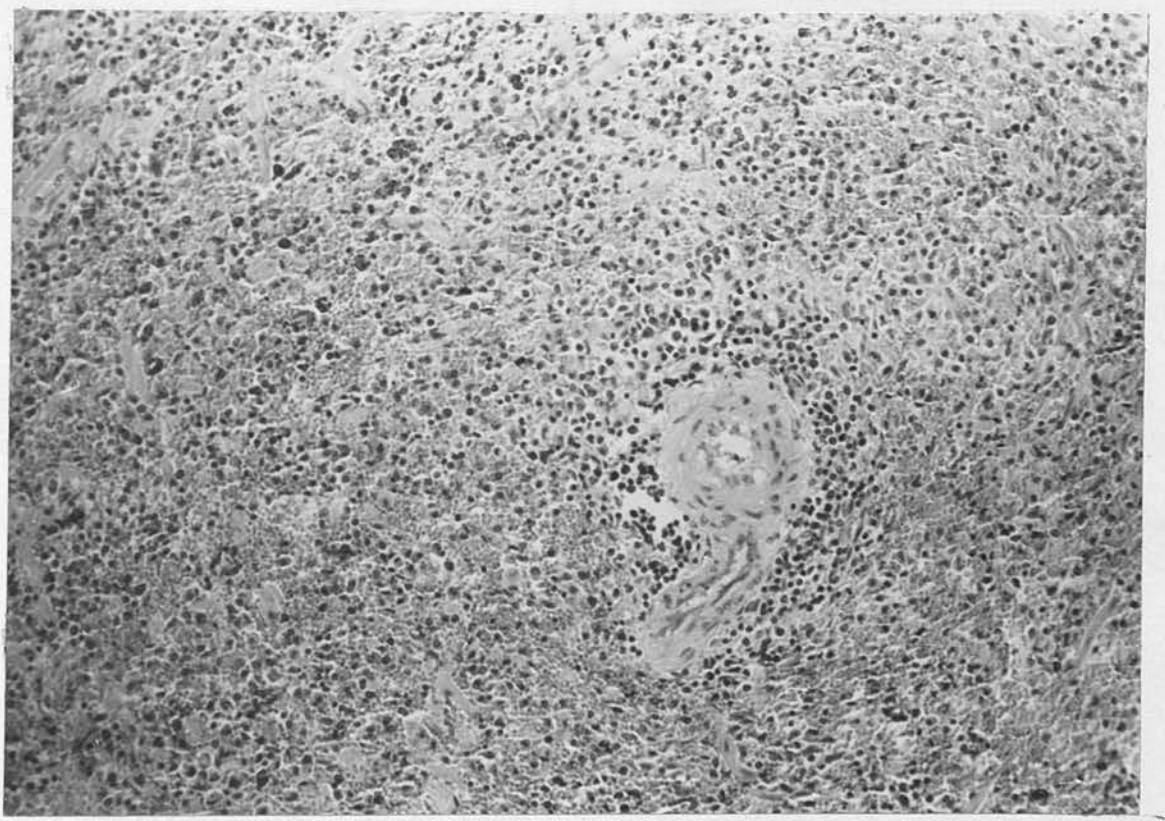


Photo 50. Endothelial swelling and hyalinization of blood vessels (spleen). H & E x 180.

with some hyalinization and obliteration of the lymphatics. Hyaline thrombi were often seen, especially in the liver and intestine producing a partial occlusion of the vascular tree.

ii) Haemopoietic System

a) Lymph Nodes

Nodes usually consisted of a poorly defined mass of loosely woven connective tissue with a few lymphocytes intertwined within its meshes. There was hypoplasia of the lymphatic elements with apparent survival of the reticulum cells. There was massive haemorrhages within the substance of the nodes espcially in the subcapsular region. Evidence of regeneration of lymphocytes was seen in some specimens but the majority showed no signs of lymphocytic regeneration. The medullary sinuses were normally filled with red blood cells; blood pigment and phagocytosed cells.

b) Spleen

The appearance of sections of spleen was often similar to that of the lymph nodes: there was pyknosis and fragmentation of the lymphocytes within the germinal centres accompanied by many macrophages full of phagocytosed red blood cells and nuclear debris. The germinal centres were often quite impossible to identify as such but ocasionally huge germinal centres were noted vaguely outlined against the parenchyma.

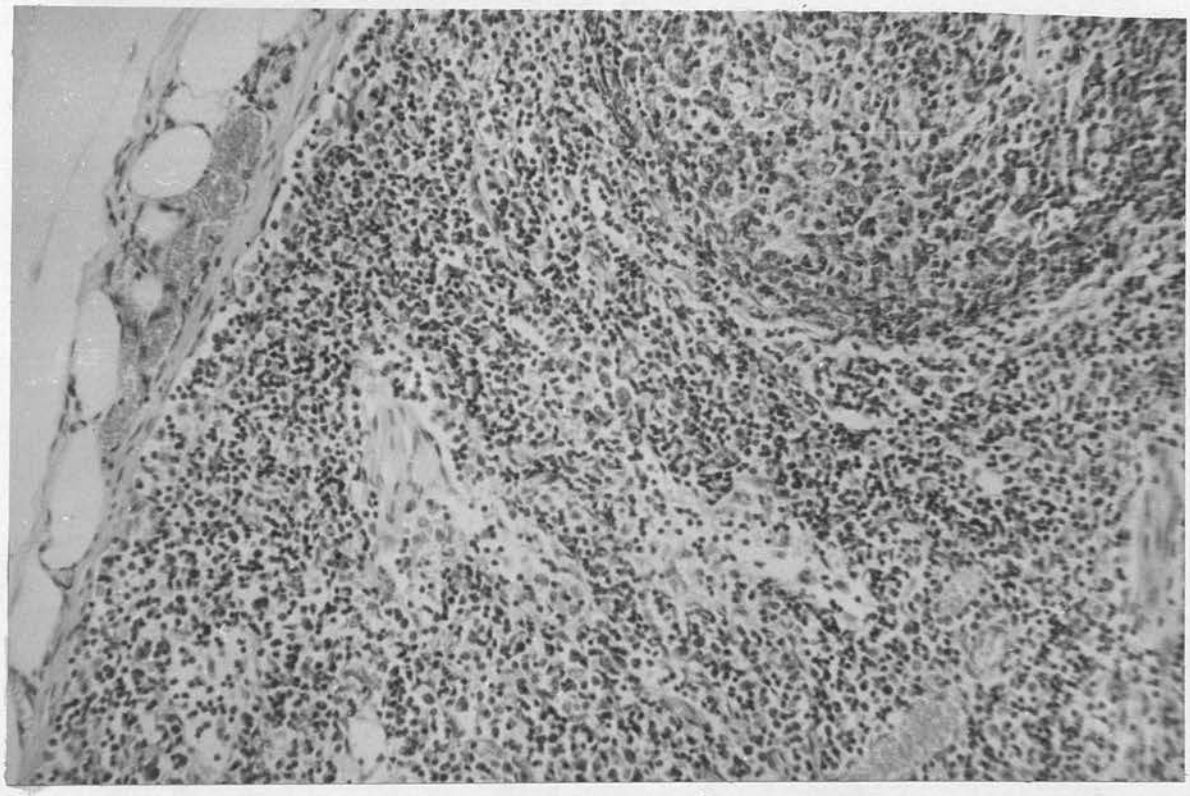


Photo 51. Lymph Node showing regeneration (Goat dying 30 days after Irradiation) H & E x 300.

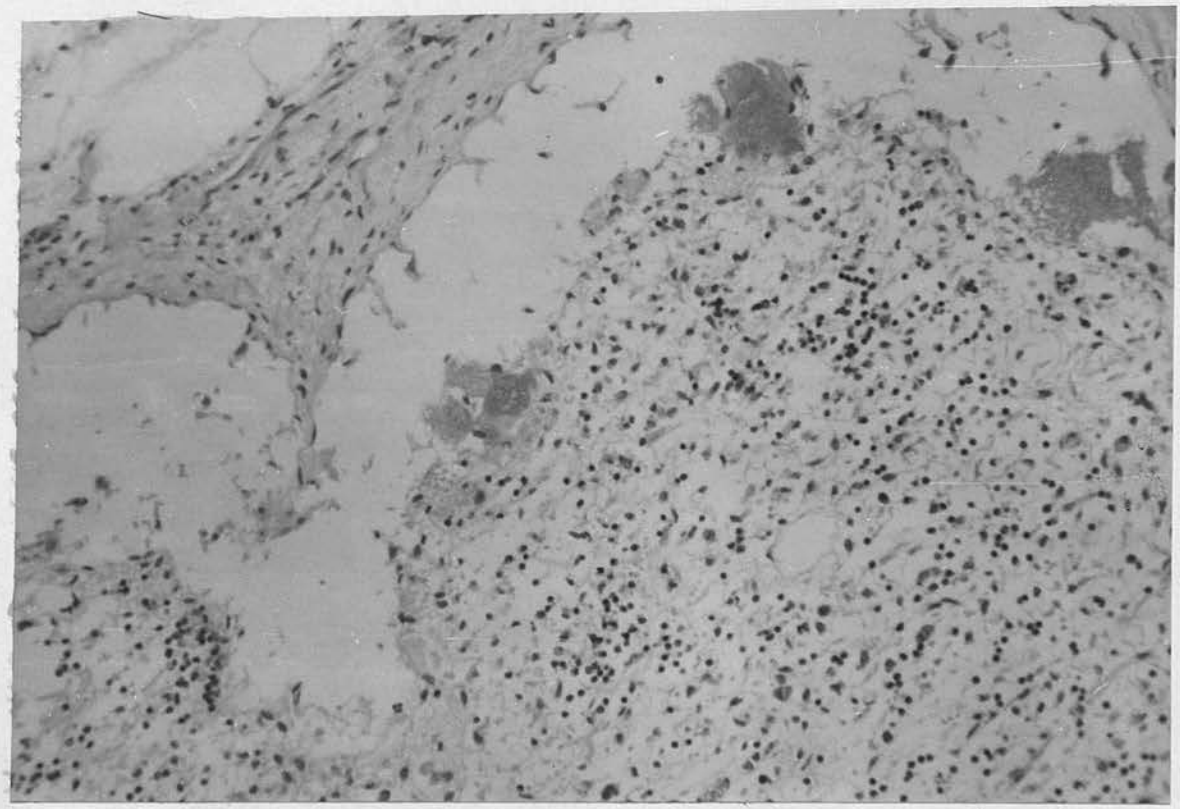


Photo 52. Lymph Node showing hypoplasia of lymphatic elements with focal degenerative areas. H & E x 300

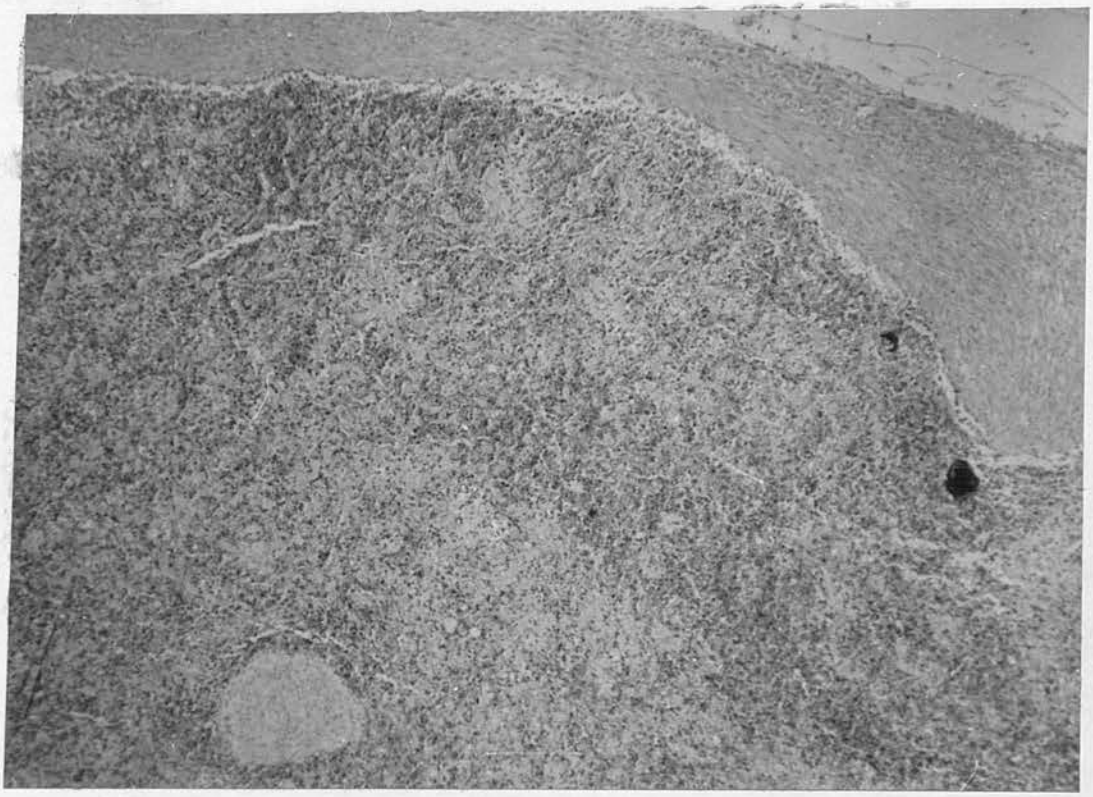


Photo 53. Spleen showing hypoplasia of cellular elements.
H & E x 100.

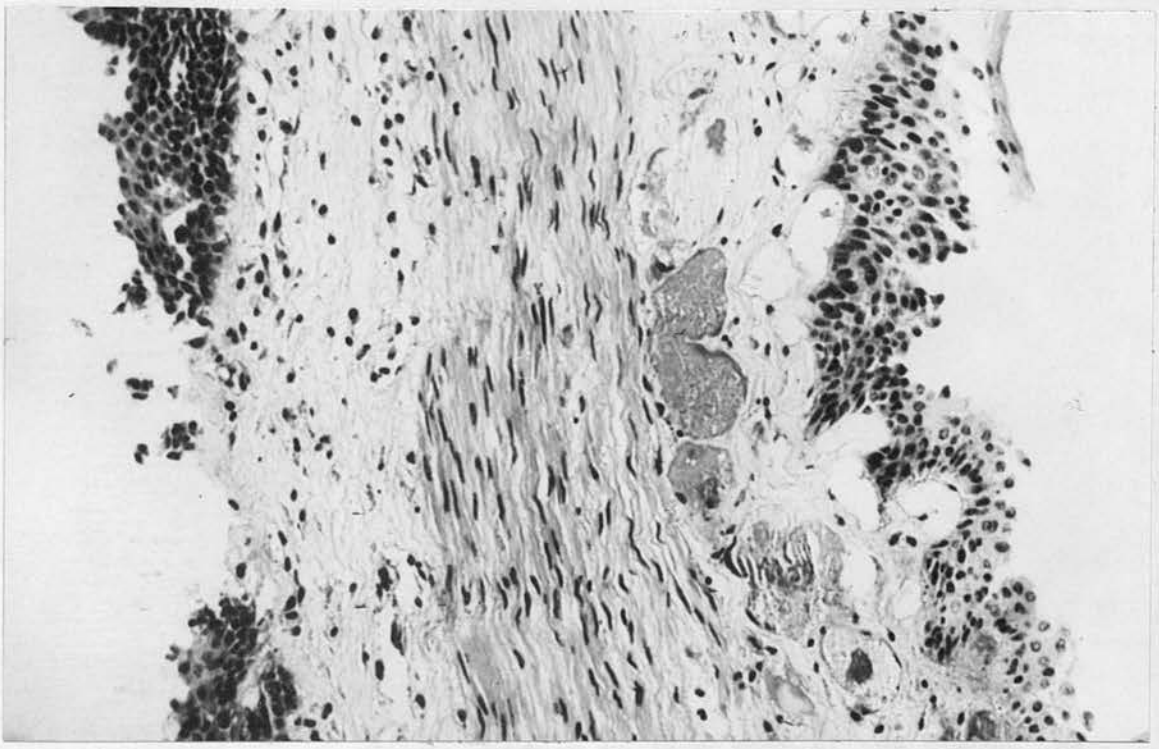


Photo 54. Omasum (epithelial erosion) H & E x 200

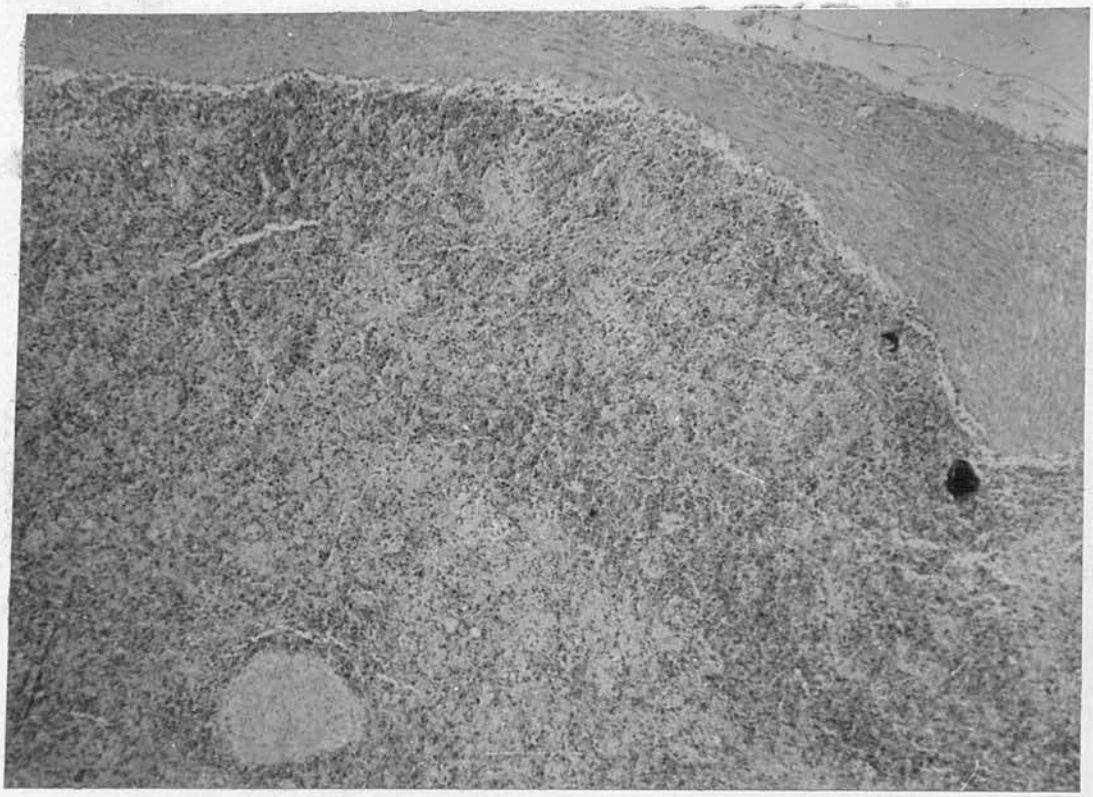


Photo 53. Spleen showing hypoplasia of cellular elements.
H & E x 100.

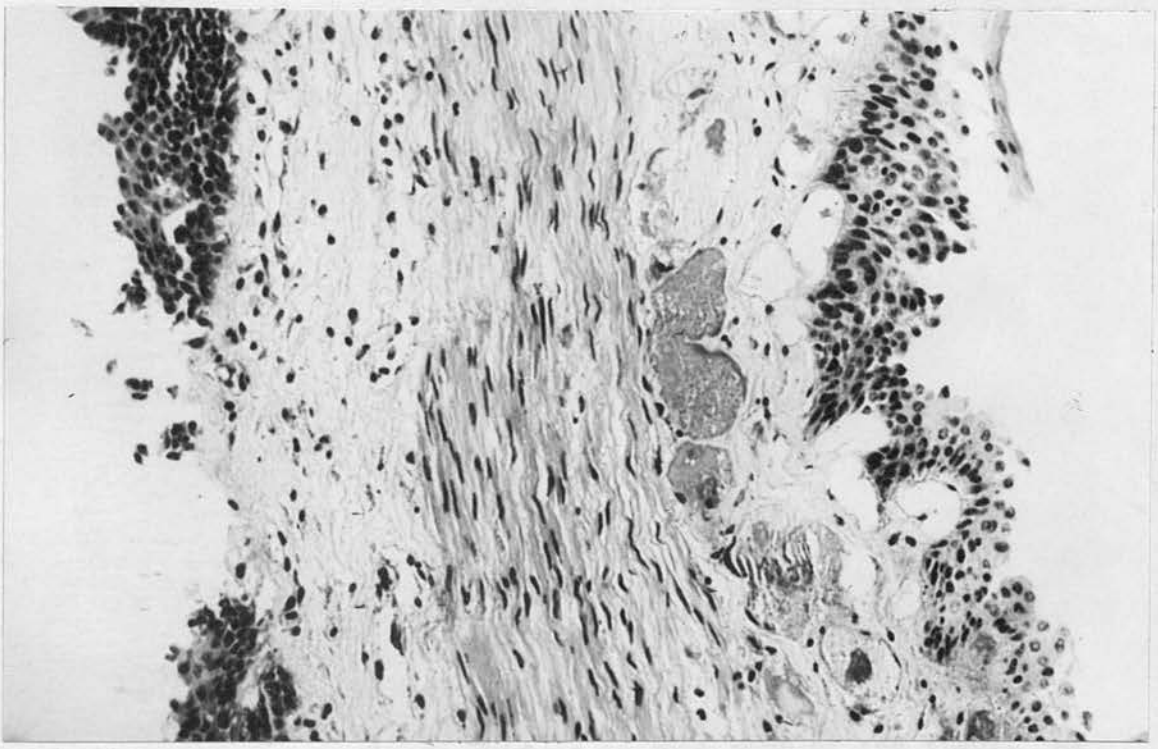


Photo 54. Omasum (epithelial erosion) H & E x 200

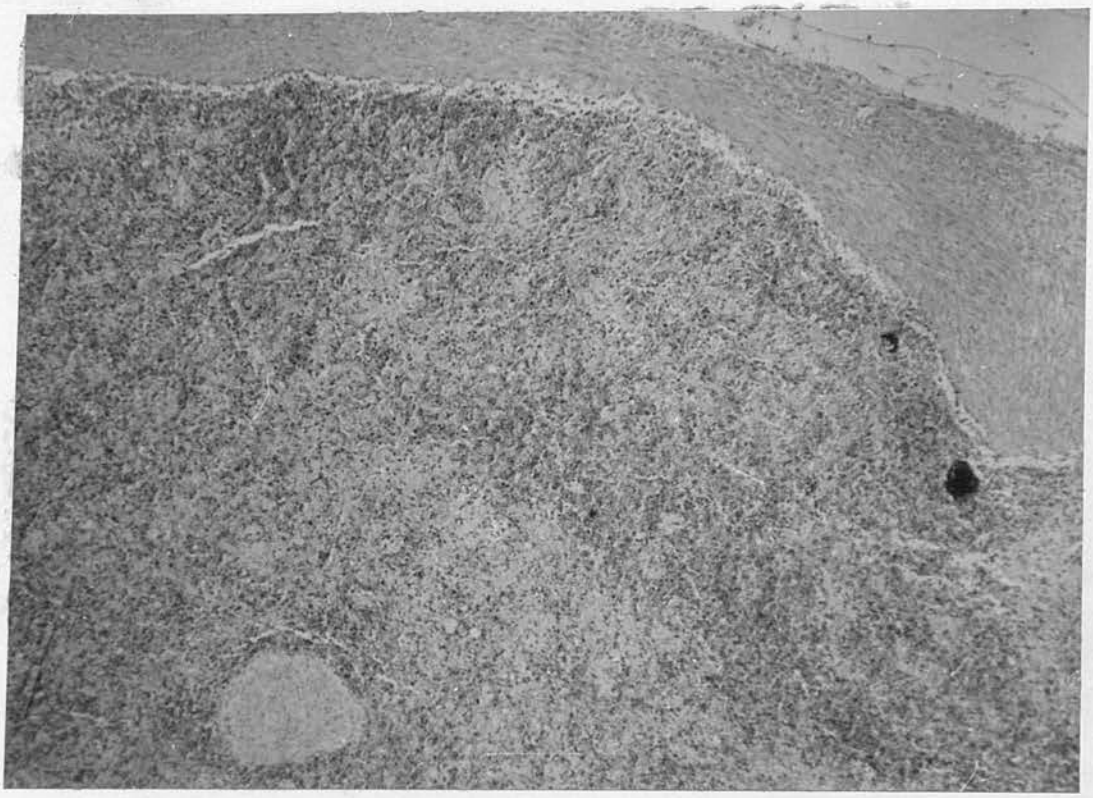


Photo 53. Spleen showing hypoplasia of cellular elements.
H & E x 100.

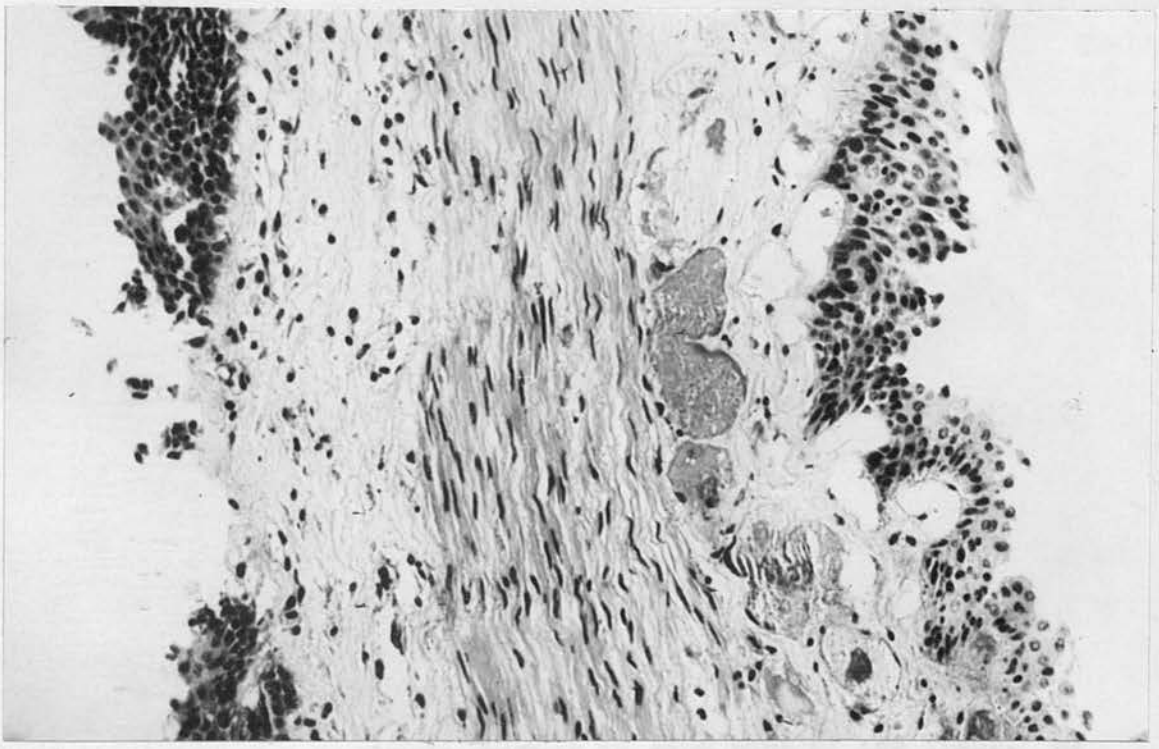


Photo 54. Omasum (epithelial erosion) H & E x 200

Considerable deposits of haemosiderin were always evident and in some specimens large numbers of plasma cells were observed. It was difficult to judge whether the fibrous tissue had increased but in some cases a distinct impression was given to this effect.

c) Bone Marrow

The normal cells were replaced by loose fatty and oedematous connective tissue in which there were a few scattered islands of more normal-looking marrow cells. The sinusoids were very prominent. In some sections and smears there was evidence of fairly large colonies of plasma cells; haemosiderin deposits were also common and often mature eosinophils were seen in more than normal numbers. In some there was complete lack of any cells and no signs of any regeneration: all that was visible was a fatty matrix with enlarged sinusoids.

iv) Alimentary System

a) Gastro-Intestinal Tract

The general effect on the gastrointestinal tract was one of degeneration of the epithelium of the mucosa accompanied by hyperaemia and haemorrhage of the wall. Occasionally a leucocytic reaction was seen but this was uncommon; ulceration was always evident in some part of the tract. By and large the rumen, reticulum and omasum did not show any signs and cellular mitosis was normally progressive in the respective germinative layers of the mucosa. Haemorrhage and oedema occurred

Table LXV

Summary of the Major Histopathological Findings in the Gastrointestinal Tract of Eleven Goats dying after Exposure to X Radiation

Tissue	Lesion	Rumen	Reticulum	Omasum	Abomasum	Small Intestine	Large Intestine
Surface Epithelium	Denudation	NIL	NIL	NIL	5	6	5
	Degeneration	NIL	NIL	NIL	8	9	8
	Metaplasia	NIL	NIL	NIL	5	7	7
	Mitosis	11	11	11	5	6	7
Glandular Epithelium	Denudation	-	-	-	NIL	NIL	NIL
	Degeneration	-	-	-	5	7	5
	Metaplasia	-	-	-	2	3	3
	Mitosis	-	-	-	5	6	6
Connective Tissue	Haemorrhage	2	2	4	11	11	11
	Oedema	2	2	4	8	9	5
	Hyalinization	NIL	1	1	4	7	4
Muscular Tissue	Haemorrhage	NIL	1	1	5	8	5
	Oedema	NIL	1	1	4	8	6
Blood Vessels	Thrombosis	1	NIL	1	8	11	7
	Endothelial proliferation	1	1	3	6	8	3
Serosa	Haemorrhages	5	NIL	NIL	4	8	4
	Desquamation	NIL	NIL	NIL	3	2	1

in the connective tissue of one or two goats but this was exceptional. The abomasum (glandular stomach), small intestine and large intestine showed the greatest histopathological changes and these were very similar in all three viscera. The surface epithelium of the mucosa often showed denudation and degeneration; the glandular epithelium usually an increase of mucin-filled cells, often slight degeneration or nothing at all; there were petechial, ecchymotic and gross haemorrhages into the connective tissue and occasionally into the muscular layers; bacterial colonies were common; blood vessels usually had obliterative thrombi with proliferation and hyalinization of the endothelium and the serosa showed telangiectatic haemorrhages. Generally speaking, the mucosa of the whole tract in some animals presented a very bizarre appearance with atypical epithelial cells, atrophy of villi, syncytial cell-forms and even fibrosis with an increased inter-glandular connective tissue. In others, regeneration had been complete showing a near normal histology.

b) Liver and Gall Bladder

The liver almost invariably showed some changes from a slight hyperaemia and congestion to a frank focal necrosis. Petechial and ecchymotic haemorrhages were common especially under the capsule. Swelling and a granular degeneration

Photo 55. Abomasum (mucosal haemorrhage, thrombosis and degeneration). H & E x 100

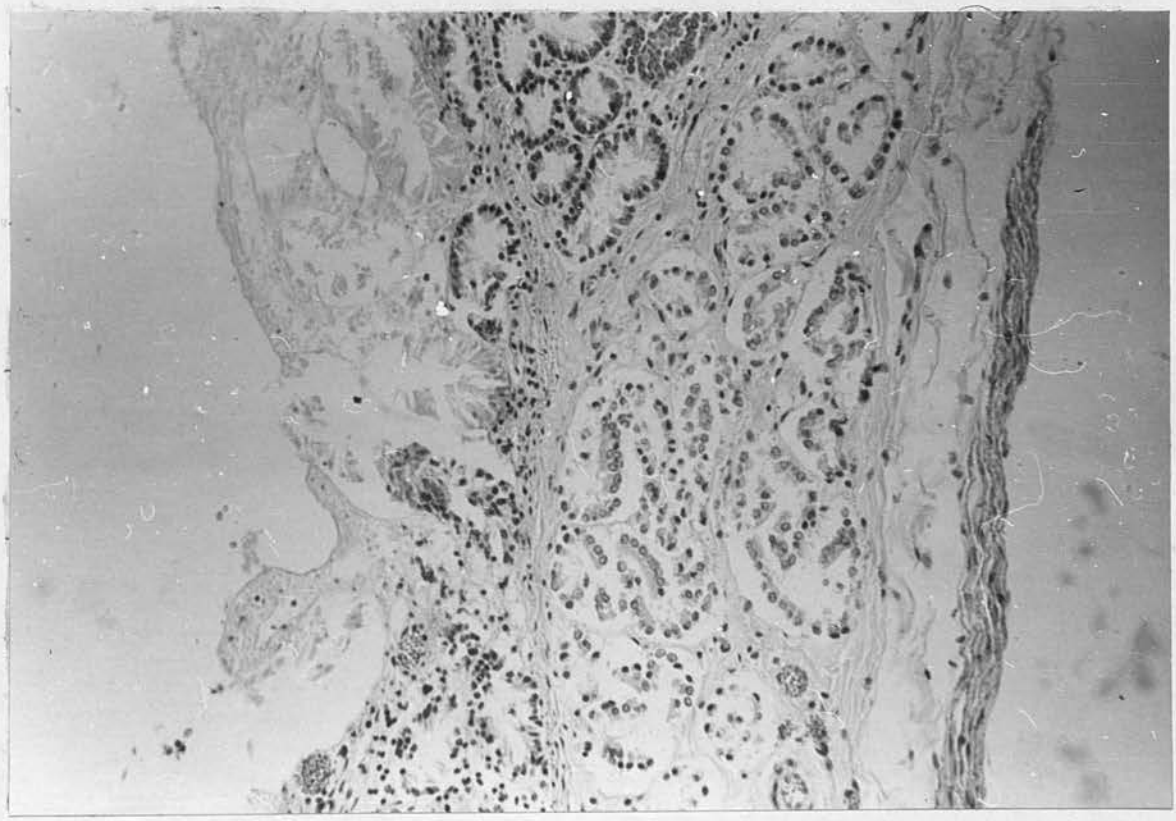


Photo 56. Abomaso-Duodenal Junction showing mucosal degeneration H & E x 250

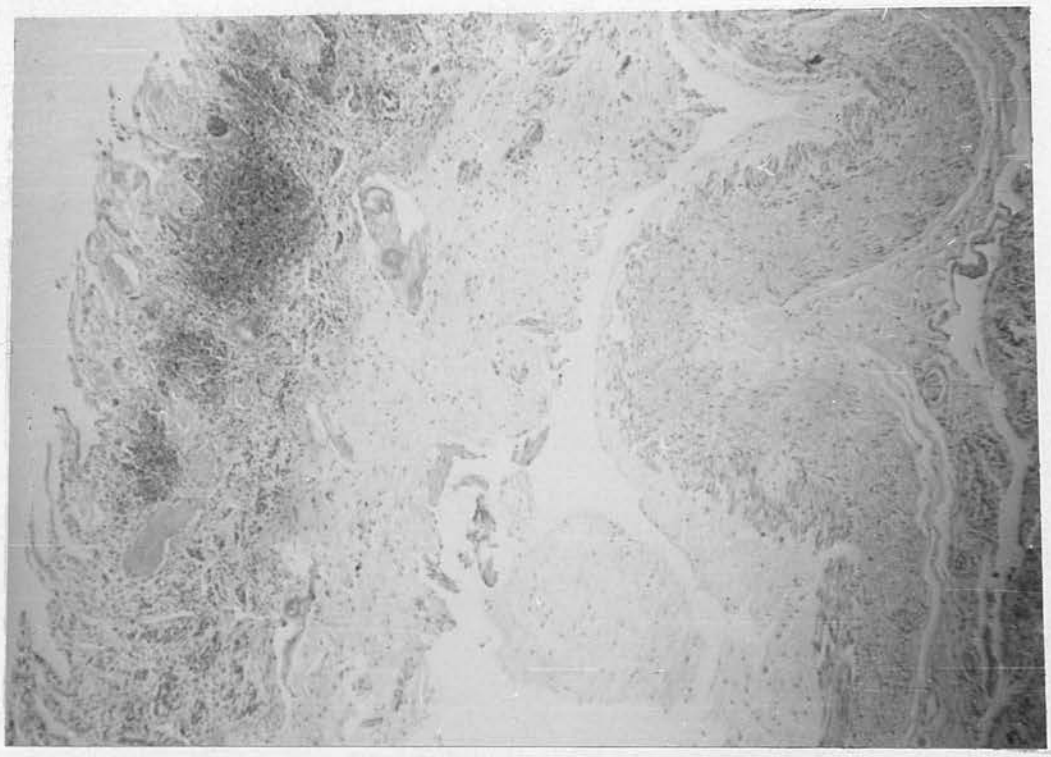


Photo 57. Small Intestine showing mucosal haemorrhage and degeneration
H & E x 180

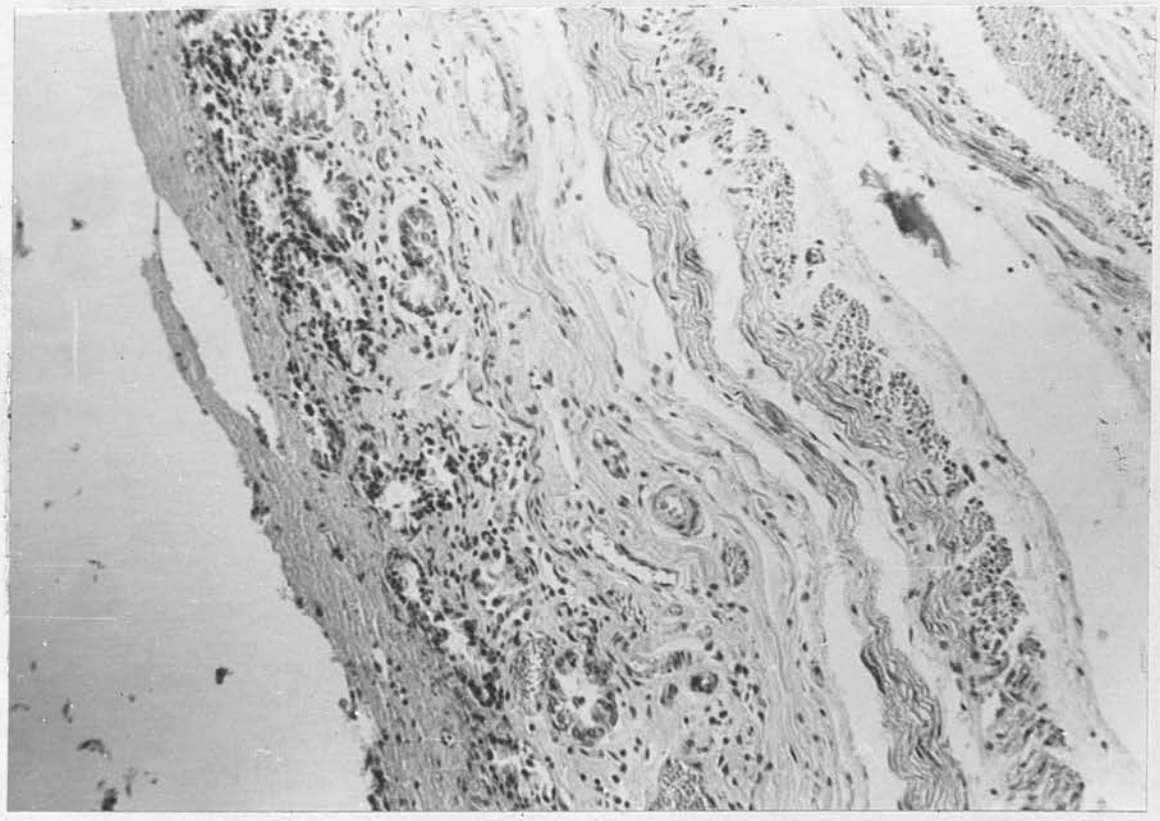


Photo 58. Large Intestine showing mucosal atrophy and degeneration
H & E x 300

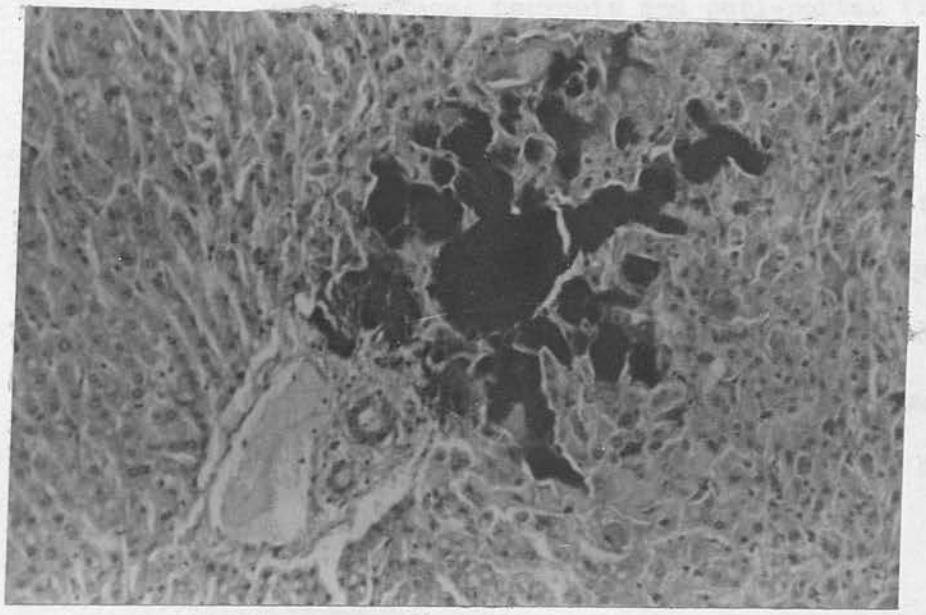


Photo 59. Liver showing focal degeneration. Picro-Mallory (Modified) x 250

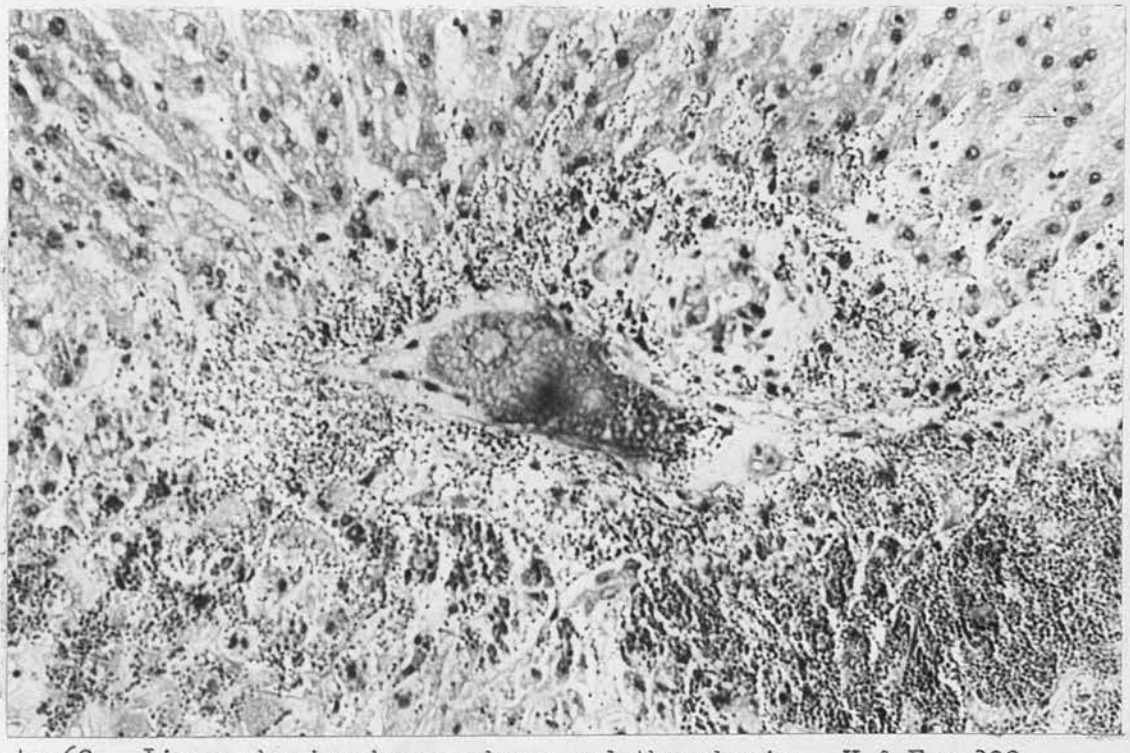


Photo 60. Liver showing haemorrhage and thrombosis. H & E x 300

of the Kupffer cells was very evident in some goats. Focal necrosis and peri-portal fibrosis was also seen. Partial occlusion of the portal and hepatic vessels by particulate complexes (probably leading to ischaemia and thus the prime cause of the necrosis) was commonly observed. In the gall bladder many goats showed oedema and haemorrhage of the epithelium. Calcareous spots were observed in the region of the gall bladder and the pancreas.

c) Pancreas

Seven goats demonstrated petechial and ecchymotic haemorrhages within the substance of the pancreas. Glandular acinous cells were considered to be atrophic and showing degeneration in three animals. Two goats showed calcareous spots within the interstitial tissue.

v) Respiratory System

a) Bronchi and Trachea

Submucosal haemorrhages were common; occasionally ecchymotic haemorrhages were seen in the connective tissue surrounding the cartilage rings. The epithelium often showed metaplasia and the cells were usually cuboidal with or without cilia; there were large numbers of mucus-containing cells and often much cellular desquamation.

b) Lungs

All stages of pulmonary damage were evident: congestion, oedema, focal atalectasis, emphysema, consolidation and haemorrhages. Often patchy atalectasis was found adjacent to areas of

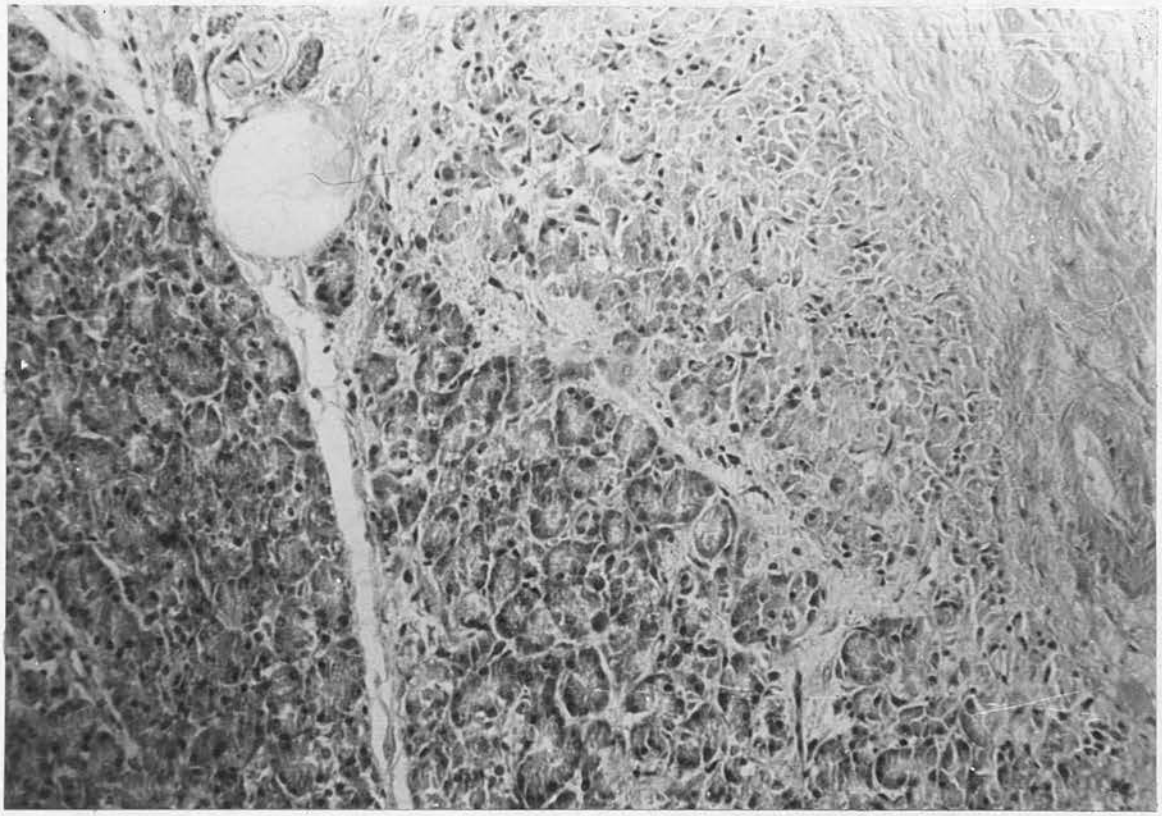


Photo 61. Pancreas (acinous degeneration and haemorrhage) H & E x 200

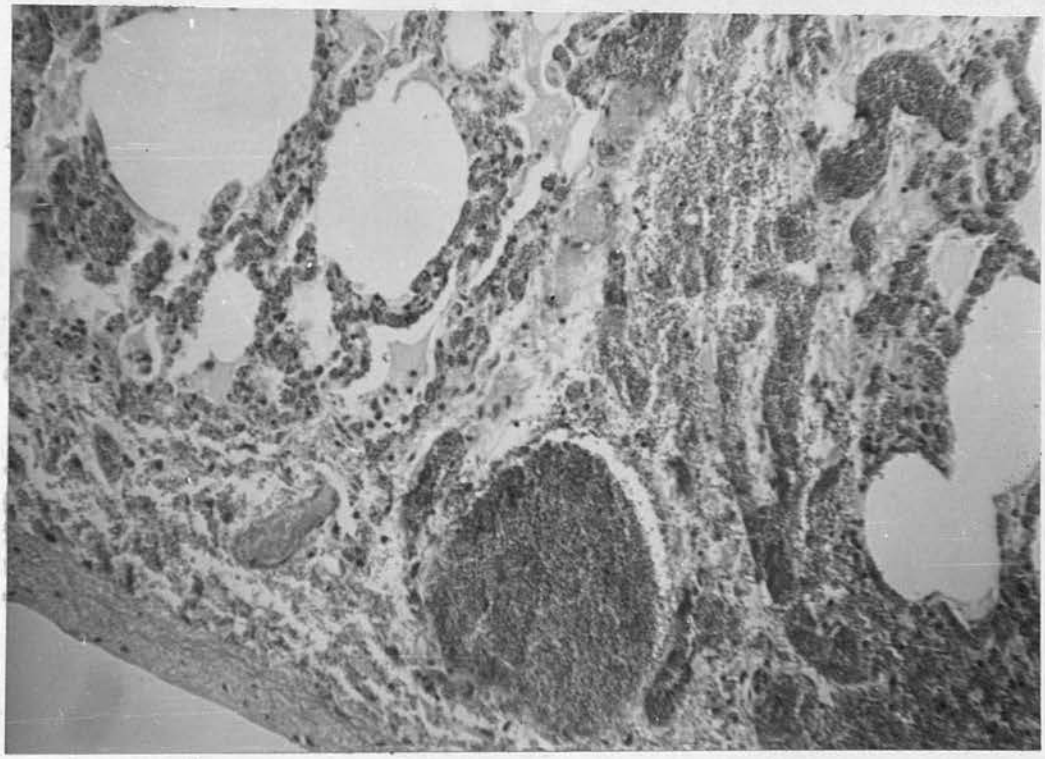


Photo 62. Lung showing haemorrhage emphysema, atalectasis and consolidation. H & E x 100

emphysema. The alveolar lining cells were sometimes bizarre and the presence of a "hyaline membrane" was often well-defined. Colonies of bacteria were often seen in areas of consolidation. Fibrosis was occasionally seen, usually around bronchioles or blood vessels. Partial occlusion of blood vessels by thrombi was often observed. Occasionally focal areas of necrosis were also seen.

vi) Genito-Urinary System

a) Kidney

The general picture in the kidneys was one of haemorrhage and focal degeneration. i) Cortex Congestion, cloudy swelling of the cortical cells, free blood within Bowman's capsule and desquamated cells in the convoluted tubules occurred in many cases. Swelling of Bowman's capsule was often seen adjacent to patches of glomerular atrophy and hyalinization. ii) Medulla Degeneration of tubular epithelium with interstitial proliferation and thickening of blood vessel walls often accompanied massive haemorrhagic lesions. Occasionally colonies of bacteria and small areas of focal necrosis were prominent with an adjacent infarct.

b) The bladder epithelium occasionally showed some swelling and desquamation and over half the cases showed some petechial haemorrhages in the wall accompanied by oedema and general hyperaemia.

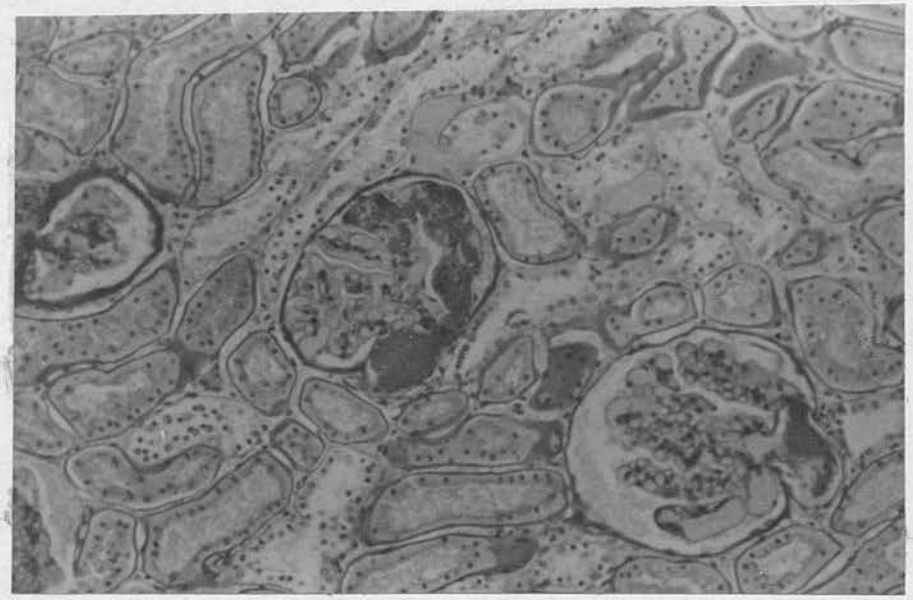


Photo 63. Kidney showing glomerular atrophy and necrotising foci Picro-Mallory (modified). x 180

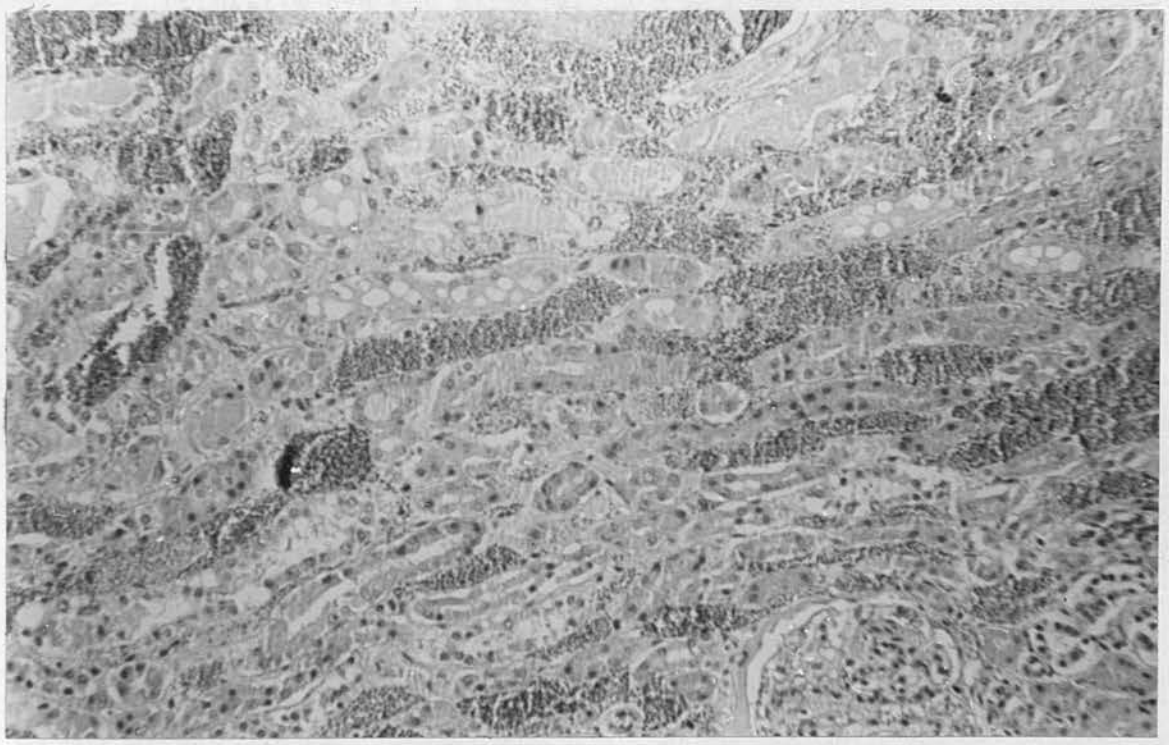


Photo 64. Kidney showing tubular haemorrhage and degeneration H & E x 200

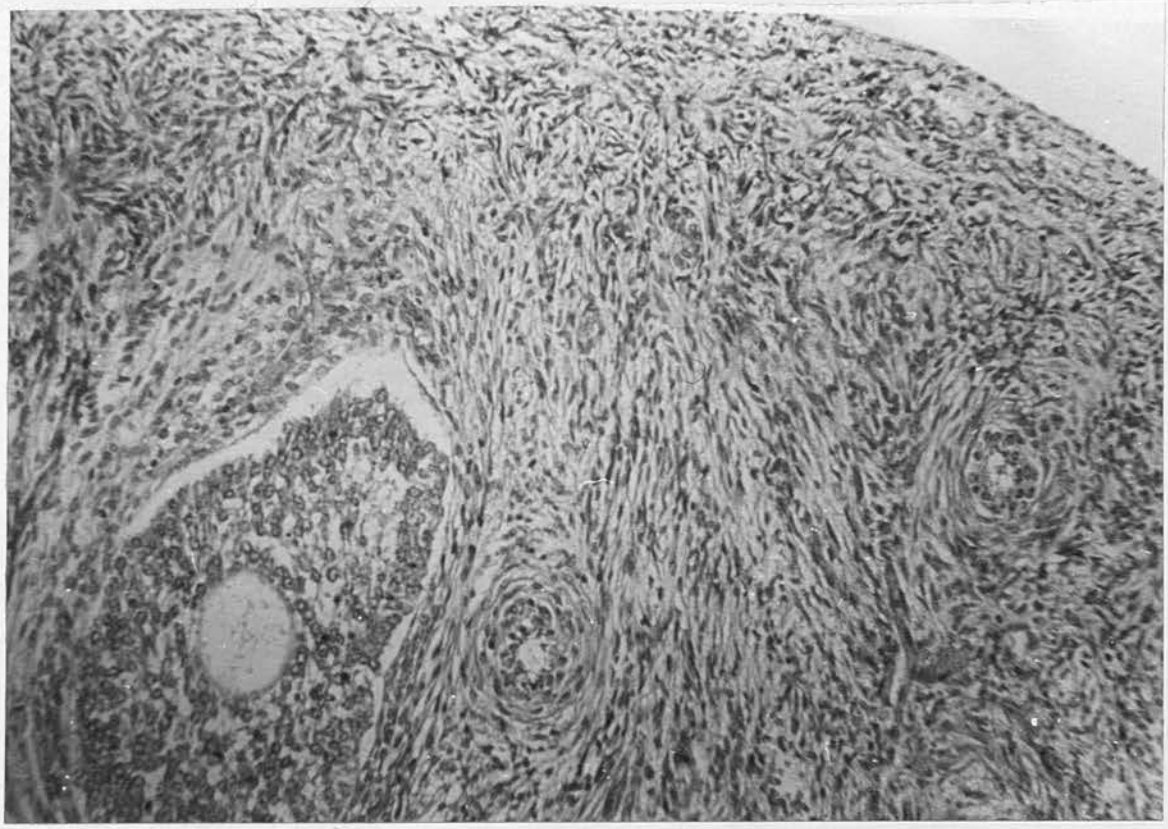


Photo 65. Ovary from goat dying after 13 days (Dose 700r) showing primitive ova and atresia. H & E x 180

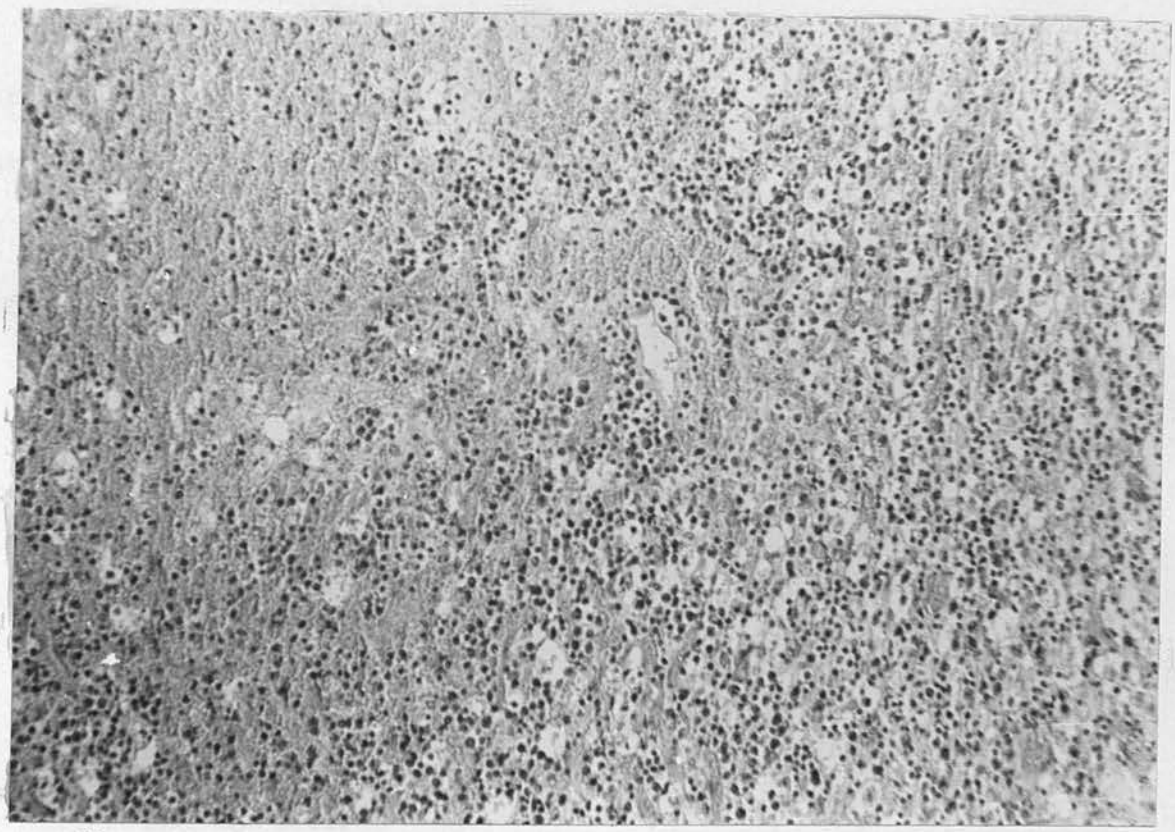


Photo 66. Adrenal showing slight haemorrhage H & E x 200

c) The ovaries of all female goats (seven in all) showed atrophic follicles and normal-looking corpora lutea. Primitive ova, however, were discernible in the dense stroma of six goats. Cysts were seen in four animals. Petechial and ecchymotic haemorrhages were seen in four goats and frank haemorrhages within the parenchyma in two goats.

vii) Endocrine System

All goats showed signs of haemorrhage (petechial or ecchymotic) within the adrenal glands and in four animals cellular degeneration was also discernible.

The thymus was atrophied in all goats.

The thyroid appeared normal in all but one goat in which there was evidence of stimulation with follicles full of colloid.

Pathogenesis of the Major Lesions Observed

To sum up, the microscopic lesions observed probably reflected directly or indirectly the destructive action of the irradiation on the various tissues of the body. These lesions were :-

a) Petechiae which frequently fused to form larger haemorrhages. These petechiae probably developed

i) Mainly from a purpura resulting from a thrombocytopenia

ii) Partly as a result of vasculo endothelial degeneration.

b) Thrombosis probably resulting from a narrowing of the vascular lumina together with the degeneration and proliferative changes of the endothelium. This proliferation leading to thrombus formation may have represented a reparative phenomenon or possibly an immunological response. Infarction was common and the obvious sequel to thrombosis was a focal necrotising degeneration.

c) Haemopoietic degeneration manifested by the development of a panleucopenia and a marrow hypoplasia was pathognomic but frequently its presence was masked by haemorrhages and/or by a secondary bacterial infection. This probably gave rise to the observed enterotoxaemic lesions.

4. Effects of X Irradiation on the Semen of Goats

The object of this study was to discover the effects of whole body X radiation on the semen of goats.

The techniques and procedures adopted have been published already (Wilkins 1963) in a preliminary study undertaken as a pilot experiment on the characteristics of the semen of twelve normal goats of various ages. (Appendix VI

It was found that a wide range of variation existed between the goats and it was therefore considered desirable that in the subsequent experiments involving irradiated goats each animal should act as its own control. In addition, one normal regular control was set up and maintained throughout the experimental period; this control was subjected to semen examination at identical periods as the goats undergoing irradiation i.e. at weekly intervals for one month before irradiation and for nearly six months after irradiation.

A summary of the methods employed is included here to facilitate the interpretation of the tables and graphs.

Collection of Semen

Throughout the study semen collection was effected by the use of a single bipolar rectal electrode with an electrical stimulator which was either mains or battery operated. The semen was collected in a plastic funnel to the end of which was attached a conical graduated tube. (Photos 67 and 68).

The Volume of semen was measured and its general appearance noted; three characteristics were recorded, viz watery, mucinous, or creamy. Viscosity, opacity and motility of the sperm were arbitrarily rated on a scale ranging from 0 to 4 as a maximum.

Live and abnormal sperms were determined from semen smears stained with Nigrosin/Eosin or Ziehl-Neelsen stains.

Sperm concentration was measured using a Neubauer haemocytometer for the counting of the spermatozoa and the pH determined with BDH Marrow Range indicator papers.

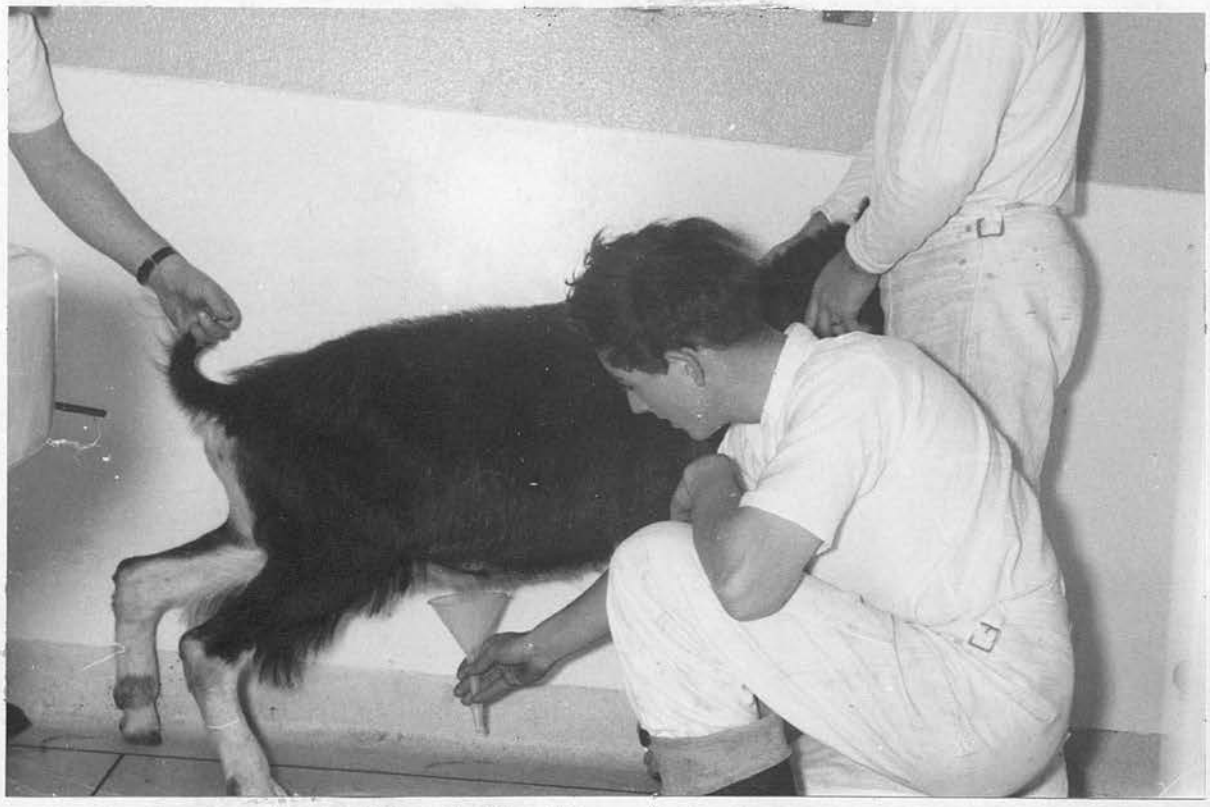


Photo 67. Collection of Semen

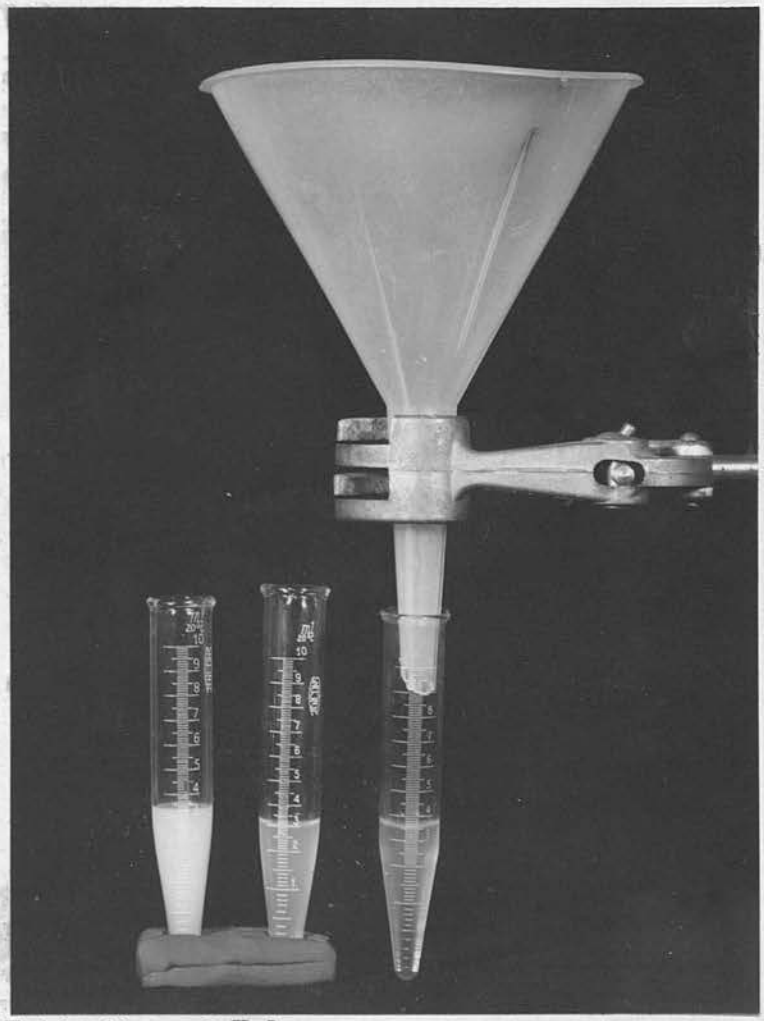


Photo 68. Estimation of Volume and Appearance of Goat Semen

All collections and determinations took place in a warmed laboratory at a temperature not less than 30°C and all apparatus was kept in an incubator at a constant temperature of 37°C. Examinations of the semen were carried out immediately after collection.

The results are dealt with under two main headings, namely,

i) general semen characteristics

ii) the interrelationship of spermatozoa values

One hundred and thirty-three semen samples were examined during the experiment. The distribution of these examinations were as follows:-

Radiation Dose	No. Pre-Irr.	No. Post-Irr.	Total
400r	4	21	25
500r (M3)	4	24	28
500r (M1)	4	23	27
600r	4	21	25
	16	89	105
Control examined at same times	4	24	28
Total irradiated and control	20	113	133

i) General Semen Characteristics

The detailed findings are presented in a series of tables and graphs (Tables LX to LXIV and Figures 37 to 49).

a) Volume

There was a tendency for the quantity of semen ejaculated to increase after the second month both in the control and the irradiated goats. This was probably due to the animals becoming favourably conditioned to the regular weekly electrical stimulus.

b) Appearance

The semen of the irradiated goats tended to be more mucinous and less opaque than in the control goat.

c) Reaction

The pH of the semen in both the control and irradiated goats was usually alkaline.

d) Sperm Motility

Motility of the spermatozoa was generally more sluggish after irradiation with minimum movement seen between the second and fourth months. Motility was usually stronger in creamy semen.

e) Sperm Production

Total sperm production per ajaculate remained at about the same level over the first 2 months after irradiation. Thereafter between the second and fourth months a depression occurred in all irradiated goats; this was followed by an increase during the fifth and sixth months after irradiation, at which time the semen of all irradiated goats appeared normal.

f) Sperm Concentration

In the control goat the sperm concentration showed fairly regular peak values at approximately 10 week intervals but the monthly trend was even. The picture in the irradiated goats was as follows:-

i) In the goat irradiated at 400r there was a severe depression on the first day after irradiation followed by a rise at 1 week and another more dramatic one at 4 weeks; thereafter there was a general depression for the next 8 to 10 weeks. The trend was fairly even for 1½ months after irradiation followed by a severe fall, lowest at 2½ - 3 months.

ii) In the goat irradiated at 500r (M1) there was a severe depression on the first day after irradiation which lasted for 2 weeks after which it rose to a peak at 4 weeks. There was then a general depression for the next 14 weeks followed by a smaller rise which continued to the 22nd week. The monthly trend was a step-like fall for $3\frac{1}{2}$ months after which there was a rise.

iii) In the other goat irradiated at 500r (M3) there was a severe depression on the first day after irradiation followed by a peak rise at 1 week. A depression set in at the 3rd week which lasted for 3 weeks after which there was a gradual rise to a peak values at the 13th and 15th weeks. The monthly trend was a fairly severe fall for $1\frac{1}{2}$ months followed by a very gradual rise over the next $4\frac{1}{2}$ months.

iv) In the goat irradiated at 600r there was a peak rise on the first day after irradiation followed by a severe depression at 1 week which lasted for a week. Another peak rise at 3 weeks was followed by a depression which lasted for 10 weeks. The monthly trend consisted of a small initial rise then a gradual fall over the next 2 months followed by a slow rise over the next 3 months.

g) Live and Abnormal Sperm

The percentage of live to dead sperm began to fall from the day of irradiation in all irradiated goats and the depression was at its lowest level between the second and fourth months after irradiation. The percentage of abnormal sperms was greatest in the goat irradiated at 600r, less in those at 500r and least of all in the goat irradiated at 400r. There was a tendency for tail abnormalities to increase with time. Normality was achieved about the sixth month after irradiation.

Table LX

Effects of X Irradiation on Goat Semen

Control

Period	Volume (ml)	Appearance	Viscosity	Opacity	pH	Sperm Motility	Sperm Conc. (Mils/ml)	Total Sperms (Mils)	Live Sperm (%)	Abnormal Sperms %	
										Head & Neck	Tails
				"PRE-IRRADIATION"							
28 days	2.0	Creamy	0	4	8.2	4	1425	2850	94	2	2
21 days	2.5	"	0	4	8.2	4	1300	3250	87	1	1
14 days	2.0	"	0	4	7.6	4	1300	2600	85	4	16
7 days	2.0	"	0	4	7.6	4	1200	2400	84	3	7
Mean	2.1	-	0	4	7.9	4	1306	2775	87.5	2.5	6.5
				"POST-IRRADIATION"							
1 day	1.5	Creamy	0	4	7.0	4	1665	2500	85	2	4
7 days	2.0	"	0	4	7.3	4	1125	2250	75	3	5
14 days	1.5	"	0	4	7.6	4	1165	1750	65	2	2
21 days	3.0	"	0	3	8.2	3	1000	3000	85	3	3
28 days	3.0	Wat & Cr.	0	4	8.5	2	1015	3245	90	1	3
1st Month	2.4	-	0	3.75	7.9	3.3	1076	2561	79	2.2	3.2
34 days	2.0	Creamy	0	4	8.2	3	800	1600	88	2	4
42 days	3.0	"	0	3	7.6	4	935	2805	80	3	7
49 days	3.0	"	0	4	7.6	4	865	2595	60	4	6
57 days	4.5	Wat & Cr.	0	4	6.7	4	1065	4793	87	1	1
2nd Month	3.1	-	0	3.8	7.5	3.8	916	2948	78.7	2.5	4.5
64 days	3.0	Creamy	0	4	7.0	3	1015	3245	80	1	19
71 days	4.5	Muc & Cr.	1	3	8.2	4	1375	6188	80	3	7
78 days	5.0	Wat & Cr.	0	3	8.2	3	650	3250	90	2	3
84 days	4.5	"	0	3	7.6	4	365	1643	95	1	1
3rd Month	4.2	-	0.25	3.3	7.7	3.5	851	3582	86	1.75	7.5
92 days	3.5	Wat & Cr.	0	3	8.2	2	765	2677	75	4	4
99 days	3.5	"	0	4	7.6	2	630	2205	80	3	3
105 days	4.0	"	0	4	6.7	3	900	3600	55	4	6
112 days	4.0	Creamy	0	4	6.7	3	1000	4000	75	4	4
4th Month	3.75	-	0	3.75	7.6	2.5	819	3120	71	3.75	4.25
119 days	4.0	Creamy	0	4	7.6	4	875	3500	80	3	3
125 days	5.0	"	0	4	7.6	4	980	4900	85	1	3
132 days	3.5	"	0	4	8.0	3	745	2608	90	1	3
140 days	4.0	Wat & Cr.	0	4	7.6	4	830	3320	94	1	2
5th Month	4.1	-	0	4	7.7	3.75	858	3582	87	1.5	2.75
147 days	5.5	Creamy	0	4	8.2	3	1490	8195	85	2	2
154 days	4.0	"	0	4	7.6	4	1250	5000	54	2	8
161 days	4.0	"	0	3	7.0	3	625	2500	93	1	2

Table LXI — Effects of X Irradiation on Goat Semen

Goat M 10 — Dose 400r

Period	Volume (ml)	Appearance	Viscosity	Opacity	pH	Motility of Sperm	Sperm Conc. (Mils per ml)	Total Sperms (Mils)	Live Sperms %	Abnormal Sperms % Head & Neck	Abnormal Sperms % Tails
				"PRE-IRRADIATION"							
30 days	2.0	Creamy	0	4	7.0	4	430	860	86	4	2
22 days	2.0	"	0	4	6.7	4	1340	2680	67	13	2
14 days	2.5	"	0	4	7.0	4	960	2400	75	5	5
7 days	2.0	"	0	3	6.4	2	750	1500	79	5	5
Mean	2.1	-	0	3.75	6.8	3.5	870	1860	77	6.75	3.5
				"POST-IRRADIATION"							
1 day	1.0	Watery	0	0	8.2	4	150	150	85	1	1
8 days	1.25	Creamy	0	4	7.9	4	1000	1250	91	6	1
15 days	1.0	Watery	0	1	8.2	0.5	22	22	18	21	19
21 days	4.0	Creamy	0	4	7.6	4	1160	4640	99	1	1
28 days	4.0	"	0	2	6.7	4	1600	6400	93	2	2
1st Month	2.6	-	0	2.8	7.6	3.1	945	1578	75	7.5	5.75
35 days	4.0	Creamy	0	3	6.7	4	1160	4640	90	3	2
39 days	3.0	"	0	2	8.2	4	1240	3720	80	4	3
49 days	3.0	"	0	1	8.2	4	420	1260	60	4	4
57 days	4.5	Watery	0	1	8.5	0.5	300	1350	3	4	4
2nd Month	3.6	-	0	1.8	7.9	3.1	780	2742	58	3.75	3.25
64 days	3.75	Watery	0	3	8.2	1.0	230	863	26	4	4
71 days	4.5	"	0	3	8.2	1.5	300	1350	15	4	2
78 days	3.25	"	0	0	8.2	NIL	NIL	NIL	NIL	NIL	NIL
86 days	2.0	"	0	0	8.2	1.0	10	20	50	2	4
3rd Month	3.4	-	0	1.5	8.2	0.9	135	558	23	2.5	2.5
92 days	2.5	Creamy	0	4	8.5	4	340	850	53	1	2
101 days	2.5	"	0	4	7.3	4	1150	2875	85	2	6
105 days	3.75	"	0	4	6.7	4	1400	5250	62	2	2
113 days	2.75	Watery	0	0	8.2	NIL	14	38.5	25	31	7
4th Month	2.9	-	0	3	7.7	3	726	2253	56	9	4.2
120 days	3.0	Watery	0	1	8.2	1	87	260	40	18	7
127 days	4.5	Creamy	0	4	8.2	2.5	510	2295	73	1	72
134 days	6.5	Watery	0	3	8.2	4	420	2730	85	1	2
140 days	5.25	Creamy	0	4	6.7	4	2000	10,500	58	0	36
5th Month	4.8	-	0	3	7.8	2.9	754	3946	64	5	29

Table LXII — Effects of X Irradiation on Goat Semen

Goat M 3 — Dose 500r

Period	Volume (ml)	Appearance	Viscosity	Opacity	pH	Motility of Sperm	Sperm Conc. (Mils/ml)	Total Sperms (Mils)	Live Sperms %	Abnormal Sperms %	
										Head & Neck	Tails
				"PRE-IRRADIATION"							
28 days	2.5	Creamy	0	4	8.2	2.0	1920	4800	82	9	0
21 days	2.0	"	0	4	8.2	4.0	1800	3600	86	7	1
14 days	2.2	"	0	4	8.2	3.0	980	2156	90	5	2
7 days	2.5	"	0	4	8.2	4.0	1260	3150	85	6	1
Mean	2.3	-	0	4	8.2	3.3	1490	3427	86	6.75	1
				"POST-IRRADIATION"							
1 day	1.5	Mucinous	4	0	8.5	0.5	3	4.5	1	20	27
6 days	1.6	Creamy	0	4	8.2	3.0	2080	3328	85	7	3
14 days	2.0	Mucinous	2	4	7.6	3.0	1840	3680	60	15	5
21 days	2.5	"	4	1	8.2	1.0	9.5	23.75	88	10	4
28 days	1.75	"	3	2	7.6	2.0	115	201.25	84	12	1
1st Month	1.96	-	2.25	2.75	7.9	2.25	1011	1808	79	11	3.25
35 days	2.0	Mucinous	2	2	8.0	1.0	125	250	85	7	3
39 days	1.75	"	4	0	8.2	1.0	40	70	85	5	2
49 days	1.75	"	4	3	8.2	2.0	400	700	93	2	2
56 days	4.5	Watery	0	2	8.5	2.0	200	900	80	10	5
2nd Month	2.5	-	2.5	1.75	8.2	1.5	191	480	86	6	3
63 days	3.0	Watery	0	3	8.2	2.0	260	780	85	7	4
69 days	3.0	Creamy	0	4	8.2	2.0	500	1500	85	6	4
74 days	3.75	"	0	4	8.5	3.5	520	1930	80	3	13
81 days	3.0	Mucinous	3	4	7.9	2.0	660	1980	79	1	2
3rd Month	3.2	-	0.75	3.75	8.2	2.4	485	1547	82	4.25	5.75
91 days	2.75	Creamy	0	4	6.7	4.0	1180	3245	96	1	2
99 days	2.5	Mucinous	4	0	8.2	0.5	8	20	84	2	13
105 days	3.0	"	2	4	7.3	4.0	800	2400	93	0	1
110 days	2.5	"	2	3	8.2	3.0	210	525	90	0	3
4th Month	2.7	-	2	2.75	7.6	2.9	550	1548	91	0.75	4.75
117 days	1.75	Watery	0	2	8.2	4.0	220	385	96	0	0
124 days	2.5	Mucinous	2	2	7.9	2.0	500	1250	92	1	1
132 days	3.0	Creamy	0	4	6.5	4.0	1300	3900	83	0	2
140 days	5.5	Wat & Cr.	0	3	6.7	4.0	650	3575	80	0	1
5th Month	3.2	-	0.5	2.75	7.3	3.5	668	2278	88	0.25	1
148 days	4.25	Wat & Cr.	0	4	6.7	3.0	550	2337.5	68	1	1
154 days	4.5	"	0	4	7.0	3.0	555	2500	85	1	2
161 days	4.5	Muc & Cr.	1	4	7.3	4.0	500	2250	94	1	1

Table LXIII — Effects of X Irradiation on Goat Semen

Goat M 1 — Dose 500r

Period	Volume (ml)	Appearance	Viscosity	Opacity	pH	Sperm Motility	Sperm Conc. (Mils/ml)	Total Sperms (Mils)	Live Sperm %	Abnormal % Head & Neck	Abnormal % Tails
				"PRE-IRRADIATION"							
30 days	2.0	Creamy	0	4	6.7	1.5	650	1300	94	1	2
21 days	2.0	"	0	4	6.7	3.0	710	1420	90	2	2
14 days	1.75	"	0	4	6.7	2.0	750	1312	95	2	3
7 days	2.5	"	0	4	7.0	1.5	600	1500	85	3	4
Mean	2.1	-	0	4	6.8	2.0	677	1383	91	2	2.75
				"POST-IRRADIATION"							
1 day	1.5	Watery	0	1	8.5	0.5	9	13.5	5	13	19
9 days	2.5	Watery	0	0	7.9	0.5	11	27.5	10	9	13
16 days	2.2	"	0	0	8.2	2.0	15	33	94	1	1
23 days	3.0	"	0	3	6.5	3.0	150	450	70	6	4
30 days	3.0	Creamy	0	4	7.3	4.0	660	1980	91	2	5
1st Month	2.7	-	0	1.75	7.5	2.4	209	622.5	66	4.5	5.75
37 days	3.0	Creamy	0	4	7.9	3.0	325	975	79	4	4
45 days	3.0	Wat. & Cr.	0	3	8.2	2.5	210	630	79	3	4
49 days	3.5	"	0	2	8.2	2.0	150	525	75	10	15
56 days	3.0	"	0	2	8.2	1.5	160	480	70	10	20
2nd Month	3.1	-	0	2.75	8.1	2.25	211	652	76	6.75	10.75
66 days	2.75	Mucinous	2	0	8.2	0.5	43	118	64	8	38
73 days	1.5	Muc. & Cr.	2	2	8.2	NIL	NIL	NIL	NIL	NIL	NIL
81	2.0	Muc & Cr.	1	1	8.2	NIL	NIL	NIL	NIL	NIL	NIL
87 days	2.5	"	1	1	8.2	NIL	7	17.5	38	10	14
3rd Month	2.2	-	1.5	1.0	8.2	0.1	12.5	34	25	4.5	13
94 days	2.5	Muc & Cr.	1	1	8.2	NIL	6	15.0	25	17	8
101 days	2.0	Mucinous	3	1	8.5	NIL	NIL	NIL	NIL	NIL	NIL
109 days	2.0	"	1	1	8.5	NIL	15	30	25	18	12
116 days	2.0	Watery	0	1	8.5	NIL	14	28	10	15	10
4th Month	2.1	-	1.25	1	8.4	NIL	8.8	18	15	12.5	7.5
123 days	2.5	Watery	0	1.5	8.2	1.0	100	250	65	3	5
130 days	3.5	Muc & Cr.	2	2	8.2	1.5	140	490	72	1	3
137 days	4.25	"	1	3	8.2	4.0	370	1572	83	1	2
142 days	3.5	"	1	3	8.2	4.0	250	875	80	1	2
5th Month	3.4	-	1	2.4	8.2	216	215	797	75	1.25	3
147 days	3.0	Muc & Cr.	1	4	8.2	4.0	915	2745	90	2	2
156 days	2.5	Wat & Cr.	0	4	8.2	4.0	1100	2750	88	1	2

Table LXIV

Effects of X Irradiation on Goat Semen

Goat No. M 9

Dose 600r

Period	Volume (Ml)	Appearance	Viscosity	Opacity	pH	Sperm Motility	Sperm Conc. (Mils/ml)	Total Sperms (Mils)	Live Sperm %	Abnormal %	
										Head & Neck	Tails
					"PRE-IRRADIATION"						
29 days	1.4	Watery	1	0	8.5	1.5	100	140	65	8	7
22 days	1.1	"	0	0	8.5	1.0	75	82.5	65	7	8
14 days	1.4	"	1	0	8.5	1.0	50	70	60	3	3
6 days	2.5	"	1	0	8.5	1.0	100	250	60	6	4
Mean	1.6	-	0.75	0	8.5	1.1	81	136	62.5	6	5.5
					"POST-IRRADIATION"						
1 day	2.0	Creamy	0	4	8.2	2.0	790	1580	29	54	1
8 days	2.5	Watery	0	0	8.3	0.5	2.5	6.25	10	50	15
15 days	2.5	Mucinous	1	1	8.3	0.5	6.0	15	5	55	15
22 days	2.75	Creamy	0	3	8.2	4.0	720	1980	77	0	2
29 days	2.5	Mucinous	3	1	8.2	NIL	160	400	NIL	51	5
1st Month	2.5	-	1	1.25	8.2	1.25	222	600	23	39	9.25
36 days	2.5	Mucinous	3	1	8.2	NIL	130	325	10	45	5
42 days	2.5	"	3	1	8.2	NIL	86	215	5	50	10
50 days	3.5	"	3	1	8.2	NIL	51	178.5	NIL	29	14
57 days	2.5	"	3	0	8.2	NIL	4.5	11.25	NIL	25	10
2nd Month	2.75	-	3	0.75	8.2	NIL	68	182	3.75	37.3	9.7
64 days	3.75	Mucinous	3	0	8.2	0.5	3	11.25	22	5	7
70 days	2.5	"	2	2	8.2	NIL	NIL	NIL	NIL	NIL	NIL
77 days	2.5	"	3	1	8.2	NIL	NIL	NIL	NIL	NIL	NIL
85 days	2.5	"	4	0	8.5	NIL	NIL	NIL	NIL	NIL	NIL
3rd Month	2.8	-	3	0.75	8.3	0.1	0.75	2.8	5.5	1.25	1.75
94 days	5.25	Muc & Cr.	2	3	8.5	1.0	230	1207.5	20	3	9
100 days	2.5	"	2	2	8.2	1.0	80	200	25	2	3
107 days	3.0	"	2	2	8.2	3.0	370	1110	90	1	1
115 days	5.75	"	4	2	8.5	3.0	110	632.5	78	3	3
4th Month	4.1	-	2.5	2.25	8.4	2.0	197.5	787.5	53.25	2.25	4.0
121 days	4.0	Wat & Cr.	0	1	8.5	0.5	30	120	20	2	0
126 days	3.5	"	0	2	8.5	1.0	150	525	35	1	2
134 days	5.0	"	0	1	8.5	1.0	140	700	52	2	3
142 days	4.5	Muc & Cr.	4	3	8.8	2.5	210	945	90	1	1
5th Month	4.3	-	1.0	1.75	8.6	1.25	132.5	572.5	49.25	1.5	1.5

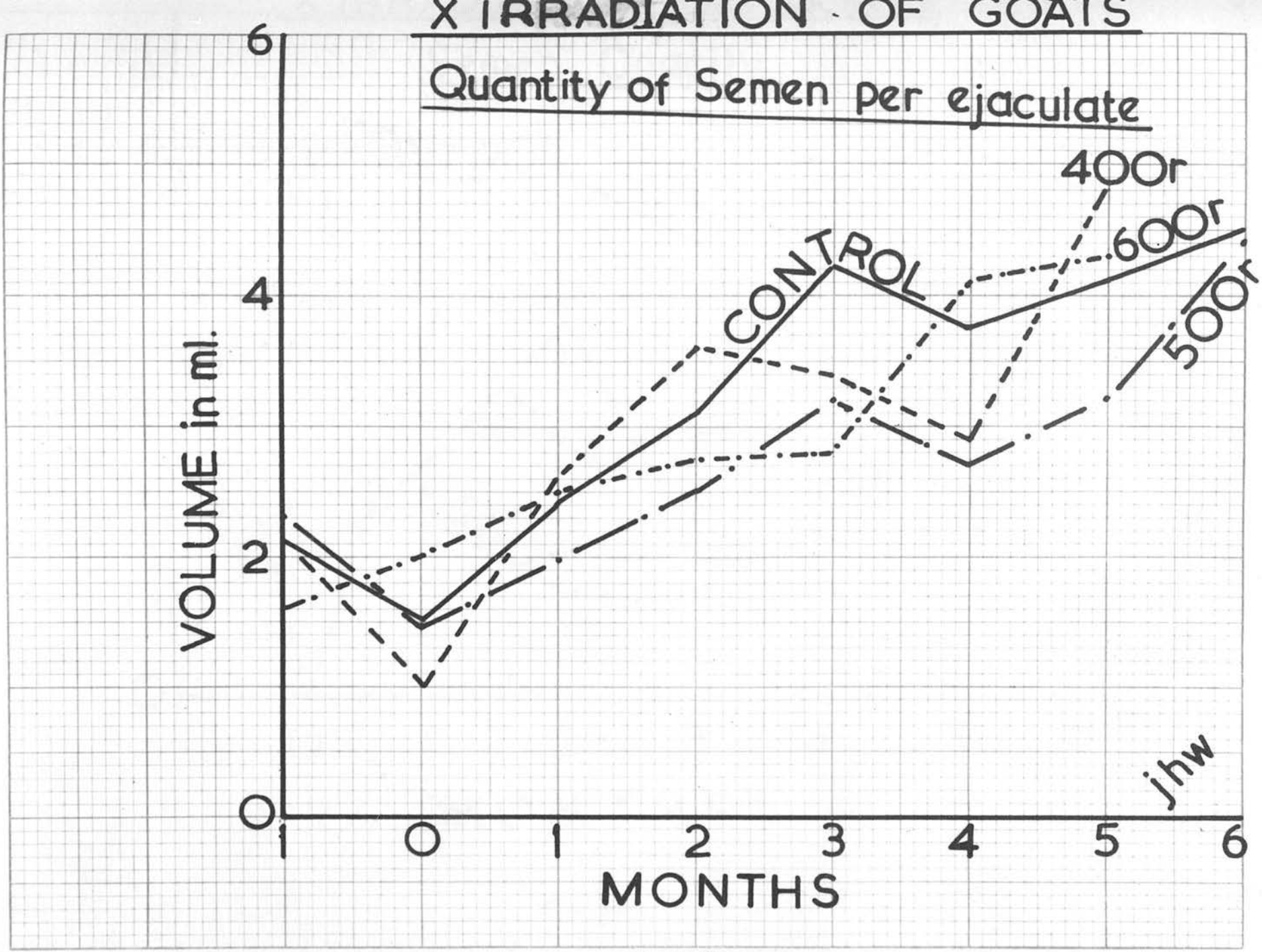

X IRRADIATION - OF GOATS
Quantity of Semen per ejaculate
400r
600r
500r
CONTROL
VOLUME in ml.
6
4
2
0
1
0
1
2
3
4
5
6
MONTHS
jhw

Fig. 37

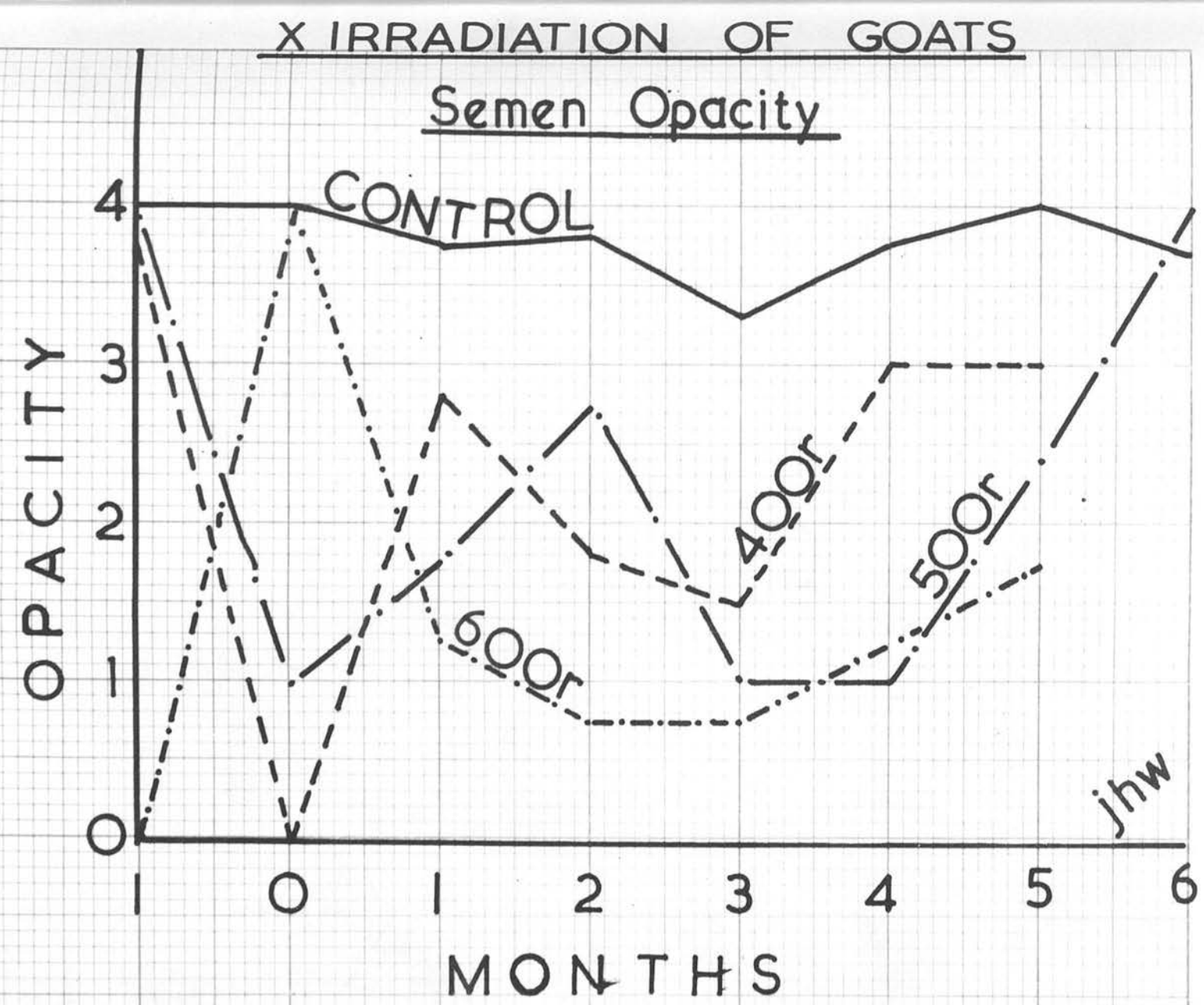

X IRRADIATION OF GOATS
Semen Opacity
CONTROL
400r
500r
600r
jhw
OPACITY
4
3
2
1
0
1 0 1 2 3 4 5 6
MONTHS

Fig. 38

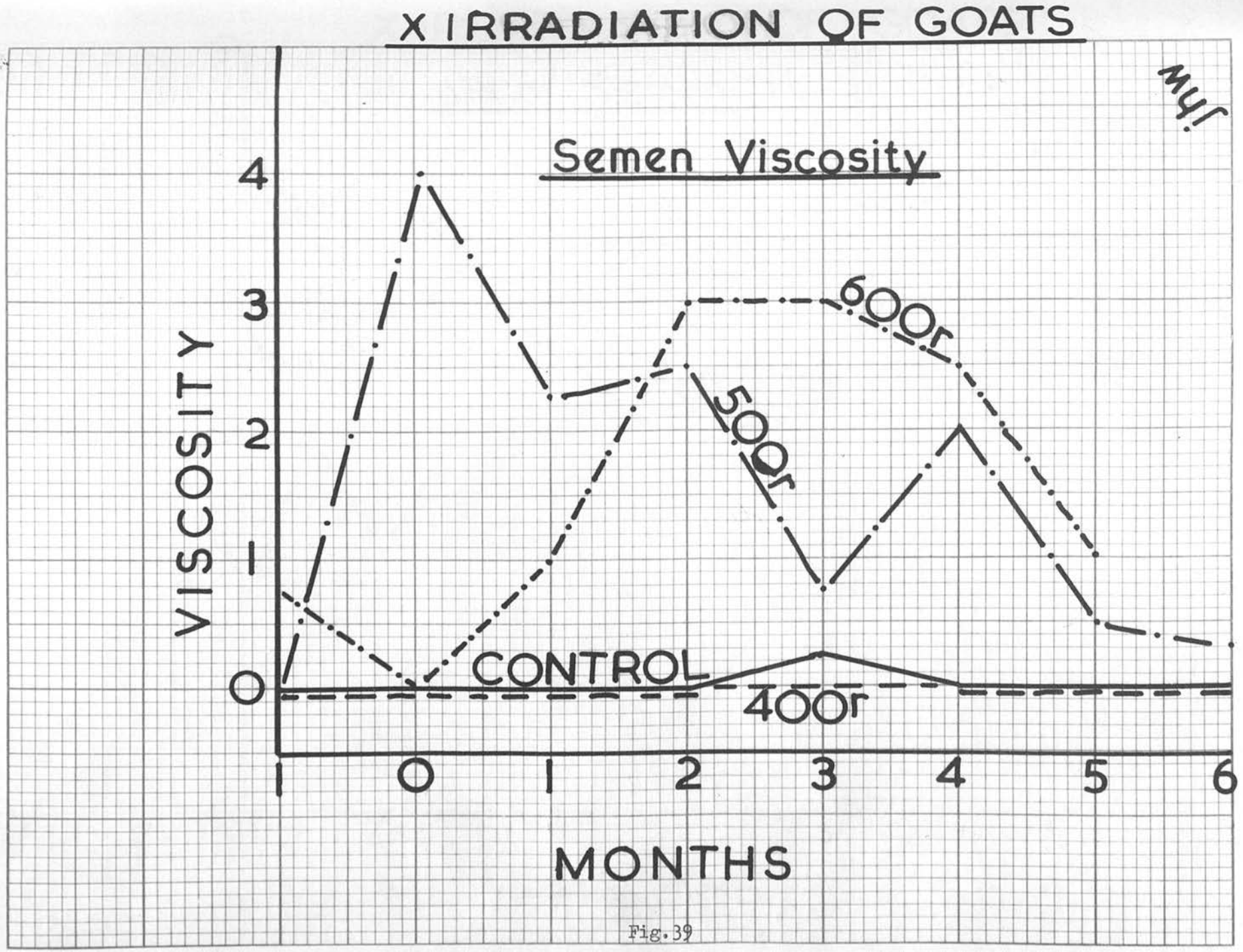
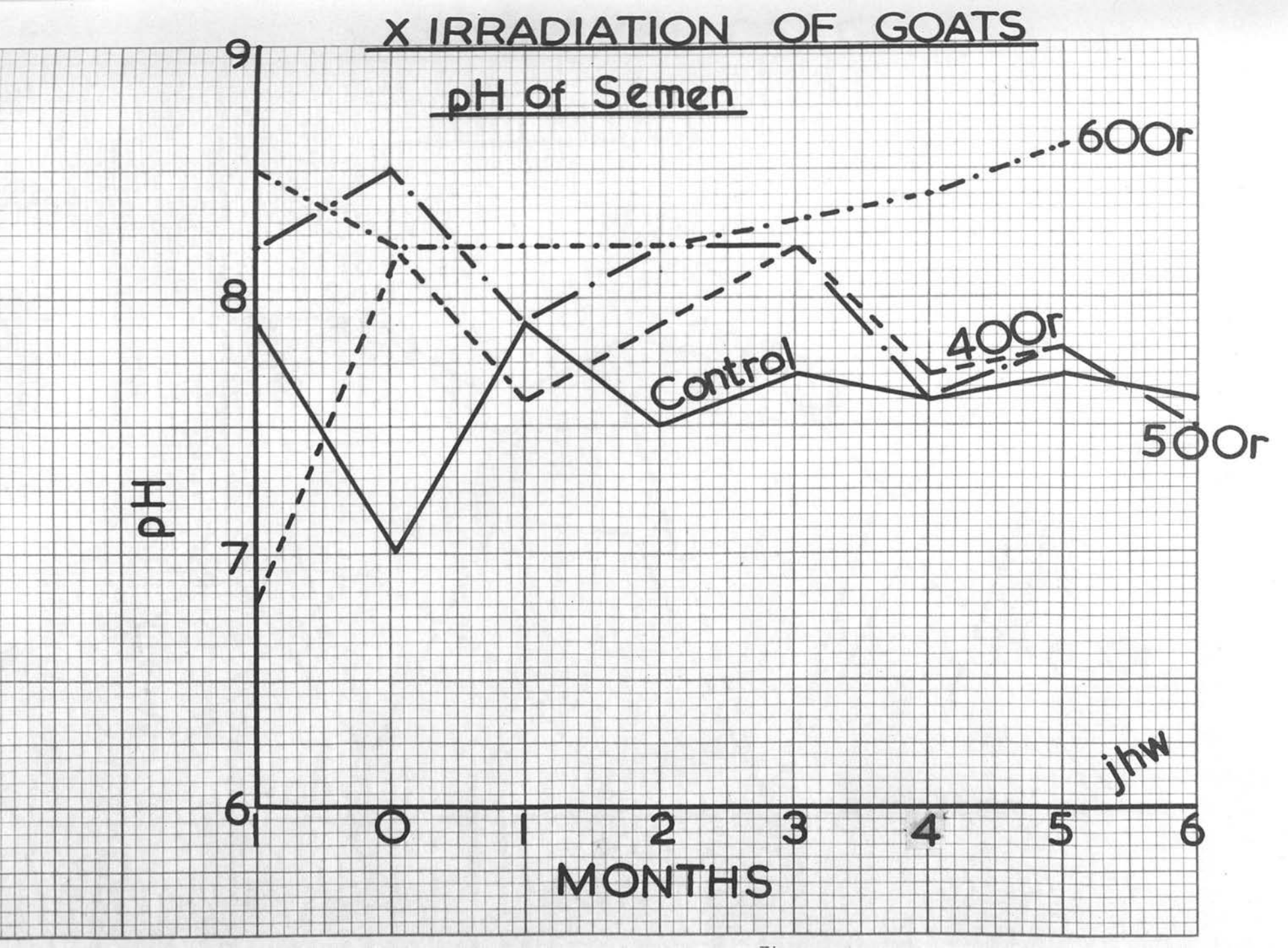

X IRRADIATION OF GOATS
Semen Viscosity
VISCOSITY
4
3
2
1
0
600r
500r
CONTROL
400r
1
0
1
2
3
4
5
6
MONTHS

Fig.39


X IRRADIATION OF GOATS
pH of Semen
600r
400r
Control
500r
pH
9
8
7
6
0
1
2
3
4
5
6
MONTHS
jhw

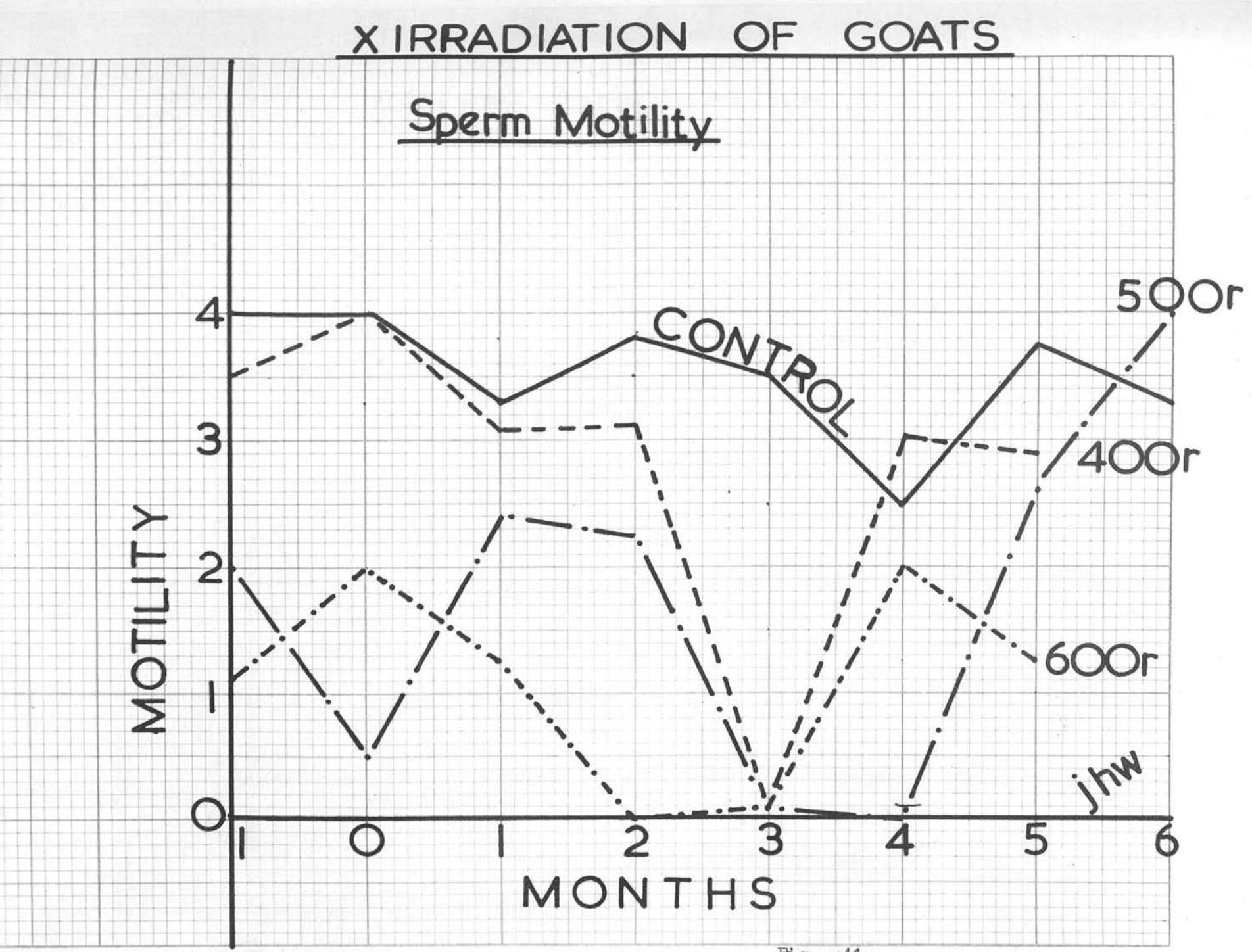

X IRRADIATION OF GOATS
Sperm Motility
CONTROL
500r
400r
600r
jhw
MOTILITY
4
3
2
1
0
1 0 1 2 3 4 5 6
MONTHS

Fig. 41

SPERM CONCENTRATION

Fig. 43

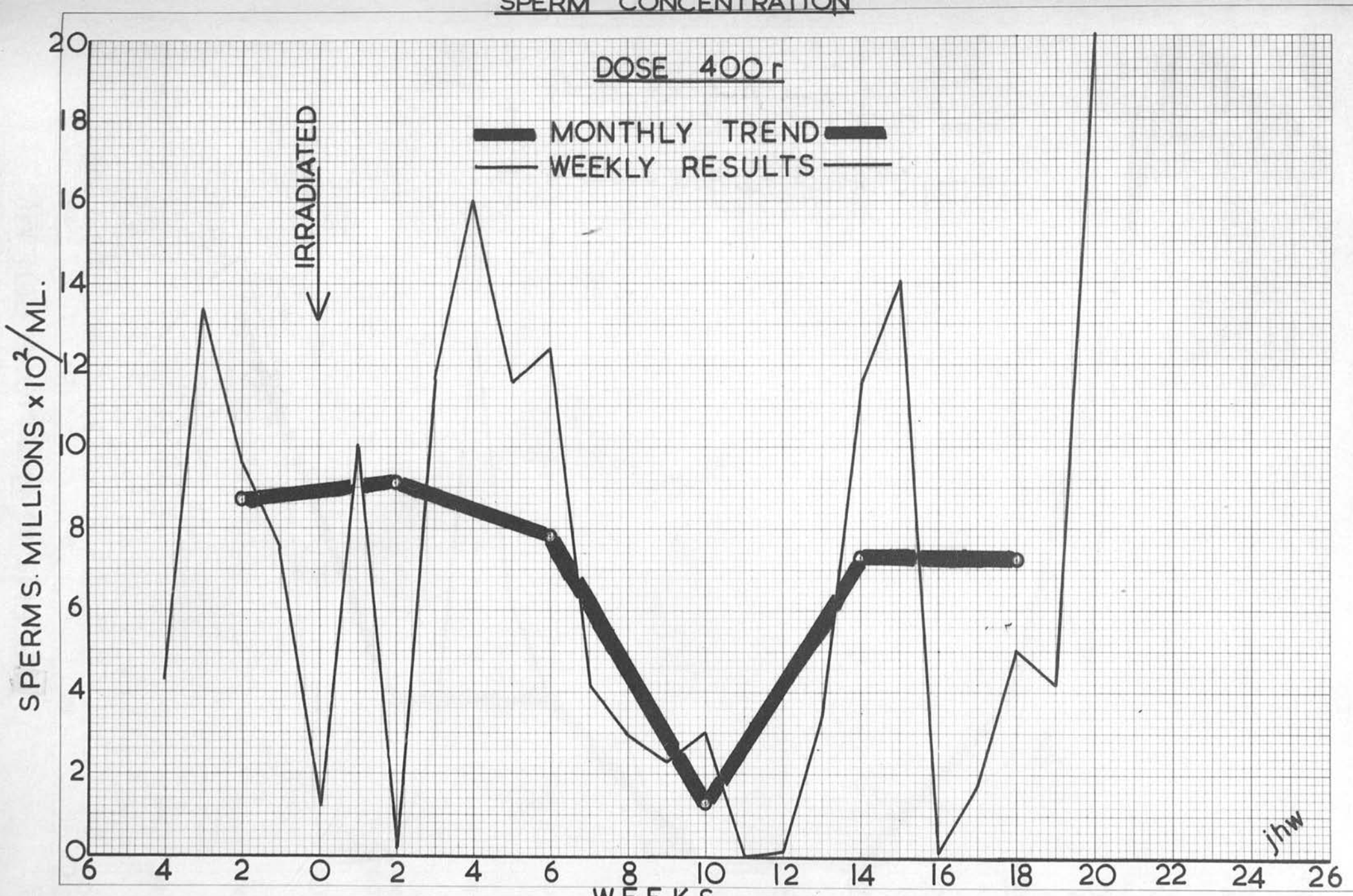
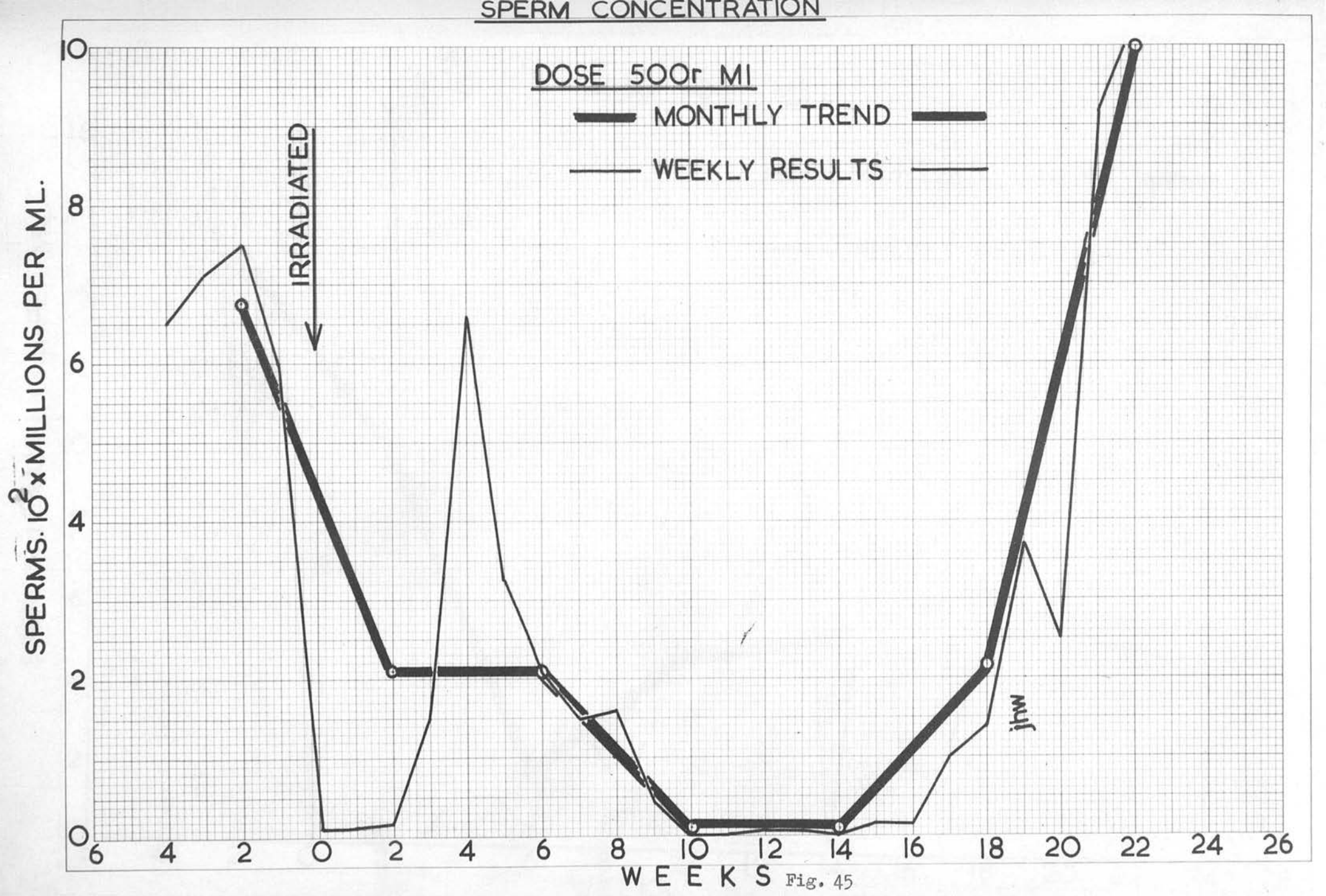

SPERM CONCENTRATION
DOSE 400 r
MONTHLY TREND
WEEKLY RESULTS
IRRADIATED
SPERMS. MILLIONS x 10^2/ML.
0
2
4
6
8
10
12
14
16
18
20
6
4
2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
WEEKS
jhw

Fig. 44


SPERM CONCENTRATION
DOSE 500r MI
MONTHLY TREND
WEEKLY RESULTS
IRRADIATED
SPERMS. 10^2 x MILLIONS PER ML.
0
2
4
6
8
10
6 4 2 0 2 4 6 8 10 12 14 16 18 20 22 24 26
WEEKS
jhw
Fig. 45

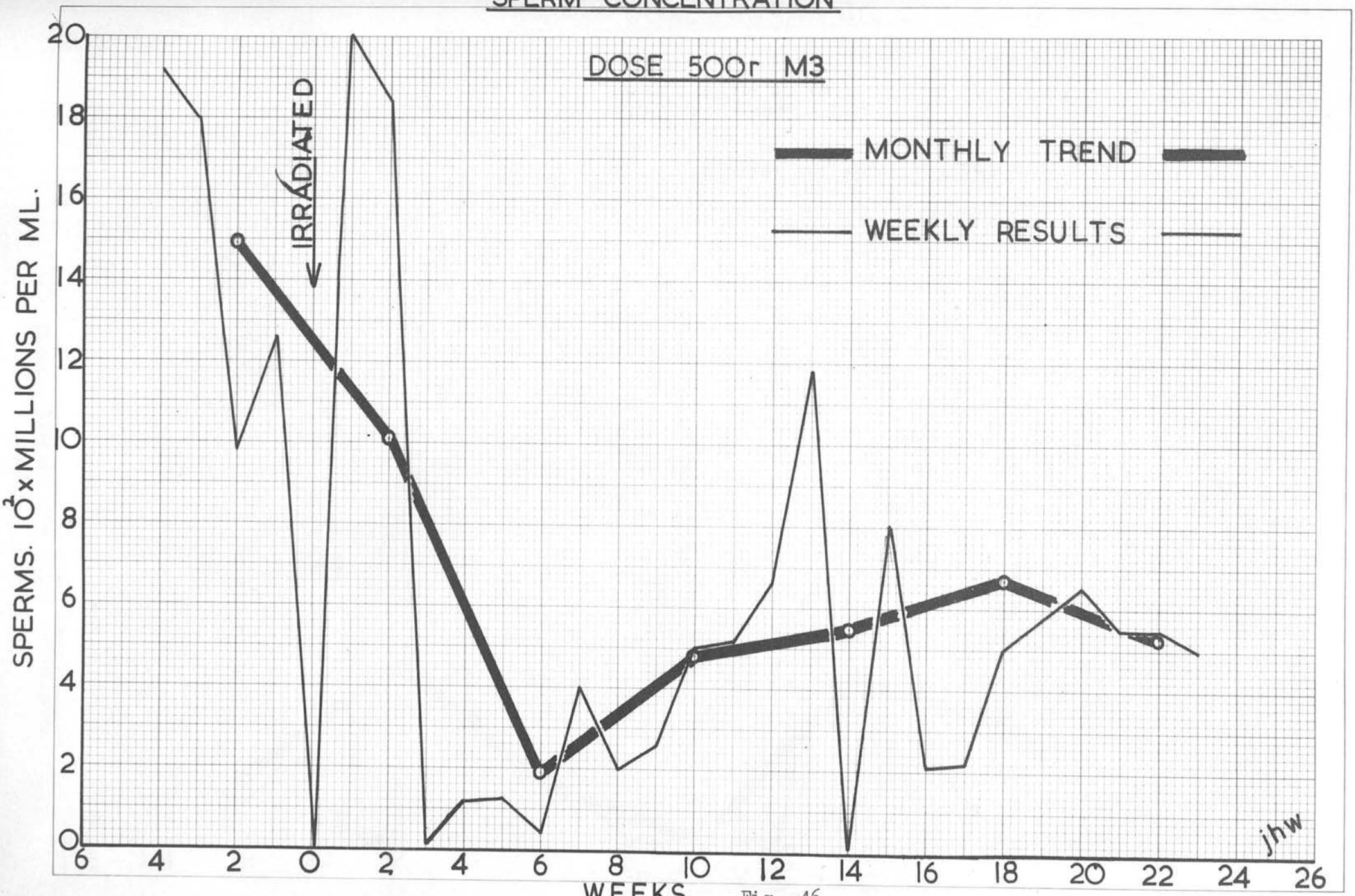

SPERM CONCENTRATION
DOSE 500r M3
IRRADIATED
MONTHLY TREND
WEEKLY RESULTS
SPERMS. 10^3 x MILLIONS PER ML.
0
2
4
6
8
10
12
14
16
18
20
6 4 2 0 2 4 6 8 10 12 14 16 18 20 22 24 26
WEEKS
jhw

Fig. 46

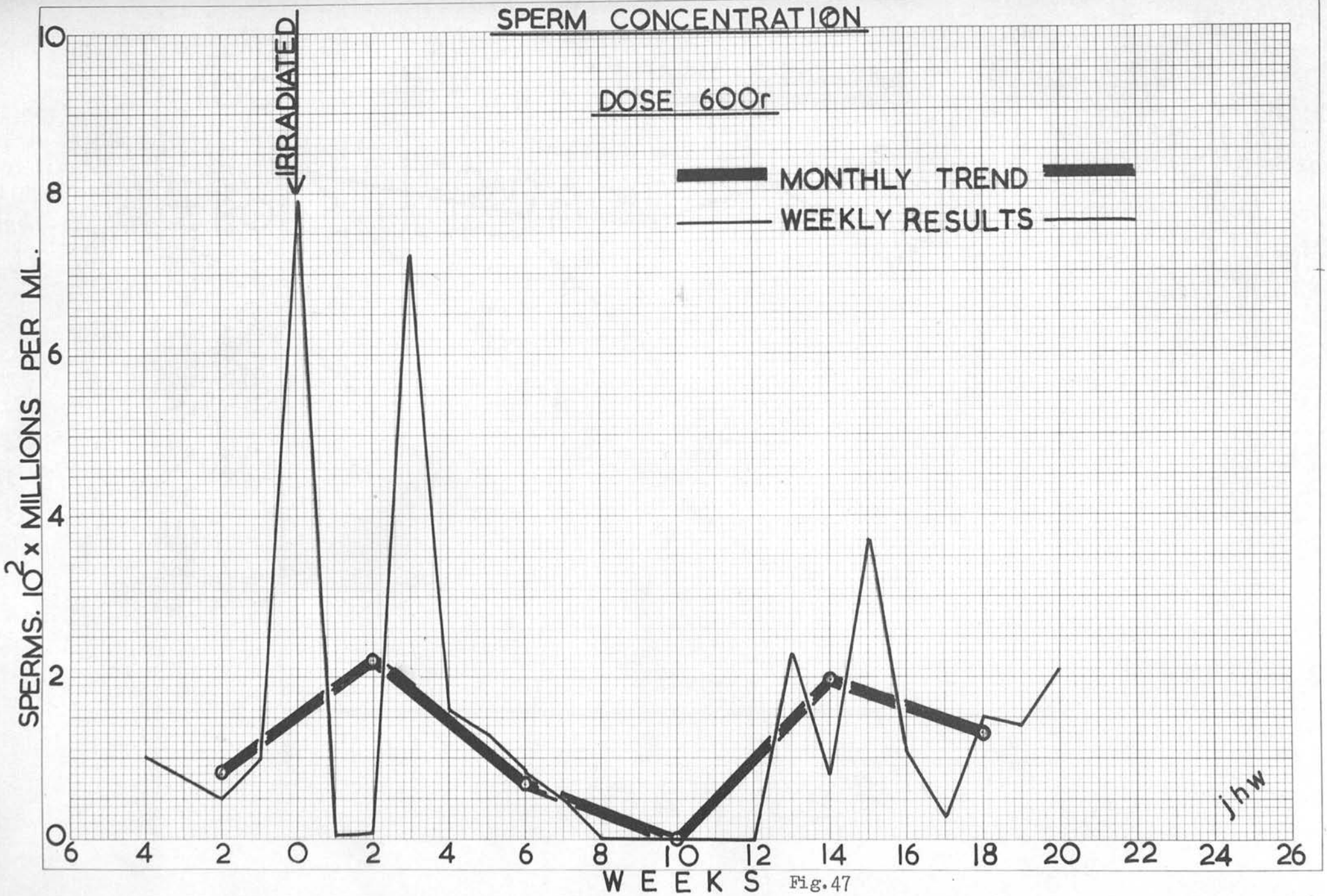

SPERM CONCENTRATION
DOSE 600r
IRRADIATED
MONTHLY TREND
WEEKLY RESULTS
SPERMS. 10^2 x MILLIONS PER ML.
0
2
4
6
8
10
6 4 2 0 2 4 6 8 10 12 14 16 18 20 22 24 26
WEEKS
jhw

Fig.47

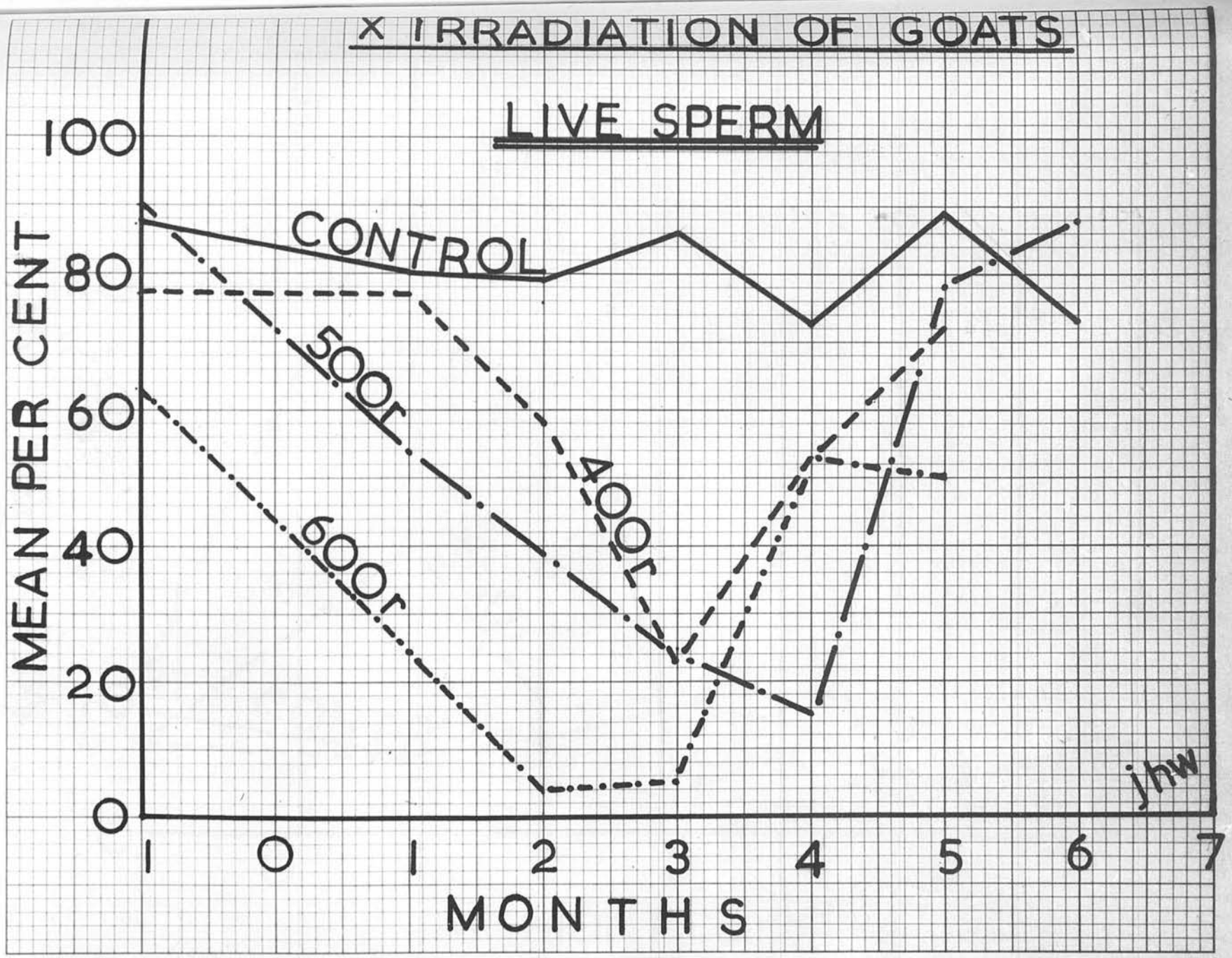

X IRRADIATION OF GOATS
LIVE SPERM
MEAN PER CENT
100
80
60
40
20
0
CONTROL
500r
400r
600r
1 0 1 2 3 4 5 6 7
MONTHS
jhw

Fig. 48

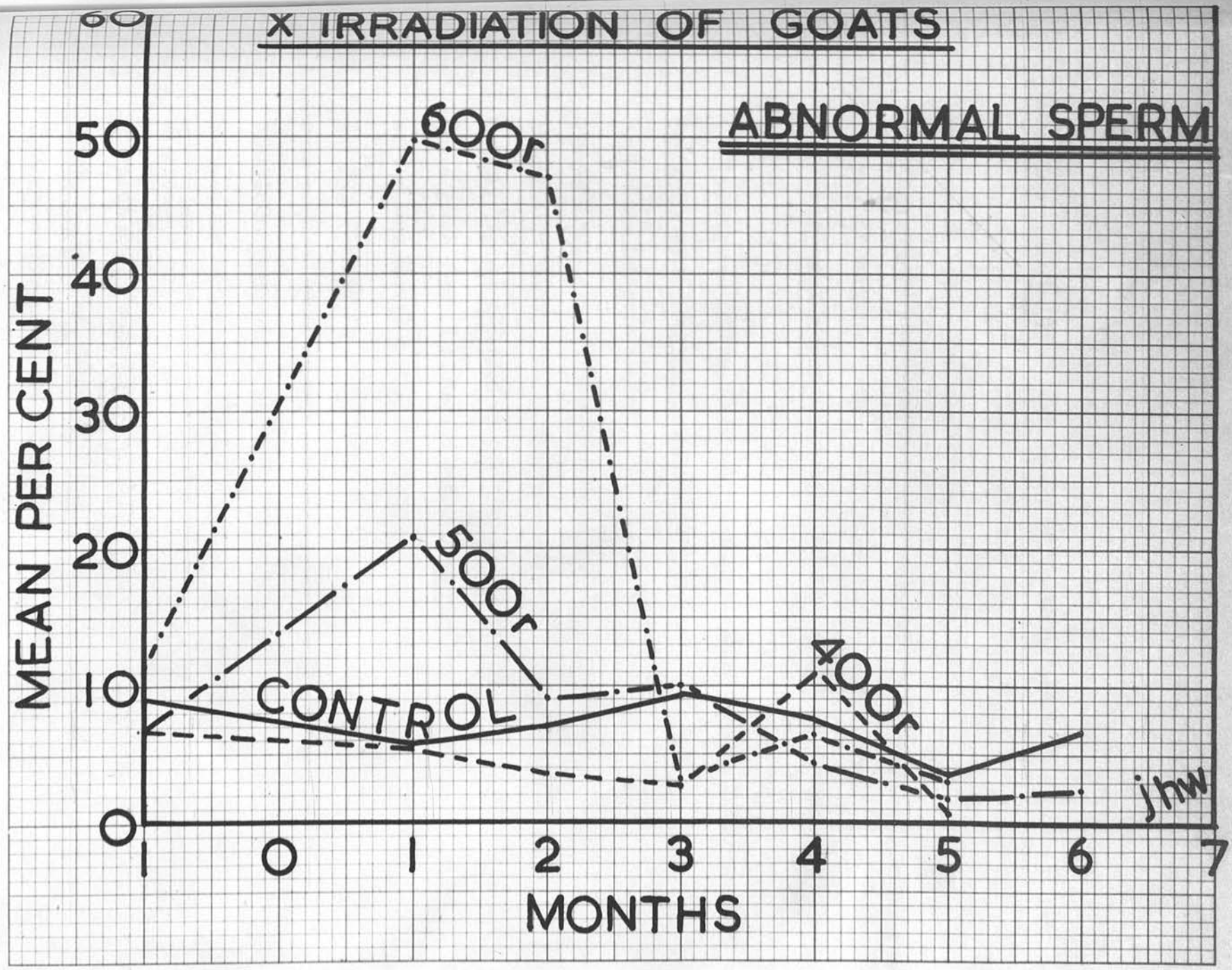

X IRRADIATION OF GOATS
ABNORMAL SPERM
600r
500r
400r
CONTROL
MEAN PER CENT
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7
MONTHS
jhw

Fig.49

ii) Interrelationships of Spermatozoa Values

The data on sperms have been partitioned into three groups

a) Total sperm production per ejaculate in millions

b) Total per cent live sperm

c) Total per cent abnormal sperm

For each group of data, the overall differences between the goats at the different levels of radiation, including the control, and the overall differences between the monthly figures including the month pre-irradiation were simultaneously tested for significance by an Analysis of Variance. Four weekly examinations constituted a "month" making one complete pre-irradiation and five complete post-irradiation months.

The figures for the first day post-irradiation and those for the 6th month post-irradiation which were incomplete were ignored so as to render the Analysis easier.

The same Analysis of Variance also tested the significance of the interaction between period and dose of radiation i.e. whether the monthly figures depended on the dose of radiation, or whether the results for a given dose of radiation depended on the time. Where the Analysis of Variance showed a significant difference between the monthly figures, the mean figures for the individual months i.e. averaged over all goats, were compared with the mean pre-irradiation figure using t-tests. Similarly, where the Analysis of Variance showed a significant difference between the figures for the different levels of radiation, the mean figures for the individual goat i.e. averaged over all months, were compared with the mean control figures also using t-tests.

If the Analysis of Variance showed no overall difference no individual testing was considered necessary.

a) TOTAL SPERM PRODUCTION PER EJACULATE

TABLE LXV

Analysis of Variance

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Between Goats	4	130.69	32.67	16.52	VHS
Between Periods	5	14.94	2.99	1.51	N.S. at 5%
Interaction	20	41.42	2.07	1.05	N.S. at 5%
Residual	90	177.99	1.98		
Total	119	365.04			

D.F. = Degree of Freedom (n-1); S.S. = sum of squares; M.S. = mean significant (S^2); V.R. = Variance Ratio; VHS = Very highly significant; N.S. = Not significant;

The overall differences between the values for the control and the irradiated goats were very highly significant ($P \ll 0.1\%$) but the differences between the monthly figures were not significant at 5%. There was no evidence to suggest an interaction between goats and periods; in other words the monthly values did not depend on the dose or vice versa.

TABLE LXVI

Tests for Differences between Irradiated Goats and Control

Dose (r)	Goat No.	t 90	Significance Level
400	M 10	1.70	N.S. at 5%
500	M 3	3.07	S at 1%
500	M 1	6.18	VHS
600	M 9	6.69	VHS

S = Significant; NS = Not Significant; VHS = Very highly significant;

There was no evidence to suggest that the goat irradiated at 400r differed in any way from the control; there was, however, a significant difference ($0.002 < P < 0.01$) between the first goat at 500r (M3) and the control. The second goat at 500r (M1) and the goat at 600r showed a very highly significant difference ($P \ll 0.001$) from the control.

b) TOTAL PERCENT LIVE SPERM

TABLE LXVII

Analysis of Variance

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Between Goats	4	5070.88	12675.97	32.19	VHS
Between Periods	5	12233.94	2446.79	6.21	S at 0.1%
Interaction	20	24445.02	1222.25	3.10	S at 0.1%
Residual	90	35445.75	393.84		
Total	119	122828.59			

D.F. Degree of Freedon (n-1); S.S. = sum of squares; M.S. = mean significant (S^2); V.R. = Variance Ratio; VHS = Very highly significant; S. = Significant.

The overall differences between the values for the control and irradiated goats were very highly significant ($P << 0.1\%$). The differences between the monthly figures were also significant ($P < 0.1\%$) and so were the interaction between goats and periods i.e. the monthly values depended critically on the radiation dose and vice versa.

TABLE LXVIII

Tests for Differences between individual Monthly Means of live sperm and the pre-irradiation Mean

Time	t 90	Significance Level
1st Month	1.63	NS at 5%
2nd Month	2.22	S at 5%
3rd Month	4.82	S at 0.1%
4th Month	2.77	S at 1%
5th Month	-	NS

S = Significant; NS = Not Significant;

There was no evidence to suggest that the mean value for the first month differed significantly from the pre-irradiation mean. However, there was a significant difference ($0.02 < P < 0.05$) in the second month, a highly significant difference ($P < 0.001$) in the third month and a very significant difference ($0.002 < P < 0.01$) in the fourth month. There was no evidence to suggest that the mean for the fifth month differed significantly from the pre-irradiation mean.

TABLE LXIX

Tests for Differences between Irradiated Goats and Control

Dose (r)	Goat	t 90	Significance Level
400	M 10	3.97	S at 0.1%
500	M 3	0.63	NS at 5%
500	M 1	4.07	S at 0.1%
600	M 9	9.38	VHS

S = Significant; NS = Not Significant; VHS = Very highly significant;

There was a highly significant difference ($P < 0.001$) in the per cent live sperm values between the goats irradiated with 400r and one of the goats (M 1) irradiated with 500r, and the control. There was no evidence to suggest that the other goat irradiated at 500r (M 3) differed from the control, but the goat irradiated at 600r showed a very highly significant difference ($P << 0.001$) from the control.

c) TOTAL PER CENT ABNORMAL SPERM

TABLE LXX

Analysis of Variance

Source of Variation	D.F.	S.S.	M.S.	V.R.	Significance Level
Between Goats	4	5887.45	1471.86	11.55	V.H.S.
Between Periods	5	1922.74	384.55	3.02	S at $2\frac{1}{2}\%$
Interaction	20	14039.30	701.97	5.51	S at 0.1%
Residual	90	11472.38	127.47		
Total	119	33321.87			

D.F. = Degree of Freedom (n-1); S.S. = sum of squares; M.S. = mean significant (S^2); V.R. = Variance Ratio; VHS = Very highly significant; S = Significant;

The overall differences between the values for the control and irradiated goats were very highly significant ($P << 0.1\%$). The differencies of the monthly figures were also significant ($1\% < P < 2\frac{1}{2}\%$). There was also a highly significant interaction between goats and periods ($P < 0.1\%$) i.e. that the monthly values depended critically on the radiation doses and vice versa.

TABLE LXXI

Tests for Differences between individual Monthly Means and the Pre-Irradiation Mean

Time	t 90	Significance Level
1st Month	0.41	NS at 5%
2nd Month	0.19	NS at 5%
3rd Month	2.21	S at 5%
4th Month	1.75	NS at 5%
5th Month	2.07	S at 5%

S = Significant; NS = Not significant;

There was no evidence to suggest that the mean values for the first, second and fourth months differed, significantly from the pre-irradiation value. However the mean for the third and fifth months differed significantly ($0.02 < P < 0.05$) from the pre-irradiation mean.

TABLE LXXII

Tests for Differences between Irradiated Goats and Control

Dose (r)	Goat	t 90	Significance Level
400	M 10	2.05	S at 5%
500	M 3	0.24	NS at 5%
500	M 1	1.62	NS at 5%
600	M 9	5.98	VHS

S = Significant; NS = Not significant; VHS = Very highly significant;

There was a very highly significant difference ($P << 0.001$) in the per cent abnormal sperm between the goat irradiated at 600r and the control; also the goat at 400r showed a significant difference ($0.02 < P < 0.05$) from the control.

Discussion

It is well known that the spermatogonia of the testicles are the most sensitive of all mammalian cells to irradiation. Albers-Schonberg (1903) first demonstrated that injury after X radiation consisted of a decrease in spermatogenesis and probable damage to the epithelium of the seminferous tubules. Phillip (1904) made the earliest observations in man, quickly followed by Brown and Osgood (1905), when they reported azoospermia and oligonecrospermia after therapeutic irradiation. Different species of animals probably possess varying proportions of the comparatively radioresistant primordial cells (Warren, 1943) of the germinative layer. Heller (1948) found that in rabbits after an exposure dose of 800r complete regeneration of sperm elements occurred in 4 months whereas in mice it took 3 months after 400r (Eschenbrenner et al, 1950.)

In this study the following general conclusions were drawn from the data obtained and from the statistical analysis carried out:-

i) In these particular goats doses of X radiation of the order of 400r to 600r had a profound effect on sperm pnysiology.

ii) The total number of spermatozoa per ejaculate was reduced after irradiation but the extent of the decrease did not depend on the radiation dose employed.

iii) There was commonly a lag period of at least one month between irradiation and the occurrence of observable effects in the semen.

iv) The first noticeable effect was a decrease in the proportion of live to dead sperms in the ejaculated semen. This effect was shown at the second month after irradiation became more profound in the third month and continued into the fourth month: thereafter there was a gradual improvement to normality.

v) The extent of the increase of dead to live sperms depended directly on the size of the radiation dose.

vi) Abnormal sperms increased significantly in number in the third month after irradiation and the extent of these increases depended directly on the size of the radiation dose employed.

vii) Sperm counts and live sperms began to increase, and the number of abnormal sperms decrease, after the fourth month post-irradiation.

viii) The semen characteristics of goats subjected to doses of 400r to 600r whole body X radiation returned to normal at about 6 months post-irradiation.

The gradual increase in total sperm counts and live sperm together with the concomitant decrease of abnormal forms pointed to a progressive regeneration of germinal epithelium. This observation was later reinforced by a fertility test carried out on one of the goats (M3) which had been exposed to a radiation dose of 500r. This goat served two female goats as follows :

i) M3 X Unirradiated female (F 51)

The female goat was a British Toggenberg, aged 5 years. She was served on 8th October 1962 and gave birth on 7th March 1963 to two kids, one male and one female, both of which were normal. Confinement and recovery were uneventful and normal. Time of Service : 116 days after irradiation of male goat with 500r.

ii) M3 X Irradiated female (F 30)

The female goat was a British Saanen, aged 5 years and was served on 10th October 1962. She was a survivor of a bilateral

exposure of 600r X radiation on 26th April 1962. She gave birth on 10th March 1963 to three kids, one of which was still born. No gross abnormality was observed in this kid. The two surviving kids, one male and one female, were robust healthy and normal. Confinement was normal but recovery was slightly abnormal in that there was incomplete separation of the placenta, remnants of which were still being voided three to four days after. Time of Service : 118 days after irradiation of male goat with 500r; Time of covering : 167 days after irradiation of female goat with 600r.

It may be reasonable to infer from this very limited experiment that a progressive recovery of sperm elements in the male goat had occurred. It was also possible that the irradiation had sufficiently damaged the ovarian follicles of the second female goat (F 30) to bring about the death of one kid and the retention of the after-birth. However, it was more probable that the abnormalities observed were perfectly natural. Testicular atrophy which occurs in man and other animals in about 4 weeks after irradiation was not clinically observed; however, some hypoplasia and a degree of subsequent recovery could be inferred from the data obtained. It is probable that at the lower radiation doses damage to the germinal epithelium was not sufficient to cause the death of much of the pre-sperm elements. Whereas at the higher doses a larger proportion of these elements were destroyed or damaged thus resulting in a lowered sperm production for a longer period of time and the appearance of a larger percentage of dead and abnormal spermatozoa. Nevertheless, the differences may be due simply to individual idiosyncrasies and could possibly be better demonstrated in a larger series of animals.

5. Effects of X irradiation on the Tuberculin and Johnin Intradermal Tests in Goats

a) Normal Goats

Results of the tuberculin, johnin and complement fixation tests in normal goats are given in Appendix III.

Tuberculosis

Out of a colony of 60 adult goats and 59 goatlings there were 7 positive reactors to the intradermal tuberculin test and 4 doubtful reactors which showed circumscribed reaction swellings without a surrounding oedema. Two reactors on post-mortem showed the presence of tuberculous lesions in the mesenteric lymph nodes and the avian type of Mycobacterium tuberculosis was recovered in each case. It was therefore confidently assumed that the other 5 reactors were also infected with the bacillus. In every avian reactor the reaction to johnin was also positive showing a highly significant correlation. In cattle, a reaction to avian tuberculin is generally accepted as indicating either infection with avian tuberculosis or a non-specific reaction due to Johnes Disease. Naturally acquired tuberculosis in goats is rare. According to Griffith (1928) this is due more to factors of environment than any inherent constitutional resistance. There are not more than twenty cases of avian tuberculosis recorded in goats in Great Britain since 1914. Lesslie et al (1960) reported 10 reactors in a herd of 85 home bred goats. Two of these were autopsied and well-established lesions were seen in the mesenteric lymph nodes. The percentage of reactors reported by them viz 11.7% was the same as in the series reported here.

Johnes Disease (Paratuberculosis)

Johnes Disease is essentially a disease of the ruminant and the goat can become affected. It is caused by the Mycobacterium johnei and, in

"negative" to the complement fixation test; four showing positive reactions to the intradermal tests were "doubtful" to the complement fixation test. The one goat which was "positive" to the complement fixation test was negative to the intradermal tests. Sixteen goats which were "doubtful" to the complement fixation test were also negative to the intradermal tests. Three which were negative to the complement fixation test were "doubtful" to the intradermal tests. (Table LXXIII).

There appears to be no correlation between the results of the complement fixation tests and the results of the intradermal tests. In no case where there was a positive or a doubtful complement fixation test result was there any evidence of clinical, bacteriological or histological signs of Johnes Disease. In addition the complement fixation test for Johnes Disease in goats is apparently not able to pick out the non-specific reactor to avian tuberculosis. From this it was concluded that the test was of little value in diagnosing either the presence of Johnes Disease or tuberculosis in goats.

Table LXXIII

A Comparison of the Intradermal and Complement Fixation Tests in Goats

Complement Fixation Test		Intradermal Tuberculin and Johnin Test		
Result		Positive	Doubtful	Negative
Positive	1	Nil	Nil	1
Doubtful	21	4	1	16
Negative	38	3	3	32
	60	7	4	49

b) Irradiated Goats

Out of the 20 goats which were irradiated with X rays in this study 3 were positive and 1 was "doubtful" to the intradermal tuberculin and johnin tests before irradiation. Tables LXXIV and LXXV give the results of the skin measurements of the two survivors before and at periods after irradiation: the other two reactors did not survive.

Table LXXIV

Goat No. C 240 Irradiated with 500r

Period	SKIN MEASUREMENTS (in mm)				
	Before Test	After 72 hrs			Remarks
	Normal	Avian	Mammalian	Johnin	
Before Irradiation	3	10	19	10	Extensive oedema Positive
After Irradiation					
7 weeks	4	4	11	4	Slight oedema Positive
13 weeks	4	4	4	4	Negative
6 months	4	4	4	4	Negative
9 months	3	3	3	3	Negative
14 months	3	3	3	3	Negative
18 months	3	3	3	3	Negative

Table LXXV

Goat No. W 302 Irradiated with 400r

Period	SKIN MEASUREMENTS (mm)				
	Before Test	After 72 hrs			Remarks
	Normal	Avian	Mammalian	Johnin	
Before Irradiation	4	11	6	10	Oedema Positive
After Irradiation					
8 weeks	3	6	3	6	Circum-scribed Doubtful
14 weeks	3	3	3	3	Negative
6 months	3	3	3	3	Negative

Discussion

Lennox et al (1952) investigated the suppression of the tuberculin reaction in rabbits by whole body irradiation. They found that irradiation of the tuberculin sensitive animal temporarily renders this reaction either negative or markedly weaker. It is interesting to note that in this very limited study in goats a similar effect occurred. In addition the suppression of the reaction in goats was very prolonged i.e. over 18 months. The cause of this suppression is a matter of speculation. Patt et al (1947) presented evidence that irradiation causes hypersecretion of cortisone and many observers consider that this is the cause of the suppression of the tuberculin reaction. Long and Miles (1950) showed that cortisone suppresses or weakens the tuberculin reaction in guinea pigs. The oedema of the normal positive local reaction is supposedly suppressed by the steroids formed incidentally after irradiation. Another possible cause of the suppression of the tuberculin reaction in the sensitive animal is the massive destruction of lymphocytes which occurs after irradiation and which may upset the sensitivity-balance. Lymphocytes are the most sensitive cells to tuberculin and it is possible that so many tuberculin-sensitive cells are destroyed that it prevents or modifies the classic reaction.

Summary

i) The object of this work was to discern and describe the response of the adult goat, maintained under laboratory conditions, to whole body X irradiation at median lethal doses.

ii) One hundred and nineteen goats and goatlings were maintained in a specially-built animal farm at Harwell in which modern laboratory facilities had been added. The housing, care and management of this colony are described in some detail. Twenty adult goats of various breeds, age and sex were selected from this colony for the experiments.

iii) Some of the main average physiological features of the normal goat as observed in the whole colony were found to be as follows:-

a) Body length . . . . . 110 cms (3 ft 6 ins)

b) Body weight . . . . . 61.2 Kg. (135 lbs)

c) Rectal temperature . . . . . 38.7°C (101.7°F)

d) Daily water consumption . . . . 2070 ml (3.6 pints)

e) Respiratory frequency . . . . . 22 per minute

f) Heart rate . . . . . 109 per minute

g) Daily urine output . . . . . 1500 ml (2.6 pints)

iv) The helminth burden of adult goats bought in randomly from dealers was shown to be mainly of strongyloid spp. Helminthiasis was controlled effectively by treatment with methyrdine or phenothiazine and did not complicate the observed effects of irradiation.

v) A new analgesic drug named phencyclidine was introduced and was used successfully on goats throughout the study both alone and in combination with other sedative agents. Phencyclidine was shown to have a slight protective effect on the mortality of mice exposed to X irradiation.

vi) The intradermal tuberculin test was effective in diagnosing tuberculosis in goats. The non-specificity of the test especially with regard to johnin and avian tuberculin was confirmed. The complement fixation test was of no value in diagnosing the presence of either Johne's Disease or tuberculosis in goats. The intradermal tuberculin reaction was suppressed by irradiation.

vii) Radiation dose was measured as the exposure air dose in roentgens and the exposure was bilateral in all cases. The main factors in the irradiation were 250 kV, 14 mA, Half-Value Layer 1.2 mm Cu. and an average dose rate of 13 roentgens per minute. The target-to-midline distance was 215 cms. in all cases.

viii)The LD50 surface air dose for adult goats was estimated to be 621.7r with a 95% confidence interval of 535.5r to 721.9r.

The LD50 tissue dose to the whole animal was estimated to be 465r.

The LD50 dose at the mid-line was estimated to be 375r.

ix) The mean survival time of decedents was:-

19.5 days for dose range 400r to 2000r.

22.0 days for dose range 400r to 700r.

x) The usual clinical syndrome consisted of intermittent anorexia, apathy, knuckling-over in the front legs, swelling of the face and head, cessation or lessening of water consumption, progressive but slight weight loss in non-survivors, loss of outer hairs of coat in some, and a terminal precipitous rise in the rectal temperature. Other signs included tachycardia and arrhythmias and murmurs, respiratory distress with occasional discharges from nose and mouth especially terminally, recumbency with abdominal pain shown by teeth grinding and a profound forlorn malaise just prior to death. Generally speaking diarrhoea, rhinorrhoea and epilation were not common.

xi) Irradiated goats showed the following changes:-

a) Loss in Body weight at
7 days post irradiation . . . . . 6% in non-survivors
3% in survivors

b) Rectal Temperature . . . . . in non-survivors a terminal rise to a mean of 106.4°F

c) Daily food and water intake . . . . cessation or profoundly reduced

d) Respiratory Frequency . . . . . increased initially and terminally

e) Heart Rate . . . . . increased initially and terminally

f) Daily urine output . . . . . reduced to a mean of 650 ml

There was a direct correlation between the rise in temperature and depression of circulating granulocytes in the blood.

xii) Urinalysis showed red blood cells, haemoglobin, sugar and bilirubin in significant quantities in many irradiated goats. The glycosuria and the haemoglobinuria were considered to be due to renal damage and toxaemia as a direct or indirect result of irradiation. The glycosuria was also considered to be indicative of an enterotoxaemia due to Clostridium welchii as a sequel to gastrointestinal damage caused by irradiation.

xiii)Haematological examinations were carried out on conventional lines by well tried methods with the following general results:-

a) Normal healthy goats showed wide individual variations in their blood pictures.

b) The destructive effects of total whole body X irradiation on the blood-forming tissues in adult goats were reflected in consistent profound changes in the peripheral blood. These changes included an extreme radiosensitivity of the leucocytes especially the mononuclear cells.

c) Within 24 hours the mononuclear and eosinophil counts fell precipitously almost to zero whilst the neutrophils increased to values between 200% and 300% above control counts. Thereafter the neutrophils dropped profoundly.

d) The red blood cell counts, haemoglobin content and haematocrit (PCV) values began decreasing after the 9th day post irradiation. Recovery commenced after the 60th day.

e) The platelet count, after an ephemeral initial increase, began to decrease after the 5th day post irradiation. Recovery was very slow.

xiv) Anaemia was normochromic and normocytic and was of all grades of severity. Anisocytosis, poikilocytosis and polychromasia were common. This picture, together with occasionaly circulating normoblasts coupled with a leucopenia, a thrombocytopenia and a general haemorrhagic diathesis was not unlike that of other diseases seen in ruminants, such as bracken poisoning, making differential diagnosis an important item.

xv) Haemorrhages were associated with a thrombocytopenia and coagulation defects. The recovery from the coagulation defect was more rapid than the return of platelets to the blood of irradiated goats.

xvi) A satisfactory method of bone marrow aspiration from the sternum was evolved. Marrow cells of the goat were found to be similar to those of other mammals especially sheep. No evidence was found in normal goats to suggest any significant differences in the general composition of the bone marrow as far as sex, age or weight was concerned. In irradiated goats neither dose nor time after irradiation could be correlated to the relative myeloid and erythroid elements.

xvii) A suppression of erythroblastic activity in the bone marrow occurred between the 2nd and 7th day after irradiation. Thereafter normality was slowly achieved in survivors whereas in non-survivors suppression was complete and, in addition, leucoblastic activity was

severely reduced. Goats which died early showed marrow aplasia whereas those dying later manifested regenerative reparative foci.

xviii) Mitotic activity in the bone marrow ceased in non-survivors within 2 to 7 days after irradiation and was severely reduced in survivors. In survivors normality appeared to be reached in 80 days.

xix) At post mortem multiple haemorrhages were observed in all non-survivors and were seen most prominently in the gastrointestinal tract, lungs, lymph nodes, trachea and heart; oedema was most commonly seen in the lungs and mucosal ulceration in the large intestine. Histologically, the most pronounced and commonest lesions observed were atrophy of lymphoid and bone marrow tissues, generalized haemorrhages and oedema. Colonies of bacteria with little or no cellular reaction together with focal necrotising degenerations were seen in most visceral organs. Intra- and extra-vascular particulate complexes which were PAS-positive were noted commonly as were the swelling and hyalinization of the vascular endothelium of many organs.

xx) Pyloric spasm and stenosis was sometimes observed at post mortem. The correlation of the observed reduction of water intake and the general effects of water depletion in the goat after total body irradiation warrants further research.

xxi) There was evidence to suggest that the clinical syndrome and post mortem lesions produced in goats after irradiation were the result in part of an enterotoxaemia. The term "toxic" death was used in cases where toxaemia appeared to be predominant. This aspect requires further research.

xxii) The functional cardiac changes clinically observed after irradiation were considered to be probably due to alterations in conduction caused by the observed collection of foreign material around the nervous and endothelial tissue of the heart.

xxiii) The effects on the heart were considered to be very important. The term "cardiac" death has been coined because of the common finding of arrhythmias and murmurs after irradiation. It is considered that this aspect requires further research.

xxiv) Sperm production was reduced in irradiated goats but the reduction did not depend on the radiation dose employed.

xxv) The percentage of live sperms in ejaculated semen was less in irradiated goats and the decreases depended on the radiation dose.

xxvi) The percentage of abnormal sperms in ejaculated semen was greater in irradiated goats and the increases depended on the radiation dose.

xxvii) Sperm characteristics were back to normal in 5 to 6 months after irradiation.

Conclusion

The general conclusion from this study was that the biological response of the adult British goat to whole body X irradiation at median lethal doses was similar to that of other animals of comparable size. The macroscopic and microscopic findings revealed generalized haemorrhages together with tissue cellular damage especially of the blood-forming organs and the mucosa of the alimentary tract. The clinical signs and symptoms reflected these lesions.

In this thesis on some of the general effects of X radiation on goats much has been said only to imply that there is more to be said and a lot more to be done.

An attempt has been made to say and do some of it; facts have been observed, they have been classified in an empirical but generally accepted manner, the role of X radiation as a biological insult in goats has been assessed and finally conclusions have been drawn. Ex omnibus verbis est eliciendus sensus qui interpretatur singula.

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COMING EVENTS

September

2nd (Sat.). STREATLEY '58 CLUB REUNION. Annual Dinner at "Ye Miller of Mansfield."

3rd to 9th (Sun. to Sat.). 79th Annual Congress and Exhibitions of the British Veterinary Association at Oxford.

7th (Thurs.). ROYAL VETERINARY COLLEGE ASSOCIATION. Annual Dinner during the B.V.A. Congress at St. Edmund's Hall, Oxford.

GLASGOW VETERINARY SCHOOL ALUMNUS ASSOCIATION. Dinner at the Mitre Hotel, Oxford, 7 p.m.

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the date on which disease has been confirmed, followed by the postal address and local authority.

Anthrax

July 26th, 1961. T. J. Reed, Yard Farm, Upottery, Honiton, Devon (Parish: Upottery). (Cattle.) DEVON.

July 31st, 1961. Mrs. M. A. Smith, Bridge House Farm, Laneshawbridge, Colne, Lancs. (Parish: Colne). (Cattle.) LANCASHIRE.

Fowl Pest

July 25th, 1961. L. J. V. Jasper, Hall Farm (Barham) Ltd., Barham, Ipswich, Suffolk. EAST SUFFOLK.

July 25th, 1961. T. B. Liversey, New Wicken House Farm, Withnell, Chorley, Lancs. LANCASHIRE.

July 25th, 1961. W. Cockerill, 7, Worsley Street, Rising Bridge, Accrington, Lancs. Disease at: Rising Bridge Holding, Accrington, Lancs. LANCASHIRE.

July 25th, 1961. W. Cockerill, 15, Oak Avenue, Rising Bridge, Accrington, Lancs. Disease at: Top of the Bank Holding, Rising Bridge, Accrington, Lancs. LANCASHIRE.

July 25th, 1961. H. Yarrow, No. 2, Council Houses, Ely Road, Little Thetford, Ely, Cambs. ISLE OF ELY.

July 26th, 1961. J. Holden, Brimmicroft Farm, Houghton, Preston, Lancs. LANCASHIRE.

July 26th, 1961. E. P. Myerscough, Bent Farm, Withnell, Chorley, Lancs. LANCASHIRE.

July 26th, 1961. C. H. Mason, Crossfield Farm, Withnell, Chorley, Lancs. LANCASHIRE.

July 27th, 1961. J. Hessell, No. 5, The Cottages, Grange Farm, Great Limber, Grimsby, Lincs. LINCOLN, PARTS OF LINDSEY.

July 28th, 1961. J. Marginson, Quarry Bank Farm, Houghton, Preston, Lancs. LANCASHIRE.

July 28th, 1961. W. J. Snook, Green Valley Farm, Ubbeston, Halesworth, Suffolk. EAST SUFFOLK.

July 28th, 1961. Mrs. M. E. Money, 13, Council Houses, Little Thetford, Ely, Cambs. ISLE OF ELY.

July 29th, 1961. E. Gillett, 5, Council Houses, Little Thetford, Ely, Cambs. ISLE OF ELY.

July 30th, 1961. Miss E. Sansome, The Hazels, Wicken Road, Clavering, Saffron Walden, Essex. ESSEX.

July 30th, 1961. P. Cammidge, 333, Benfleet Road, South Benfleet, Essex. ESSEX.

July 31st, 1961. M. W. Rumsey, The Avenue, Eye, Suffolk. EAST SUFFOLK.

July 31st, 1961. F. J. Bassingham, Vineyard, Little Thetford, Ely, Cambs. ISLE OF ELY.

July 31st, 1961. B. Goodwin Ltd., Merton Farm, Little Bury, Saffron Walden, Essex. ESSEX.

APPENDIX I

Annexure (i)

Letters to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

SCIENTIFIC

The Effect of a New Analgesic Induction Agent on Goats

Sir,—A problem which I have had to solve recently has been to render adult goats immobile and quiet for periods of 2 hours or more. Anaesthesia by volatile agents requires continued attention throughout the period and was to be avoided if possible. Barbiturates by themselves are too short-acting, produce copious salivation, are solely hypnotic possessing little if any analgesic effect, and repress respiratory and cardiovascular systems considerably.

A new analgesic agent called Sernyl has been used with satisfactory results. This drug is undoubtedly the most potent general analgesic agent which has yet been used in human clinical medicine. It has the unique advantage of causing no depression of either the cardiovascular or respiratory functions and can be safely used. In monkeys doses of 0.3 to 0.6 mg. per kg. intravenously induced a cataleptoid state lasting up to 1 hour during which time major surgery could be performed without difficulty whilst the animals appeared to be awake and alert. The oral administration to cats and dogs produced similar results (Johnstone, Evans & Baigel, 1959).

Sernyl is a white solid, freely soluble in water and ethanol. Its chemical name is 1-(1-phenyl cyclohexyl) *piperidine* monohydrochloride. Parke, Davis & Co. kindly supplied it in solution, 100 mg. per ml.

I used it on adult goats by intramuscular injection in doses ranging between 0.5 mg. and 16 mg. per kg. bodyweight. The accompanying table summarises the major clinical effects.

In general, animals went down within 1 to 10 minutes of injection depending on the dose. At the lowest dose goats remained standing though quite immobile and in 5 to 20 minutes quietly subsided on to their sides. During this induction there was no period of excitation. Absence of reaction to painful stimuli was noticed at all dose rates. Muscular tonicity especially of the neck and limbs occurred. Vertical nystagmus, except at the highest dose rate, was a noticeable feature. A characteristic was that pharyngeal and laryngeal reflexes appeared not to be depressed: the risk of respiratory obstruction seemed small and intratracheal intubation was not necessary. When the animals recovered and regained their feet they began eating at once.

Another valuable feature is that a preliminary dose of Sernyl reduces the quantity of barbiturate necessary to produce surgical anaesthesia.

My general impression was that Sernyl is a new safe analgesic induction agent which may be of value in many veterinary medical procedures encountered in general practice.

The manufacturers emphasise that Sernyl is still undergoing clinical trials, and cannot yet be recommended for a number of species. It is contra-indi-

TABLE I
EFFECT OF SERNYL ON THE GOAT

Mg. per kg. intramuscularly	0·5	0·75	1	1·5	2	4	6	10·4	16
Locomotion...	Moored to floor after slight ataxia	Ataxia	Ataxia, moored to spot	Nil	Nil	Nil	Nil	Nil	Nil
Muscular tonus	Subcutaneous fibrillation. Normal	Slightly hypertonic legs and neck	Slightly hypertonic neck and limbs	Hypertonic legs and neck	Hypertonic legs and neck	Hypertonic legs and neck	Hypertonic legs and neck	Hypertonic	Hyperto
Time to go down from injection	8 to 50 mins.	20 mins.	5 to 15 mins.	5 to 10 mins.	3½ to 8 mins.	5 mins.	2 mins.	1½ mins.	1 min.
Jaw movements	Slight	Slight	Yes +	Yes +	Yes ++	Yes ++	Yes +++	Yes +++	Yes ++
Eyelids ...	Open	Open	Open	Open	Open	Open	Open	Open	Open
Pupil ...	Slightly dilated	Slightly dilated	Dilated +	Dilated +	Dilated ++	Dilated +++	Dilated +++	Dilated +++	Dilated +++
Corneal reflex	+	+	+	+	+	+	Slightly depressed	Suppressed	Suppresse
Visual response	+	+	?	?	?	?	No	No	No
Respiration...	Normal	Normal	Slightly increased	Increased	Increased	Increased	Increased	Increased	Increased
Pulse ...	Normal	Normal	Slightly increased	Slightly increased	Slightly increased	Increased	Increased	Increased	Increased
Temperature	Normal	Normal	Normal	Slightly increased	Slightly increased	Slightly increased	Slightly increased	Slightly increased	Normal
Involuntary voiding of faeces and urine	Yes	No	Yes	No	No	No	No	No	No
Analgesia ...	Very slight	Slight	Slight	Slight	More ?	+	++	+++	++++
Anaesthesia...	—	—	—	—	—	—	—	Slight	Slight
Duration till on feet	1 to 1½ hrs.	2 hrs.	2 to 3 hrs.	3 hrs. +	3½ to 4 hrs.	6 to 8 hrs.	8 to 12 hrs.	10 to 14 hrs.	12 to 18 hr
Salivation ...	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight

cated in small rodents which become excited, in dogs which may convulse at anaesthetic doses, and in horses which show anxiety and restlessness.

Yours faithfully,
J. H. WILKINS.

Medical Research Council,
Radiobiological Research Unit,
Harwell,
Didcot,
Berks.

July 7th, 1961.

Reference

JOHNSTONE, M., EVANS, V., & BAIGEL, S. (1959). *Brit. J. Anaes.* **31**. 433.

Antigenic Relationship of Some Bovine Viral Diarrhoea-mucosal Disease Viruses from the United States, Great Britain, and West Germany

Sir,—Several viruses which appear to be members of the viral diarrhoea-mucosal disease complex have been isolated in various parts of the world; however, the interrelationship of most of these agents has not been determined. As a preliminary step in this direction, we have initiated serum neutralisation studies to investigate the antigenic relationship of several members of this group.

Neutralisation tests were conducted against a bovine viral diarrhoea virus (Oregon C24V) originally isolated by Gillespie *et al.* (1960), from a field case of the disease in Oregon, U.S.A. The antisera used in our tests came from calves exposed experimentally to various viral diarrhoea-mucosal disease agents isolated in New York by Baker *et al.* (1954); in Iowa by Ramsey (1956); in North Dakota by Schipper and Noice (1959); in Indiana by Pritchard (1955); in England by Huck (1957); in West Germany by Stöber (1959); and in Scotland by Dow *et al.* (1956).

Oregon C24V virus was propagated in bovine embryonal kidney tissue culture. It had a titre of 1×10^6 plaque forming units per millilitre. Sera, previously heated for 30 minutes at 56° C., were diluted 1:10, 1:100, and 1:1,000; mixed with virus to give approximately 350 plaque forming units of virus per millilitre; and incubated at 25° C. for 1 hour. The per cent. of virus neutralised was quantitated by a plaque assay system using bovine testicular cell cultures as described by Kniazeff and Pritchard (1960).

All of the post-inoculation sera neutralised in high dilution the test virus (Table I). All tests were conducted on paired pre- and post-inoculation sera excepting those from Scotland, for which pre-inoculation samples were not available. The pre-inoculation sera were negative for all of the animals included in the table. However, among the sera from North Dakota, Iowa, and England there were specimens with low pre-inoculation virus neutralising titres, precluding the conclusion that the convalescent serum titre definitely resulted from infection with

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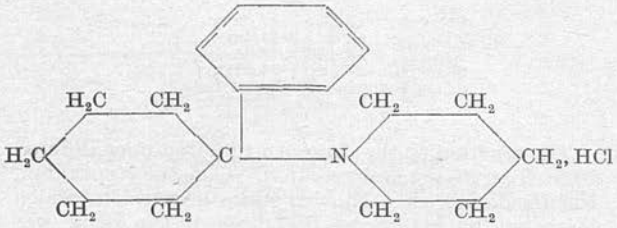
EFFECTS OF PHENCYCLIDINE ON THE RADIOSENSITIVITY OF MICE

By Lieut.-Colonel J. H. WILKINS and J. H. BARNES

Medical Research Council, Radiobiological Research Unit, Harwell, Berks.

PHENCYCLIDINE (Cl-395) I-(I-phenylcyclohexyl) piperidine monohydrochloride is a white solid, freely soluble in water and ethanol. It was synthesized in the Detroit Laboratories of Parke, Davis and Co. about four years ago.



The drug has undergone investigation in several animal species[1–6], revealing potent analgesic properties. It can be administered orally, intramuscularly or intravenously. The drug completely blocks somatic sensory perception[7] and causes vasomotor and respiratory stimulation. It acts on the central nervous system by stimulation or depression, the effect varying according to the species of animal and dosage used: excitement is predominantly shown in rodents whereas in most other species depression is the principal effect. Phencyclidine cannot be regarded as a hypnotic or a sedative, and is best classified as a psychotropic drug.

Previous studies with goats[5] showed promising results. As it was intended to use the drug as an induction analgesic agent in certain radiation experiments it was necessary to discover whether phencyclidine possessed any protective effects. With this objective in view experiments with mice were undertaken:

Experiments I and II were designed to discover the general effects of the drug on mice before and after irradiation. All injections were given intraperitoneally. Female mice of *R* stocks, weighing between 20 and 25 g, were used at the age of 14 weeks.

Experiment I. Effects at various dose-levels in unirradiated *R* mice

2 mg/kg and below: no apparent effect.

4–14 mg/kg: after a few minutes disoriented. In lower part of range, some signs of excitement. In higher part of range, often unable to stand. Effects lasting about 1 h.

20 mg/kg: after a few minutes, very unsteady and depressed. There was recovery after about 2 h.

80 mg/kg and higher: convulsions, collapse and death.

At the higher dose-levels, œdema of the lungs was noticeable at post-mortem.

The dose giving the greatest degree of depression was 20 mg/kg.

Experiment II. Effects at various dose-levels in lethally irradiated *R* mice

Groups of ten mice each were given respectively 2, 5, 20 and 60 mg/kg of the drug intraperitoneally. 15 min later they were exposed, in groups of five, to 1,025 r. X-irradiation, which normally gives more than 95 per cent mortality in controls within 30 days. Ten controls were included.

The conditions of the X-irradiation were as follows: 250 kV, 15 m.amp.; half-value layer, 1·2 mm copper; distance to underside of partitioned box, 60 cm: dose-rate, 73·6 r./min.

All five treatments (that is, controls, 2, 5, 20 and 60 mg/kg) were represented in each box during each irradiation.

Controls:	all died 8–13 days
2 mg/kg:	all died 9–13 days
5 mg/kg:	all died 5–12 days
20 mg/kg:	all died 6–14 days
60 mg/kg:	one died on first day; remainder died 7–24 days

The reaction to the drug of irradiated mice differed from that of the unirradiated. Generally speaking, the irradiated mice showed more depression, which increased in extent as the dose of the drug was increased.

2 mg/kg:	showed little effect
5 mg/kg:	caused disorientation with difficulty in standing
20 mg/kg:	partial collapse with depression
60 mg/kg:	more collapsed with greatest depression

It was decided that to detect any protective effect LD_{50} doses of X-irradiation would be necessary.

Experiment III. Effect of phencyclidine on the $LD_{50(30)}$ of mice exposed to X-irradiation

The mice were randomized with regard to sex, weight and age. Groups of ten mice were irradiated at a dose-rate of 54·5 r./min so as to receive a total dose of 650 r., 725 r., 800 r., 875 r. and 950 r. respectively in one fraction.

Control mice received 0·3 ml. of saline intramuscularly and the treated groups 0·3 ml. of a diluted solution of phencyclidine, so that each mouse received an equivalent of 4 mg/kg.

Irradiation took place 0·5 h after the injections and the X-ray beam was directed upwards towards the partitioned boxes. Treated and control mice were irradiated simultaneously.

The conditions of irradiation were as follows: 250 kV., 14 m.amp.; half-value layer, 1·2 mm copper; distance to underside of boxes, 72 cm; dose-rate, 54·5 r./min.

Table 1 and Fig. 1 give the mortality response of control and treated groups.

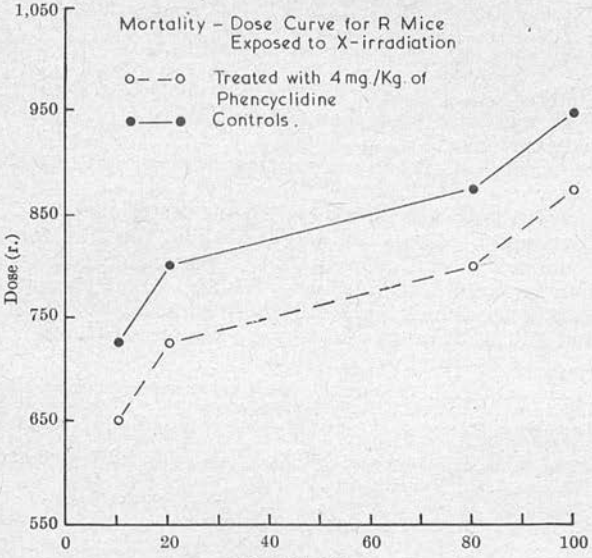


Fig. 1

Table 1. 30-DAY MORTALITY RESPONSE

Dose (r.)	Controls	Phencyclidine-treated
650	1/10	0/10
725	2/10	1/10
800	8/10	2/10
875	10/10	8/10
950	10/10	10/10
$LD_{50(30)} \pm S.E.$	752·5 r. ± 15·8 r.	828·0 r. ± 15·9 r.
Approx. 95 per cent limits of $LD_{50(30)}$	724 r. 785 r.	799 r. 861 r.

Table 2 gives the cumulative mortality over the period of 30 days.

Table 2. 30-DAY CUMULATIVE MORTALITY

Days after irradiation	Controls No. dead	Treated with phencyclidine (Cl-395) No. dead
8	5	—
10	3	—
11	1	1
14	16	13
15	1	3
16	2	2
18	1	—
21	1	1
28	—	1
30	1	—
Total	31	21

The $LD_{50(30)}$ for mice for this experiment was calculated to be as follows:

Controls: 752·5 r.
Phencyclidine-treated: 828·0 r.

The $LD_{50's}$ are significantly different ($P < 0{\cdot}001$). It was concluded that phencyclidine at 4 mg/kg increased the $LD_{50(30)}$ by 75·5 r. and had a slight protective effect on the mortality response of *R* mice.

We thank the Director, Army Veterinary and Remount Services, the War Office and the Director, Radiobiological Research Unit, Harwell, for permission to publish this article. We also thank Messrs. Parke, Davis and Co., who kindly supplied the drug, and Mr. M. J. Corp, who arranged the irradiations.

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APPENDIX II

RESTRAINT BY SEDATION IN GOATS

LIEUTENANT COLONEL J. H. WILKINS, B.SC., M.R.C.V.S., RAVC.

In general, the volatile anaesthetic agents are unsuitable for ruminants. They provoke copious salivation and bronchial secretion in addition to lessening or abolishing the swallow and cough reflexes, thus increasing the danger of inhalation pneumonia. However, the increasing use of goats for physiological study has led to the development and extensive use of cyclopropane and halothane in closed circuit anaesthesia (Jones *et al*, 1952; Wright *et al*, 1962). The use of non-volatile agents such as chloral hydrate (Yokozawa, 1953) pentobarbitone sodium (Gimbo, 1954; Whitrock, 1955) and thiopentone sodium (Titchen *et al*, 1949) have been reported but have not been extensively used in goats. Phencyclidine alone appeared to possess certain drawbacks (Wilkins, 1961), but it was considered that the simultaneous employment of phencyclidine with barbiturates or promazine would be an improvement.

As the specific problem in this study was one more of restraint rather than of anaesthesia, it was deemed important in recording the results to define the observed effects precisely only with regard to time. However, an attempt was made at the same time to assess systemic effects which were arbitrarily divided into three main kinds, namely sedation, narcosis and anaesthesia. Definitions of the terms used to describe the effects are considered necessary and may be summarised as follows :

"Anaesthesia" was employed when there was loss of consciousness, motor function and sensory perception. For convenience three degrees were recognized, namely, deep, medium and light.

"Narcosis" indicated severe cerebral depression ranging from imminent loss of consciousness to hypnosis. As before, three degrees were recognised but all manifested one common factor, namely, recumbency.

(i) Deep : the goat was always recumbent and in a state of deep sleep from which it could be roused only with great difficulty.

(ii) Medium : the goat was always recumbent and in a state of sleep from which it could be easily roused.

(iii) Light : the goat was recumbent usually with open eyes and could sometimes stand if helped but could not itself maintain the standing position.

"Sedation" was used to indicate light cerebral depression ranging from a profound stupor to an unawareness of surroundings. Again, three degrees were recognized but all had one common factor, namely, ability to stand and to maintain the standing position.

(i) Deep : the goat was usually recumbent but quite able to maintain the standing position if helped up and to walk unsteadily if forced to do so.

(ii) Medium : the goat was often found recumbent but stood up if forced to do so and could walk with only slight unsteadiness.

(iii) Light : the goat could stand up easily but had a vacant glassy stare in its eyes. If forced to do so it would walk fairly steadily and was amenable to man-handling.

Results and Discussion

(*The detailed results are presented in a series of tables* (*Tables I to VI*)).

Sedative Drugs Used Alone

Anaesthethesia

The barbiturates produced only comparatively short-lived anaesthetic effects whereas promazine had no effect.

TABLE I

Effects of Sodium Pentobarbitone on Goats

Goat No.	Wt Kg	Dose mg/Kg	Route	Time in hours: Anaesthesia D. M. L.	Narcosis D. M. L.	Sedation D. M. L.	Total Time Hrs.
F29	61	3	i.v.	– – –	– – –	¼ ⅛ ⅛	½
C315	68	4	i.v.	– – –	– – ⅛	⅛ ¼ ¼	¾
C234	74	6	i.v.	– – –	– ⅛ ⅛	¼ ¼ ¼	1
F36	60	8	i.v.	– – –	¼ ⅛ ½	– ¼ ¼	1⅛
F298	55	8	i.v.	– – –	⅛ ⅛ ¼	⅛ ¼ ¼	1⅛
C229	100	10	i.v.	– – –	⅛ ⅛ ¼	¼ ¼ ½	1½
F38	72	12	i.v.	– – –	¼ ¼ ¼	¼ ½ ½	2
F36	60	15	i.v.	– – ¼	¼ ¼ ¼	½ ½ ½	2½
F298	60	15	i.v.	– – ½	½ ¼ ¼	½ ½ ½	3
C234	75	15	i.v.	– – ⅛	½ ⅛ ⅛	¼ ¼ ½	1⅞
F29	60	15	i.v.	– – –	¼ ⅛ ⅛	⅛ ¼ ½	1⅜
F36	60	20	i.v.	– ⅜ ⅛	¼ ⅛ ½	¼ ½ ½	2⅜

D—Deep M—Medium. L—Light. i.v.—intravenous.

TABLE II

Effects of Sodium Pentobarbitone plus Phencyclidine on Goats

Goat No.	Wt. Kg	Dose and Route: Sodium P. Barb. mg/kg	Phen-cycl. mg/kg	Time in Hours: Anaesthesia D. M. L.	Narcosis D. M. L.	Sedation D. M. L.	Total Time Hrs.
C298	45	8 iv	2 im	– – ½	1 ½ ½	1 ½ ½	4½
F30	80	8 iv	0.5 im	– – ¼	¼ ½ 1	1 ½ 1	4½
F2	70	6 iv	1 im	– – –	¾ ¼ 1	½ ¼ ½	3¼
F48	75	6 iv	1 im	– – –	1 ¼ 1	½ ½ ½	3¾
M3	73	6 iv	0.5 im	– – –	⅛ ½ ½	¾ ½ ½	2⅞
F1	66	6 iv	0.5 im	– – –	¼ ¼ ½	½ ½ ½	2½
F3	71	6.5iv	0.75im	– – –	½ ¼ ¼	¾ ½ 1	3¼
F47	70	6.5iv	1 im	– – ⅛	¼ ¼ ¼	½ ½ ¾	2⅜
F45	70	6.5iv	1 im	– – –	¼ ½ ¼	1 1¼ 1	4¼
F49	73	6.5iv	1 im	– – –	– ½ ½	¾ ¾ 1	3½
C107	66	3.0iv	0.5 im	– – –	¼ ¼ ¼	½ ½ ½	2¼
C315	70	10.0iv	1 im	– – ½	¾ ¾ ½	1 ½ ¾	4¾

D—Deep. M—Medium. L—Light. iv—intravenous. im—intramuscular.

TABLE III

Effects of Sodium Thiopentone on Goats

Goat No.	Wt. Kg	Dose mg/kg	Route	Time in Hours: Anaesthesia D.	Anaesthesia M.	Anaesthesia L.	Narcosis D.	Narcosis M.	Narcosis L.	Sedation D.	Sedation M.	Sedation L.	Total Time Hrs.
F48	75	6.5	i.v.	–	–	–	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$	$1\frac{1}{8}$
C107	66	7.5	i.v.	–	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$	$1\frac{1}{2}$
C229	100	5.0	i.v.	–	–	–	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	1
C229	100	2.5	i.v.	–	–	–	–	–	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$
C118	110	4.5	i.v.	–	–	–	–	–	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	1
C298	45	11.0	i.v.	–	–	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$2\frac{1}{2}$
C229	100	10.0	i.v.	–	–	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	1	$\frac{1}{2}$	$3\frac{3}{4}$
F48	75	5.0	i.v.	–	–	–	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$1\frac{1}{2}$
C107	66	3.0	i.v.	–	–	–	–	–	–	–	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{3}{4}$
C118	110	8.5	i.v.	–	–	–	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	2
C298	45	5.5	i.v.	–	–	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$1\frac{3}{8}$
C118	110	12.0	i.v.	–	–	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$2\frac{3}{4}$

D—Deep. M—Medium. L—Light. i.v.—intravenous.

TABLE IV

Effects of Sodium Thiopentone plus Phencyclidine on Goats

Goat No.	Wt. Kg	Dose and Route: Sod. Th. mg/kg	Phen. mg/kg	Time in Hours: Anaesthesia K.	Anaesthesia M.	Anaesthesia L.	Narcosis D.	Narcosis M.	Narcosis L.	Sedation D.	Sedation M.	Sedation L.	Total Time Hrs.
F48	75	6.5iv	1 im	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	1	$2\frac{3}{4}$
C107	66	7.5iv	1 im	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	1	3
C229	100	5.0iv	1 im	–	–	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{3}{4}$	$2\frac{1}{2}$
C118	110	4.5iv	1 im	–	–	–	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	2
C234	70	7.0iv	1 im	–	–	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{3}{4}$	$2\frac{3}{4}$
F46	60	8.5iv	1 im	–	–	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$	1	$2\frac{5}{8}$
F48	75	6.5iv	0.5 im	–	–	–	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$1\frac{1}{2}$
C107	66	7.5iv	0.5 im	–	–	–	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$1\frac{1}{4}$
C229	100	5.0iv	0.5 im	–	–	–	–	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}$	$1\frac{1}{8}$
C118	110	4.5iv	1.5 im	–	–	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{8}$	$\frac{1}{2}$	$2\frac{1}{2}$
C234	70	7.0iv	1.25im	–	–	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{3}{4}$	1	$3\frac{7}{8}$
F46	60	8.5iv	1.5 im	–	–	$\frac{1}{8}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	1	$3\frac{1}{8}$

D—Deep. M—Medium. L—Light. iv—intravenous. im—intramuscular.

TABLE V
Effects of Promazine hydrochloride on Goats

Goat No.	Wt. Kg	Dose mg/Kg	Route	Time in Hours: Anaesthesia D. M. L.	Narcosis D. M. L.	Sedation D. M. L.	Total Time Hrs.
F46	61	1	i.m.	– – –	– – –	– – ½	½
C229	100	1	i.m.	– – –	– – –	– ½ ¾	1¼
C298	60	2	i.m.	– – –	– – –	– ½ ¾	1¼
C229	100	2	i.m.	– – –	– – –	– ½ 1	1½
F46	61	3	i.m.	– – –	– – –	¼ ½ 1¼	2
C229	100	3	i.m.	– – –	– – –	¼ 1 1	2¼
F38	73	1	i.v.	– – –	– – –	¼ ¼ ½	1
C229	100	1	i.v.	– – –	– – –	⅛ ¼ ⅜	¾
C229	100	2	i.v.	– – –	– – –	¼ 1 1	2¼
C298	60	2	i.v.	– – –	– – –	⅛ ¾ 1⅛	2
C118	110	3	i.v.	– – –	– – ¼	½ 1 1¼	3
C315	68.5	3	i.v.	– – –	– – ⅛	½ 1¼ 1¼	3⅛

D—Deep. M—Medium. L—Light. im—intramuscular. iv—intravenous.

TABLE VI
Effects of Promazine Hydrochloride plus Phencyclidine on Goats

Goat No.	Wt. Kg	Dose and Route: Promazine Hydrochlor mg/kg	Phencycl. mg/kg	Time in Hours: Anaesthesia D. M. L.	Narcosis D. M. L.	Sedation D. M. L.	Total Time Hrs.
F266	34.5	0.5 iv	0. 5iv	– – –	– – –	½ 1 ¾	2¼
C315	68.5	1 iv	0. 5iv	– – –	– – ½	½ ¾ ½	2¼
F46	61.8	1 iv	0.75iv	– – –	– – ½	¼ ½ ½	1¾
C299	100	1 iv	0.75iv	– – –	– – ¼	½ ½ 1¾	3
C315	68.5	1.5 iv	0.75iv	– – –	– ⅛ ⅝	½ ½ ½	2¼
C298	60	1.5 iv	0.75iv	– – –	– – ½	½ ½ 1	2½
C125	100	1.75iv	1 iv	– – –	– ¼ ¼	1 1 3	5½
C118	107	1.75iv	1 iv	– – –	– ⅛ ⅛	¾ 1 2½	4⅛
C229	100	2 iv	1 iv	– – –	½ ½ ¾	1 1 2½	6¼
F266	35	2 iv	1 iv	– – –	¼ ½ ¼	¾ 1 2	4¾
F31	62	3 iv	0.75iv	– – –	¾ ½ 1	¾ 1 1¾	5¾
F50	60	3 iv	1 iv	– – ⅛	½ ½ ¾	1 ½ 1½	4⅞
C315	60.5	1.5 iv	1 iv	– – –	¼ ½ ½	1 1 1	4¼
F40	50	2 iv	1 iv	– – ⅛	½ ⅛ ½	1 1 1¼	4½
C315	60.5	1.5 iv	1 iv	– – –	½ ½ ½	1¼ ½ 1	4¼
F30	75	1.7 iv	0.75iv	– – –	¼ ¼ ½	¼ ½ ½	2¼
F27	50	1.5 iv	1 iv	– – –	½ ½ ¼	1 1 1½	4¾
W302	70	1.5 iv	0.75iv	– – –	½ ¼ ½	1 ½ 1½	4¼
W316	60.5	1.25iv	0.75iv	– – –	⅛ ⅛ ¼	½ ½ ½	2
C125	100	1.5 iv	0.75iv	– – –	¼ ¼ ½	½ ½ 1	3
M4	70	2 iv	1 iv	– – ⅛	½ ¼ ½	¾ ¾ 1¾	4⅝
F46	70	2 iv	1 iv	– – ⅛	½ ⅛ ⅛	½ ¼ 1⅞	4
F50	60	1 iv	1 iv	– – –	– – ½	½ ½ 2	3½
F40	50	1.5 iv	1 iv	– – –	– – ¼	1 ½ 2	3¾
F37	51	1 im	1 im	– – –	– – –	½ ½ 1	2
C299	100	0.75im	1 im	– – –	– – –	– ½ 2½	3
M4	70	2 im	1 im	– – –	– – –	½ ½ 2	3
W302	70	3 im	1 im	– – –	– – –	1 1 2	4
F30	75	3 im	1 im	– – –	– – –	½ ½ 2	3
F27	50	2 im	1 im	– – –	– – –	¼ ¼ 1½	2
C315	60	1 iv	1 im	– – –	– – –	¼ ¼ 1¾	2½
F266	35	2 iv	1 im	– – –	– – ½	1 1 1½	4

D—Deep. M—Medium. L—Light. im—intramuscular. iv—intravenous.

Gimbo (1954) found that a dose of 24 to 27 mg/kg of sodium pentobarbitone produced anaesthesia for 20 to 30 minutes similar to the results reported here. Whitrock (1955) introduced a method of barbiturate anaesthesia for prolonged surgical procedures in the goat using low tracheotomy combined with small intermittent intravenous injections of sodium pentobarbitone. He reported that intubation and the tracheal aspiration of secretions by the oral route was impossible. In fact intubation in the goat is not difficult and was used whenever signs of progressive anoxia or excessive salivation was shown or expected. Hyoscine hydrobromide was used as an antisialic successfully in many goats. The standard dose was 3 mg (1/20 gr.) given subcutaneously five minutes before administration of the sedative drugs.

Narcosis

The barbiturates produced narcotic effects lasting from 10 minutes to 1 hour in duration but promazine had no such effects as defined above and in the doses used.

Sedation

The barbiturates produced sedative effects as defined above for periods lasting from ½ hour to 1½ hours whereas in the case of promazine these effects were prolonged in some cases up to 3 hours.

Sedative Drugs Combined with Phencyclidine

(*a*) Sodium Pentobarbitone and Phencyclidine

The total period of the effects was approximately trebled as compared with sodium pentobarbitone alone. This applied to the narcotic and sedative effects but not to the anaesthetic effect.

(*b*) Thiopentone sodium and Phencyclidine

The total length of the effects was increased 1½ to 2 times as compared with thiopentone sodium alone. The results, however, were more consistent than with Sodium Pentobarbitone.

(*c*) Promazine hydrochloride and Phencyclidine

The total period of the effects was increased up to as much as 6 times as compared with promazine hydrochloride alone. The reaction however, was more unpredictable.

Conclusion

The general conclusion was that phencyclidine alone, although it could effectively immobilise goats for almost any desired period, was not completely satisfactory because of the muscular tonicity produced. However, this drug combined with sodium pentobarbitone, thiopentone sodium or promazine hydrochloride was capable of producing a relaxed quiet and restrained goat as required.

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ENTERSORB—A CLINICAL TRIAL

(The information contained in this article has been compiled from notes obtained from Captain T. H. R. Davies, RAVC, and Captain A. I. Robertson, RAVC.)

A clinical trial was carried out at No. 1 War Dog Training Unit, RAVC to assess the value of Entersorb in the control of canine diarrhoea.

The investigators were particularly interested in its value in the control of the irritant diarrhoeas which are commonly encountered in kennels of war dogs.

Each Entersorb tablet contains 470mg activated attapulgite with 125mg Streptomycin base (as Sulphate B.P.)

Attapulgite, when specially activated, is claimed to be many times more powerful than kaolin and to be an extremely efficient physical trap for the bacteria, toxins and irritants which cause diarrhoea. The streptomycin content is claimed to be active against most causative pathogens.

The recommended dosage rate for large dogs (60 lbs and over) is 2 tablets *t.i.d.*

Thirty-six Alsatians, showing diarrhoea in varying degrees of severity were treated over a period of four months.

Entersorb treatment was administered in the following circumstances.

Group A

10 dogs—non-specific diarrhoeas.

Group B

2 dogs—diarrhoea as a secondary complication to canine distemper.

Group C

12 dogs—*induced* diarrhoea, caused by a sudden change of diet from fresh meat to canned meat.

Group D

12 dogs—as a diarrhoea prophylactic, prior to a sudden change of diet from fresh meat to canned meat.

Results

(It is regretted that a number of the completed case histories have been lost. The information provided has been compiled from the remaining records available).

GROUP A

Ser. No.	*Dog*	*Weight*	*Condition*	*Consistency of faeces*	*Dosage rate*	*Duration of Treatment*	*Result*
A1	E362	76 lbs	Gastro-enteritis	Watery, strong, smelling, very frequent	Four tabs b.i.d. for 48 hrs. Followed by three tabs for 48 hrs.	96 hrs.	Considerable improvement after 48 hrs.
A2	2D79 RAND	62 lbs.	Enteritis	Loose, strong smelling	Two tabs t.i.d.	36 hrs.	Cured
A3	2D02 BUSTER	96 lbs.	Enteritis	Very loose	Three tabs b.i.d.	36 hrs.	Cured

Intradermal
Tuberculin (PPD) and Johnin (PPD)
Tests

Goat No.	Sex	Breed	Age	Skin Measurements (mm)				Oedema	Result	Complement Fixation Test for Johnes Disease	Subsequent History and Disposal
				Before	After 72 hours Avian	Mamm.	Johnin				
ADULTS											
F1	F	BS	6	4	4	4	4	-	-	-	
F2	F	BTS	5	3	4	3	3	-	-	Doubtful	
F3	F	BS	6	4	9	6	4	C	Doubtful	-	Neg. later tests
F4	F	BTS	4½	4	23	11	16	E.O.	Positive	Doubtful	Destroyed Bacterial cultures positive for avian type but neg. for M. johnei
M1	M	BT	3	6	6	6	6	-	-	-	Irradiated
W153	F	BTS	4	2	2	2	2	-	-	Doubtful	
C229	CM	BS	3	3	3	3	3	-	-	-	Irradiated
C234	CM	BA	3	3	3	3	3	-	-	Doubtful	Irradiated
C240	CM	BA	3	3	10	19	10	E.O.	Positive	Doubtful	Irradiated. All later tests neg.
C136	CM	BS	3	3	3	3	3	-	-	-	
C315	CM	BS	3	3	3	3	3	-	-	Doubtful	Irradiated
C118	CM	BS	3	4	4	4	4	-	-	-	Irradiated
C107	F	BS	3	3	3	3	3	-	-	-	
C125	F	BS	3	3	3	3	3	-	-	Doubtful	
C200	F	BT	3	3	3	3	3	-	-	Doubtful	Irradiated
C298	F	BT	3	3	3	3	3	-	-	Doubtful	
M6	F	BS	4½	2	3	3	2	-	-	-	
M5	F	BS	5	3	10	7	9	D.O.	Positive	-	Pos. in later tests
M3	M	BS	5½	5	5	6	5	-	-	-	Irradiated
M2	M	BS	4½	4	4	4	4	-	-	-	
C293	F	BT	3	3	3	3	3	-	-	Doubtful	
W302	F	BT	3½	4	11	6	10	S.O.	Positive	Doubtful	Irradiated. All later tests neg.
M7	F	BS	6	3	4	3	3	-	-	Doubtful	
M8	F	BS	5	4	4	4	4	-	-	-	
W316	F	BSA	3½	3	3	3	3	-	-	Doubtful	Irradiated
C175	F	BA	3	3	3	3	3	-	-	Doubtful	Irradiated
C356	F	BS	3	3	3	3	3	-	-	Doubtful	
F11	F	BT	5	3	8	6	2	C.	Doubtful	Doubtful	Doubtful in later tests
F12	F	BS	6½	3	3	3	3	-	-	Positive	
F13	F	BS	3½	3	8	3	3	C.	Doubtful	-	Neg. in later tests
F14	F	BS	3	4	4	4	4	-	-	-	Irradiated
M9	M	BA	2	5	5	5	5	-	-	-	Irradiated
M10	M	BS	3	6	6	6	6	-	-	-	Irradiated
F20	F	BSA	4	3	3	3	3	-	-	-	Irradiated
F21	F	BT	4½	2	2	2	2	-	-	-	
F23	F	BS	3	2	2	2	2	-	-	-	
F24	F	BA	2½	3	24	6	16	E.O.	Positive	-	Irradiated PM showed caseous lymph nodes. Avian type bacilli recovered. Neg for M. johnei.
F25	F	BS	3½	3	3	3	3	-	-	-	
F27	F	BS	4½	4	4	4	4	-	-	-	Irradiated
F29	F	BTS	3½	3	3	3	3	-	-	-	

Results of Tuberculin, Johnin and Complement Fixation Tests (contd)

Goat No.	Sex	Breed	Age	Skin Measurements (mm) Before	After 72 hours Avian	Mamm.	Johnin	Oedema	Result	Complement Fixation Test for Johnes Disease	Subsequent History and Disposal
F30	F	BS	4½	3	3	3	3	-	-	-	Irradiated
F31	F	AN	6	3	3	3	3	-	-	-	Irradiated
F32	F	BTS	3½	3	3	3	3	-	-	-	
F34	F	BT	2	3	3	3	3	-	-	Doubtful	
F36	F	BS	2	3	3	3	3	-	-	-	
F37	F	BS	4	3	3	3	3	-	-	-	
F38	F	BS	4½	3	3	3	3	-	-	-	Pos. in later test
F38	F	BS	5½	3	9	5	8	S.O.	Positive	-	
F39	F	BS	4½	3	3	3	3	-	-	-	
F40	F	BS	4	3	8	3	5	C.	Doubtful	-	Neg. in later test after irradiation
M41	M	BS	2	6	6	6	6	-	-	-	
M45	F	BS	3	3	3	3	3	-	-	Doubtful	
F46	F	BS	2	3	3	3	3	-	-	Doubtful	
F47	F	BTS	4½	2	2	2	2	-	-	-	
F48	F	BA	5	3	12	8	11	D.O.	Positive	Doubtful	Pos. in later test
F49	F	BT	5	2	2	2	2	-	-	-	
F50	F	BS	4½	2	2	2	2	-	-	-	
F51	F	BT	4½	3	3	3	3	-	-	-	
F52	F	BS	4½	3	3	3	3	-	-	Doubtful	

KIDS and Goatlings (aged 9 months to 18 months)

59 kids and goatlings were tested twice and were negative in all cases to both conditions.

Tests were done at 9 and 18 months.

Annexure (ii)

Analysis of Fifty Two (52) random Samples of Normal Goat Urine

Goat No.	Sex	Breed	Age (yrs)	Appear.	Sp.G.	React.	Protein	Blood	Sugar	Bilirubin	Bile Salts	Acetone	Deposits
C229	CM	BS	4	LB	1022	Alk.	-	-	-	-	-	-	Casts (Epi)
F52	F	BS	5	LP	1002	Alk.	-	-	-	-	-	-	-
F3	F	BS	6	LB	1012	Alk.	-	-	-	-	-	-	Phos.
F31	F	AN	5	LB	1022	Alk.	-	-	-	-	-	-	Casts. (Hyal) Cryst.
F29	F	BTS	4	LB	1012	Alk.	-	-	-	-	-	-	Amorp.
F37	F	BS	4½	LP	1022	Alk.	-	-	-	-	-	-	Phos.
F1	F	BS	6½	LB	1012	Alk.	-	-	-	-	-	-	Casts. (Hyal)
M5	F	BS	5½	LB	1022	Alk.	-	-	-	-	-	-	Ditto Phos.
F13	F	BS	4½	LB	1012	Alk.	-	-	-	-	-	-	Casts (Hyal)
F48	F	BA	5½	LP	1002	Alk.	-	-	-	-	-	-	Phos.
F39	F	BS	5	LB	1002	Alk.	-	-	-	-	-	-	Casts (Hyal)
F20	F	BSA	5	LB	1022	Alk.	-	+	-	-	-	-	Casts (Epi) Cryst RBCs
F14	F	BS		LP	1002	Alk.	-	-	-	-	-	-	Phos.
F30	F	BS	5	LB	1012	Alk.	-	-	-	-	-	-	Casts (Hyal) Phos.
F320	M	BA	1	LP	1006	Alk.	-	-	-	-	-	-	-
F282	M	BS	1	LP	1002	Alk.	-	-	-	-	-	-	-
F217	M	BA	1	LP	1001	Neu.	-	-	-	-	-	-	-
F318	M	BS	1	LP	1006	Alk.	-	+	-	-	-	-	RBCs
F254	M	BS	1	LP	1002	Alk.	-	-	-	-	-	-	-
F315	CM	BS		TB	1002	Alk.	+	+	-	-	-	-	RBCs Cryst
F228	M	BS	1	TP	1006	Alk.	-	+	-	-	-	-	RBCs Cryst Phos.
F104	M	BT	1	LP	1003	Alk.	-	-	-	-	-	-	Casts (Epi) Cryst.
F316	M	AN	1	LP	1016	Alk.	-	-	-	-	-	-	Amorp.
F293	M	BT	1	LP	1002	Alk.	-	-	-	-	-	-	-
F261	M	BS	1	LP	1002	Alk.	-	+	-	-	-	-	RBCs
F248	M	BS	1	LP	1002	Alk.	-	-	-	-	-	-	Cryst
22	M	BS	1	LP	1015	Alk.	-	-	-	-	-	-	-
F45	F	BS	3	LP	1017	Alk.	-	-	-	-	-	-	-
F46	F	BS	2	LB	1008	Alk.	-	-	-	-	-	-	-
F267	M	BS	1	LP	1003	Alk.	-	-	-	-	-	-	Cryst
M19	F	BS	1	LP	1005	Alk.	-	-	-	-	-	-	-
F250	M	BS	1	LB	1010	Alk.	-	-	-	-	-	-	-
F40	F	BS		TP	1004	Alk.	-	-	-	-	-	-	-
F251	M	BT	1	LP	1003	Alk.	-	-	-	-	-	-	Casts (Gran)
C125	F	BS	3	LP	1060	Alk.	-	-	-	-	-	-	Phos Cryst

Analysis of Fifty Two (52) random Samples of Normal Goat Urine (contd).

Goat No.	Sex	Breed	Age (yrs)	Appear.	Sp.G.	React.	Protein	Blood	Sugar	Bilirubin	Bile Salts	Acetone	Deposits
W302	F	BT	4½	LP	1047	Alk.	-	-	-	-	-	-	-
F32	F	BTS	3½	LP	1022	Alk.	-	-	-	-	-	-	-
F27	F	BS	6	LB	1047	Alk.	-	-	-	-	-	-	Casts (Gran)
F47	F	BTS	4½	TP	1022	Alk.	-	-	-	-	-	-	Phos.
F50	F	BS	4½	LP	1047	Alk.	-	-	-	-	-	-	Casts (Hyal)
F51	F	BT	4½	LP	1007	Alk.	-	-	-	-	-	-	-
C200	F	BT	4½	LB	1008	Alk.	-	-	-	-	-	-	-
F24	F	BS	4½	LP	1010	Alk.	-	-	-	-	-	-	-
C175	F	BA	4	LP	1009	Alk.	-	-	-	-	-	-	-
C118	CM	BS	4	LP	1010	Alk.	-	-	-	-	-	-	-
C234	CM	BA	4	LB	1018	Alk.	-	-	-	-	-	-	-
W316	F	BSA	4½	LB	1020	Alk.	-	-	-	-	-	-	Casts (Epi)
C240	CM	BA	4	LP	1015	Alk.	-	-	-	-	-	-	Casts (Hyal)
M1	M	BT	4	LB	1018	Alk.	-	-	-	-	-	-	-
M3	M	BS	6½	LB	1019	Alk.	-	-	-	-	-	-	-
M10	M	BS	4	LB	1009	Alk.	-	-	-	-	-	-	-
M9	M	BA	3	TB	1016	Alk.	-	-	-	-	-	-	Casts (Gran) Cryst

LEGEND :

M	=	Male
CM	=	Castrated Male
F	=	Female
BA	=	British Alpine
BS	=	British Saanen
BT	=	British Toggenburg
AN	=	Anglo. Nubran
BTS) BSA)	=	"British"
LP	=	Limpid and Pale Yellow
LB	=	Limpid and Brown
TP	=	Turbid and Yellow
TB	=	Turbid and Brown

Casts (Epi)	=	Epithelial Casts
Casts (Hyal)	=	Hyaline Casts
Casts (Gran)	=	Granular Casts
Cryst	=	Various crystalline deposits
Phos.	=	Phosphates
Amorp.	=	Amorphous Deposits

Goat No.	Period Post Irrad.	Appearance	S.G.	Reaction	Protein	Glucose	Blood	Bilirubin	DEPOSITS
M10	During Irrad.	Dark Straw	1017	Alk.	-	-	-	-	
M10	½ hr	Pale Straw	1015	Alk.	-	-	-	-	
M3	½ hr	Pale Straw	1015	Alk.	+ 0.3g/1000ml	-	-	-	Casts (Hyal)
W302	½ hr	Dark Red Brown	1032	Alk.	+ 2.25g/1000ml	+ 2%	+	-	V. heavy green deposit. RBCs crystals
W316	½ hr	Pale Straw Turbid	1048	Alk.	-	+	+	-	RBCs
F40	½ hr	Pale Straw Clear	1003	Acid	+ 2.5g/1000ml	-	+		Greenish granular deposit. RBCs
C240	½ hr	Ditto	1010	Alk	+ 1g/1000ml	+	+	+	RBCs Granular Casts
F24	1 hr	Dark Red Brown Turbid	1044	Alk	+ 2.5g/1000ml	+	+	-	RBCs Granular Casts
C229	1 hr	Red Brown Turbid	1002	Alk.	+ 4.5gm/1000ml	-	+	-	RBCs, Casts (Epi) and Cells
M3	2 hrs	Pale, Straw Clear	1015	Alk.	-	-	-	-	Casts (Epi)
F30	2 hrs	Dark Red Brown Turbid	1047	Alk.	+ 4g/1000ml	+	+	-	RBCs crystals
C118	2½ hrs	Beige Turbid	1042	Alk	+ 2g/1000ml	+	+	-	RBCs
F27	3 hrs	Light Red Brown Turbid	1022	Alk	+ 0.758/1000ml	-	+	-	RBC
W316	3 hrs	Light Straw Turbid	1052	Alk.	-	+	+	-	RBC Granular Casts
F315	4 hrs	Red Brown Turbid	1101	Alk.	+	+	+	-	RBC Granular Casts Crystals, Cells
F27	4 hrs	Light Brown Turbid	1027	Alk.	+	-	+	-	RBC Cells
F14	4 hrs	Pale Straw Clear	1013	Alk.	-	-	-	-	Casts (Epi)
C200	4 hrs	Light Brown Turbid	1018	Alk.	-	-	-	-	Casts (Epi)
M9	4 hrs	Pale Straw Clear	1015	Alk.	-	-	-	-	Casts (Epi)
M1	4 hrs	Dark Straw Clear	1016	Alk.	-	-	-	-	Casts (Granular)
F30	5 hrs	Pale Straw Turbid	1060	Alk.	-	+	-	+	Crystals (Uric Acid)
W316	6 hrs	Light Brown Turbid	1012	Alk.	-	-	+	-	RBCs
C118	1 day	Dark Brown Turbid	1052	Alk.	+ 2gms/1000ml	+	+	-	RBCs Cells
C234	2 days	Pale Straw Clear	1020	Alk.	+ 0.3g/100ml	+	+	+	RBCs
C118	2 days	Straw Clear	1018	Alk.	+ 0.38g/1000ml	-	+	+	RBCs Casts (Hyal)
F20	4 days	Dark Red Turbid	1031	Alk.	+ 1g/100ml	+	+	-	RBCs Casts (Hyal)
C234	5 days	Dark Red Turbid	1060	Alk.	+ 0.1g/1000ml	+	+	+	RBCs
F27	6 days	Straw Clear	1027	Alk.	-	-	-	-	Casts (Epi)

Urinalysis of Irradiated Goats (contd).

Goat No.	Period Post Irrad.	Appearance	S.G.	Reaction	Protein	Glucose	Blood	Bilirubin	DEPOSITS
F30	6 days	Dark Red Turbid	1052	Alk.	+	-	-	-	Crystals
F30	7 days	Orange Clear	1047	Alk.	+ .3g/1000ml	-	-	-	Crystals Cells
F30	8 days	Orange Clear	1062	Alk.	+ 2g/1000ml	-	-	-	Crystals Cells
F315	11 days	Dark Red Turbid	1005	Alk.	+ 6g/1000ml	-	+	+	RBCs
F30	12 days	Orange Clear	1082	Alk.	+	-	-	-	Crystals and Cells
C118	16 days	Red Brown Clear	1041	Acid	+ 4g/1000ml	-	+	+	RBC Casts (Epi)
F315	27 days	Straw Turbid	1080	Alk.	-	+	-	-	Crystals
F315	29 days	Straw Turbid	1040	Alk.	+	-	+	-	RBCs, Cells and Casts (Epi)
F315	30 days (day of death)	Light Red Turbid	1050	Acid	+ 3g/1000ml	+	+	-	RBCs, Cells and Casts (Epi)
F27	34 days	Dark Brown Turbid	1015	Alk.	+ 6g/1000ml	-	-	-	Casts (Gran)
F27	42 days	Straw Clear	1022	Alk.	+ 3g/1000ml	-	-	-	Casts (Hyal)

" Southern Warrior "

Sir,—May I seek, through the courtesy of THE RECORD, the owner of a yellow labrador dog, " Southern Warrior "?

The owner, who is a veterinary surgeon, was on holiday in Morar during October.

Yours faithfully,

444, Shields Road, ALEX. THOMSON.
Glasgow, S.1.
February 15th, 1962.

Experimental Copper Poisoning in Pigs

Sir,—I refer to a paper entitled " Experimental Copper Poisoning in Pigs " by Allen and Harding in your issue of February 10th, 1962, and very much deprecate the fact that the authors, having chosen to use a proprietary product (XF Minsal) in an experiment designed specifically to produce toxicity in pigs, have not had the courtesy to point out that the level which they required to use to achieve toxicity was greatly in excess of the rate of usage recommended by the manufacturer. I think that this is a matter which must be put right in the strongest possible terms.

The facts are these:—

The supplement in question (XF Minsal) is recommended to be included in pig foods at a maximum rate of 2½ per cent. at which level the contribution of copper to the finished food amounts to 250 p.p.m. I need hardly point out to your readers that this is an accepted level of addition and has been shown to be perfectly safe by overwhelming scientific evidence. In the experiment in question it was found that to produce toxicity a level of 1,000 p.p.m. of copper was required which meant that the mineral supplement had to be included at the rate of 10 per cent., a level which of course is 4 times the rate of usage recommended. The authors mentioned in the paper that the meal containing this level of addition was rather unpalatable to the pigs which is hardly surprising since no less than 2 cwt. per ton of minerals were included. Not only was copper included in great excess but also all other mineral elements including sodium chloride. It would also be interesting to know how the basic diet was amended to accommodate 2 cwt. of minerals or did the authors consider that such an amendment was unnecessary?

In practice no one in their right senses would include minerals at this rate and the possibility of such a level being included even in error is so remote as to be non-existent.

Yours faithfully,
for Minsal Limited,

Minsal Limited, J. CASSIDY,
Victoria Works, *Director*.
Wincham,
Northwich,
Cheshire.
February 16th, 1962.

Cysticercus tenuicollis: Unusual Site in a Goat

Sir,—According to the text-books the intermediate stage of the dog tapeworm (*T. hydatigena*) known as *Cysticercus tenuicollis* occurs normally in the peritoneal cavity of sheep, goats, cattle and sometimes in man, but occasionally is to be found in other sites (Monnig, 1956). The following case report is presented in support of this.

Subject. 6-year-old British Saanen goat bought from a dealer in April, 1961. The animal was perfectly healthy and in very good condition until February 9th, 1962, when it developed characteristic clinical signs.

The first thing noted was a frothing at the mouth. On examination, very sharp molar teeth lacerating the gums may have been the cause. However, concomitantly with this frothing, nervous symptoms developed which showed themselves in an apparent blindness, and turning of the head first one way and then to the opposite direction. Sometimes the animal would bend its head directly backwards so that the horns touched the back, and would then subside on to the floor. When this happened the goat was unable to rise by itself and had to be helped to its feet. If it were near a wall it would occasionally press its head against it. The signs were typical of " gid " or " sturdy " (*Coenurosis cerebralis*) in sheep and a tentative diagnosis was made on these lines. The animal died within 24 hours of showing the first symptoms.

On *post-mortem* examination the only pathological features were ecchymotic subendocardial haemorrhages in the left ventricle of the heart and inflammation of the abomasum (slight) and duodenum (severe). One mucoid globular vesicle of 4 to 5 cm. in diameter was found embedded in the omental fat. When the cranial cavity was opened another, similar vesicle, was extruded. No other vesicles were found in the carcase.

Identification of the bladder worm was easy and this was confirmed by the A.R.C. Field Station at Compton, Berks.

Comment. It is a mystery how the bladder worm developed within the cranial cavity without producing detectable clinical signs during the 10 months the animal was in our care.

Yours faithfully,
J. H. WILKINS.

Medical Research Council Radiobiological Research Unit,
Harwell,
Didcot,
Berks.
February 15th, 1962.

Reference

MONNIG, H. O. (1956). " Veterinary Helminthology and Entomology." 4th ed. Baillière, Tindall & Cox, London.

Measuring Sticks

Sir,—May I suggest that those who will be measuring ponies and horses for the coming showing season have their measuring sticks checked for accuracy now?

Yours faithfully,

Berkeley Lodge, E. F. BECKETT.
Blandford,
Dorset.
February 12th, 1962.

be aware that there are many in these islands with a specialised knowledge of veterinary bacteriology and pathology, and some with such specialised knowledge in the equine field, who would be willing to assist and advise their non-specialist professional colleagues in the preparation of relevant technical papers.

Yours faithfully,
P. E. MULLANEY.

Department of Veterinary Pathology,
University College,
Dublin.
February 15th, 1962.

Reference

MULLANEY, P. E. (1947). *Irish Vet. J.* **1**. No. 2.

Blood and Serum Transfusion in Cattle

Sir,—Mr. Swarbrick's technique of blood transfusion in cattle is indeed simple, and has much to commend it for use in an emergency. I feel, however, that for less urgent cases the following points might be worthy of consideration.

Whilst the size of needle used for collection is often a matter of personal taste, the smaller the hole made in the donor cow's jugular vein the better. I use needles of 2 mm. or 3 mm. bore, and make a skin incision ½ inch long. Leakage after withdrawing the needle is negligible, and a skin suture is not required. The needle is treated with silicone to prevent clotting, and a short length of polythene tubing is attached to reduce wastage and contamination to an absolute minimum.

If the anticoagulant solution is being prepared some time in advance, sodium acid citrate is preferable to sodium citrate because of its greater stability during sterilisation. If there is to be any delay between collection of the blood and transfusion, the addition of dextrose to the anticoagulant delays haemolysis of the red cells.

Prevention of clotting in the receiving bottle is naturally dependent on thorough mixing, and in deciding the composition of the citrate solution one must strike a happy medium between too much mixture—causing excessive dilution of the blood—and too little, making thorough mixing difficult. I find the following solution quite satisfactory: sodium acid citrate 8 per cent., dextrose 10 per cent. 150 ml. of this acid citrate dextrose (A.C.D.) mixture is sufficient for 2 litres of blood.

The use of several 500-c.c. or 1-pint bottles instead of one or two Winchesters has several points in its favour. It facilitates mixing of blood and A.C.D. mixture; it enables the blood to be kept warm during collection and transfusion; and it imposes less strain on the arms of the operator.

For transfusion I use a Capon Heaton disposable plastic giving set as used in most hospitals. This contains a fine-mesh filter, and a drip chamber giving constant indication of blood flow, and it is easily transferred from an empty to a full bottle without the danger of air bubbles becoming trapped.

It is unfortunate that Mr. Swarbrick does not give more details of his cases. In discussing the rate of transfusion he does not differentiate between surgical and medical cases, or discuss the important question of circulating blood volume. It is generally accepted that transfusion must be carried out very slowly when the circulating blood volume is not reduced, but clinical evidence on this point is conflicting. I have twice witnessed signs of over-transfusion in adult cows with piroplasmosis after giving 4 litres in 1½ hours. Haemoglobin levels before transfusion were 2.7 g. per 100 ml. and 2.9 g. per 100 ml. respectively.

On the other hand, I have heard of workers routinely transfusing 4 litres in 20 minutes for the same condition.

Yours faithfully,
A. H. PILL.

55, South Court Avenue,
Dorchester,
Dorset.
February 17th, 1962.

Protection Against Canine Distemper in Great Britain

Sir,—In his letter of November 7th, 1961 (**73.** 1,182), Mr. J. K. Bateman quotes figures which he believes support the view that 9 weeks old is a suitable time to vaccinate Greyhound puppies against distemper. He states there has not been one case of breakdown of immunity over the last 7½ years. Nothing is stated to imply that the dogs ever came into contact with active infection, and failing knowledge of such contact the reader is unable to judge whether, in fact, they had any immunity at all.

In her letter of November 18th, 1961 (**73.** 1,230), Miss J. O. Joshua implies that as she vaccinates at 11 weeks or more the dogs become solidly immune and states: "I can thus still record only the one genuine breakdown . . ." As she presumably does not know how many of the dogs she vaccinated met infection and how many of these succumbed and were not presented to her for treatment, her single recorded case may not be a significant figure. According to the figures given by R. E. Ablett and L. A. Baker in their letter of February 10th, 1962 (**74.** 188), 4.5 per cent. of the number of litters vaccinated at 12 weeks of age would be unlikely to be satisfactorily immunised.

While Baker's nomograph is something to work from, J. R. Gorham (1960) states: "Although there is no contrary evidence, the critical titres designated by the Cornell workers for either success or failure in puppy immunisation appear too closely delineated. Thus, there is essentially no allowable variation in the test itself or in the immunisability of the dog."

I wish to make the suggestion that all contributions on this subject should be scrutinised by a virologist to prevent needless confusion and publication of uninterpretable results.

Yours faithfully,
IAN M. LAUDER.

University of Glasgow Veterinary School,
Veterinary Hospital,
Bearsden Road,
Bearsden,
Glasgow.
February 14th, 1962.

Reference

GORHAM, JOHN R. (1960). *Advances in Veterinary Science.* **6**. 287.

the Air Research and Development Command, working as Chief of the Biomedical Branch, under the Assistant for Life Sciences, Headquarters ARDC. However, Lt. Colonel Charles M. Barnes in USAF, Aircraft Nuclear Propulsion Office, and Captain George D. Smith, Head, Bio-Technology of the National Aeronautics and Space Administration are also administering and managing extensive research and development programmes.

In summary, three types of veterinary activities in research and development have been discussed. The first and by far the most extensive, and probably the most important to the veterinary profession, is that of direct assistance in research which requires animals. This has been subdivided into several parts. Secondly, the veterinarian acts as the principle scientific investigator of certain phases of bio-medical research and reports his work in the scientific literature. And lastly, a few veterinarians act as administrators and managers of biomedical research and development programmes.

These, then, are the means by which veterinarians in research are contributing to the fruits of the tree of knowledge—another example of understanding the nature of things and making use of basic principles for man's own benefit.

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OBSERVATIONS ON NORMAL GOAT BLOOD

by

LIEUT. COLONEL J. H. WILKINS, R.A.V.C.

and

CORPORAL R. R. D. H. HODGES, R.A.M.C.

This study is based on blood obtained between March and September 1961 from 66 goats, consisting of 60 adults (48 females, 6 males and 6 castrated males) aged 3 to 5 years and 6 male kids aged 18 weeks.

The data cannot be considered as " standards " in any way but may possibly add to the general information on goat blood.

Methods

Blood examinations and counts were done on blood drawn from the jugular vein. Sequestnin was used as the anti-coagulant. Counting was done in improved Neubauer chambers. Two hundred wbcs were counted in every case. Haemoglobin was estimated as oxyhaemoglobin in the MRC grey-wedge photometer. Blood smears were stained with Leishman's stain and two hundred cells were estimated in differential counts. The packed cell volume (PCV) was arrived at using a micro-haematocrit centrifuge for ten minutes. Platelets were counted using a phase contrast microscope according to the method of Brecker and Cronkite (1950). Erythrocytic indices (MCH, MCHC and MCV) were calculated using conventional methods.

Cell Morphology

The polymorphonuclear leucocytes were indistinguishable from the cells in the peripheral blood of other animal species as described by Holman (1944) and others.

The lymphocytes were of two kinds. Large lymphocytes were often more common than small lymphocytes. The small lymphocyte consisted of a purple reticulated nucleus surrounded by a narrow ring of light blue clear cytoplasm. The large lymphocyte was often twice the size or more than the small lymphocyte. The nucleus was pinkish blue in colour, often eccentric, surrounded by an irregular very light blue clear cytoplasm.

TABLE I—60 Adult Goats

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	8.46 20.46	8.0 15.3	17.5 39.5	13.2 27.7	6.0 13.0	27.3 52.6	189 785	3.3 14.0	17.5 68.8	23.3 80.3	0.0 9.0	0.3 11.4
Mean	14.30	11.5	29.4	20.9	8.2	39.1	421	8.30	41.8	53.9	1.9	2.2
S.D.	2.8	1.7	5.2	3.0	1.6	4.2	134	2.4	14.1	15.1	1.4	2.2

TABLE II—6 Adult Male Goats

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	10.99 18.12	8.0 12.6	19.0 32.0	16.0 21.1	6.1 8.2	36.7 47.3	298 535	3.8 13.2	30.2 66.7	29.2 56.8	0.1 9.0	0.3 10.9
Mean	14.95	10.6	27.2	18.1	7.2	39.5	415	9.2	51.9	40.3	2.7	3.3
S.D.	2.4	1.6	5.2	1.7	0.8	3.6	84	3.5	11.7	9.1	1.1	3.9

TABLE III—6 Adult Castrated Male Goats

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	13.16 18.94	11.2 15.3	29.0 38.0	20.0 22.8	7.4 8.6	34.6 40.3	386 785	6.3 9.7	17.5 41.6	55.0 80.3	1.5 3.6	0.4 1.4
Mean	16.34	13.1	34.8	21.4	8.1	37.7	582	7.7	30.5	65.9	2.6	0.9
S.D.	2.1	1.2	3.8	0.8	0.5	2.1	152	1.2	8.3	9.4	0.9	0.4

TABLE IV—48 Adult Female Goats

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	8.46 20.46	8.0 14.5	17.5 39.5	13.2 27.7	6.0 13.0	27.3 52.6	189 780	3.3 14.0	20.0 68.8	23.3 77.4	0.0 7.0	0.3 11.4
Mean	13.94	11.4	28.9	21.1	8.4	39.6	400	8.2	42.4	54.1	1.7	2.2
S.D.	2.8	1.6	5.1	3.1	1.6	4.4	139	2.4	13.9	15.1	1.4	1.9

TABLE V—6 Male Kids

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	18.02 20.78	11.10 12.80	29.5 35.0	14.9 19.4	5.3 7.1	33.7 42.1	410 680	8.2 15.9	21.5 41.0	57.0 76.6	0.5 2.6	0.2 1.5
Mean	19.15	11.7	31.9	16.7	6.1	36.7	517	11.7	29.8	68.1	1.3	0.7
S.D.	1.17	0.7	1.9	1.7	0.7	2.8	88	3.1	5.4	6.7	0.8	0.5

TABLE VI—Holman (1952 ; 1956)

Statistic	R.B.C. 10^6/cmm.	Hb. gm/100ml.	P.C.V. %	M.C.V. c u	M.C.H. u ug	MCHC %	Platelets 10^3/cmm.	WBC 10^3/cmm.	Polys %	Lymphs %	Mono %	Eosins %
Range	7.9 19.9	7.5 15.7	14.0 38	15.0 35	— —	— —	— —	— —	— —	— —	— —	— —
Mean	12.6	11.5	27.7	22.4	—	42.0	—	7.7	50.3	43.7	3.6	2.3
S.D.	2.6	1.8	5.0	3.6	—	5.3	—	2.7	10.7	10.9	3.2	2.5

The differentiation of the large lymphocyte from the monocyte with a round nucleus was often difficult.

Results and Discussion

Table I to V give the results of the findings.

Table VI from Holman (1956) has been inserted as a comparison.

Perhaps the most significant item is the wide range found in certain parameters in the adult goats. This is probably accounted for by the fact that these goats had only recently been obtained from a dealer. Their state of nutrition and helminth burden differed considerably. In contra-distinction the kids and castrated males were a more homogenous group obtained through the ARC Field Station, Compton, Berks.

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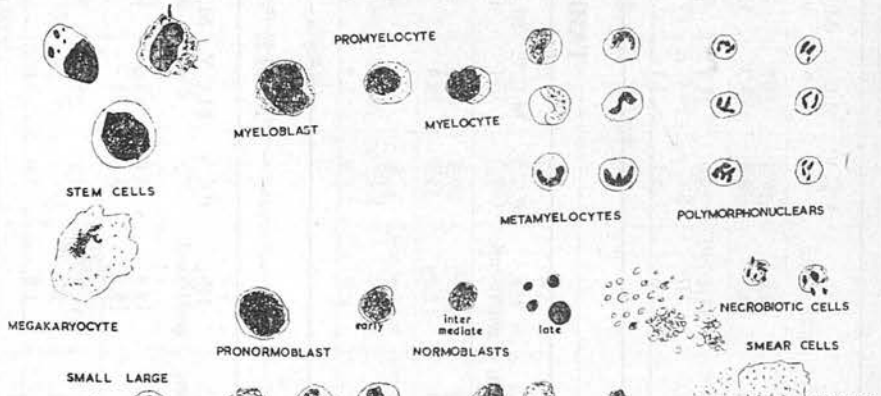
OBSERVATIONS ON THE BONE MARROW CELLS OF NORMAL GOATS

by LIEUT. COLONEL J. H. WILKINS, RAVC

A technique for the biopsy of bone marrow in goats has already been described (Wilkins, 1962).

The description to follow is based on the examination of bone marrow smears made from biopsy material and stained with Jenner-Giemsa or Leishman's stain. The cells were classified according to Ferrata and to Whitby and Britton (1957).



PRIMITIVE CELLS

Stem Cells were very scarce and two kinds were recognised.

Type (*i*) These were usually oval in shape, with a deep blue nucleus composed of a reticulum of chromatin with nucleoli often visible. The cytoplasm was very pale blue often containing darker pyknotic bodies.

Type (ii) These were round or irregular in shape with a round nucleus surrounded by a blue rim of cytoplasm. The nucleus was purple in colour and contained a number of nucleoli.

MYELOID CELLS

Myeloblasts were not common but were comparatively easy to differentiate. The nucleus was round in shape and placed centrally being surrounded by a rim of clear blue cytoplasm. It was dark blue in colour showing a reticulated chromatin network structure, and contained four to eight nucleoli. Sometimes a peri-nuclear clear area was recognizable.

Promyelocytes were uncommon. The nucleus was round and usually in the centre of the cell. It had a similar appearance to the nucleus of the myeloblast but contained fewer nucleoli. A rim of blue cytoplasm with easily visible granules served to differentiate the two types.

Myelocytes, Metamyelocytes and Polymorphnuclear Cells were classified as neutrophilic, eosinophilic or basophilic according to the staining affinity of the characteristic granules together with the shape and structure of the nucleus.

Myelocytes

The nucleus of the myelocyte was round but usually eccentric containing one or two nucleoli. Granules were distributed throughout the blue cytoplasm. Often they extended over the nucleus and obscured its outline. This was particularly noticeable in the infrequent eosinophilic and the rare basophilic myelocyte.

Metamyelocytes

For description and identification purposes these were divided into two types :—(*i*) young (*ii*) old

(*i*) **Young metamyelocytes** possessed elongated nuclei which were eccentric in position with a distinct indentation in the middle. This indentation varied in degree depending on age : the younger had a mere depression (kidney-shaped nucleus) whereas the older form looked like capital U. (U-shaped nucleus). There were no nucleoli. The cytoplasm tended to be paler than seen in the promyelocytes or myelocytes. Granules were sometimes seen lying over the nucleus and the cytoplasm of the kidney-shaped forms but were confined to the cytoplasm in the older U-shaped forms.

(*ii*) **Old metamyelocytes** were very common and possessed S-shaped or W-shaped elongated eccentric nuclei. The cytoplasm was faint pink-blue in colour and the brownish granules were often difficult to distinguish.

Polymorphonuclear Cells

These were olbulated forms and indistinguishable from the mature cells of the peripheral blood.

ERYTHROID CELLS

The main characteristic of the cells of the erythroid series was the round appearance of the nucleus, both in mature and immature forms. In addition, the nucleus occupied most of the cell leaving a thin rim of cytoplasm in the more immature forms or little or no visible cytoplasm in the older forms. The nucleus always took on a purple-blue appearance and the size became progressively smaller as maturation occurred.

Pronormoblasts

The nucleus was a course purple-blue network within which two to six nucleoli were distinguishable.

The cytoplasm consisted of a closely-fitting navy-blue ring, sometimes irregular, around the nucleus.

Early Normoblasts

The nucleus was made up of dark blue whorls of chromatin against a faintly pink background. No nucleoli were ever seen.

The cytoplasm consisted of a rim of blue around the nucleus.

Intermediate Normoblasts

The nucleus was seen as a whorl or " cart-wheel " of dark blue chromatin against a faintly pink background. Sometimes the chromatin appeared as clumps not unlike squashed-up damsons and quite characteristic.

The cytoplasm was sometimes not discernible but usually appeared as a shred of blue around the circumference.

Late Normoblasts

The nucleus was a deep pyknotic mass staining purple or black. The cytoplasm was usually not seen or appeared as a thick black rind. The cells were much smaller than any others seen.

Immature Erythrocytes

A large number of normal-sized red blood cells were always seen showing small round blue spots (punctate basophilia) and often macrocytes were observed stained a greyish-blue (polychromasia). However, reticulocytes, Cabot rings or Howell-Jolly bodies were never seen.

MISCELLANEOUS CELLS

Lymphocytes

Only a comparatively few mature lymphocytes were recognized. They were of two kinds : (*i*) **small** lymphocytes had a purplish reticulated nucleus surrounded by a narrow ring of light blue clear cytoplasm. (*ii*) **large** lymphocytes consisted of a blue to pinkish-blue nucleus with an irregular light blue clear cytoplasm, often showing a few pink granules. The differentiation of the large lymphocyte from the monocyte with a round nucleus was often difficult.

Monocytes

No immature monocytes could be recognized and only a few mature forms were seen. The nucleus was bean-shaped or treble-lobed sometimes rounded and consisted of a homogeneous light blue nucleus, surrounded by a pale grey-blue amorphous cytoplasm.

Plasma Cells

These cells were not common. The nucleus was round or flattened and placed at one end of the cell : it consisted of a blue mass in the mature cell but was pinkish-blue and reticular in structure in the immature plasma cell. The cytoplasm was pinkish-blue and often had a clear space adjacent to the nucleus.

Megakaryocytes

The nucleus was usually large and blue in colour, often lobulated and at times consisting of separate pieces.

The cytoplasm was pinkish-blue in colour with many more deeply staining small particles giving the whole structure a speckled appearance. Fractured cells extruded the particles (platelets) in a characteristic manner which showed up clearly as speckled mauve bodies.

Mast Cells

These were rare and appeared as small basophilic cells containing a lobed nucleus and a blue granular cytoplasm.

Necrobiotic Cells

These cells, in the process of destruction, showed the nucleus condensed to a pyknotic mass or consisting of a number of small discoloured spots extruding from a fractured cell-membrane.

Smear Cells

These were blue to pink masses of cytoplasm often partly enveloping red blood cells and produced in the process of making smears from the bone marrow aspirate. Their numbers were a good indication of the efficiency of smear making.

Mitotic Cells

Immature cells of both the myeloid and erythroid series showing mitosis were seen although the numbers of erythroid mitotic forms were more frequent. All phases of mitosis were observed.

RESULTS AND DISCUSSION

The percentage mean marrow cell counts of six 4-year-old adult goats is given in the accompanying Table.

The animals consisted of two females (British Toggenburgh) and four castrated males (one British Alpine and three British Saanen). One thousand cells from sternal bone marrow aspirate smears were differentiated in each case according to the classification as given in the text. The proportion of the erythroid (E) to the myeloid (M) elements varied from 3.5 to 1 to 2.0 to 1 with a mean value of 2.6 to 1. Whereas the M/E Ratio in sheep as given by Grunsell (1951, 1955) was found to vary from 0.77 to 1 to 1.68 to 1 with a mean value of 1.1 to 1, the M/E Ratio for the goat has been found in this series to vary from 0.3 to 1 to 0.48 to 1 with a mean value of 0.38 to 1. It is interesting to note that in man the ratio varies from 8 to 1 to 2 to 1 (Whitby and Britton 1957).

The incidence of lymphocytes and monocytes is also higher than in sheep. Neutrophilic metamyelocytes make up the bulk of the myeloid series in both sheep and goats.

The low incidence of eosinophilic cells is considered to be an indication of the freedom of the goats from helminth parasites.

SUMMARY

The morphology and the mean percentage of the cellular composition of the sternal bone marrow aspirates of six adult goats is given. Comparison with sheep is briefly discussed.

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EDITOR'S NOTE

Lieut. Colonel J. H. Wilkins, who is enroled as a Ph.D., student at Edinburgh University, is now working at the Radio-biological Research Unit, Harwell, on the general effects of various types of irradiation on large animals.

His articles are the first of a series that we hope to publish in the Royal Army Veterinary Corps Journal.

The work reported here is a section of a wider research programme on the effects of radiation on large animals upon which the writer is engaged under the direction of Dr. J. F. Loutit (Director, Radio-biological Research Unit,

M.R.C., Harwell) and Dr. J. R. Greening, (Director, Medical Physics Unit, University of Edinburgh).

DIFFERENTIAL BONE MARROW CELL COUNT MEANS AND RANGES FOR 6 NORMAL ADULT GOATS

Cells	Range %	Mean %
Stem Cells	0.0 to 2.0	0.65
Myeloblasts	1.4 to 1.7	1.40
Promyelocytes	0.2 to 1.1	0.50
Myelocytes (Neutrophilic) ...	0.4 to 1.8	1.40
,, (Eosinophilic)	0.3 to 0.8	0.47
,, (Basophilic)	0.0 to 0.3	0.08
Metamyelocytes (Neutro) ...	5.5 to 18.7	10.55
,, (Eosino)	0.4 to 1.8	0.93
,, (Basophilic) ...	0.0 to 0.2	0.05
Polymorphonuclears (Neutro) ...	4.4 to 14.7	8.45
,, (Eosin) ...	0.0 to 1.35	0.36
,, (Baso) ...	0.0 to 0.0	0.00
Total MYELOID (M)	18.9 to 27.5	24.15
Pronormoblasts	0.75 to 5.0	2.48
Normoblasts (Early)	3.3 to 8.2	5.43
,, (Intermediate) ...	10.8 to 26.75	19.73
,, (Late)	29.0 to 42.3	35.37
Total ERYTHROID (E)	56.5 to 68.0	63.01
Lymphocytes	1.8 to 5.5	3.50
Monocytes	0.0 to 6.0	2.00
Plasma Cells	0.0 to 0.5	0.27
Mast Cells	0.0 to 0.1	0.03
Megakaryocytes	0.0 to 3.2	0.77
Necrobiotic Cells	0.0 to 2.0	0.85
Smear Cells	1.5 to 6.6	4.74
Mitotic Forms	0.2 to 1.9	0.53
M/E Ratio	1/2.08 to 1/3. 5	1/2. 6
E/M Ratio	1/0. 3 to 1/0.48	1/0.38

CLINICAL COMMUNICATIONS

TWO INTERESTING EQUINE CASES ENCOUNTERED IN BAOR

CAPT. T. H. R. DAVIES, B.V.SC., M.R.C.V.S., R.A.V.C.

CASE 1.

History

A 13 year old polo pony was presented for examination in early March 1961. On questioning the owner it was obvious that this animal had shown intermittent lameness in its near fore leg, during the preceding 12 months. There was no evidence of injury or blemish to either leg or foot. However, the animal did appear " pottery " and so a high plantar nerve block was done on the near fore leg, using " Diethylamino dimethylacet-anilide."[1] After 10 minutes rest this animal became distinctly lame on its off fore leg.

It was decided to X-ray both near fore (Fig. 1) and off fore legs (Fig. 11) in a cranio-caudal plane. The results are obvious—a rarefying pedal ostitis being apparent.

Woolley Park Farm, Bradford-on-Avon; Dairy House Farm, Hilperton, Trowbridge; No. 3 Site, Nettlebury, Netts Road, Shrewton (Feb. 19).

Yorks. Barlow Post Office, Barlow, Selby (Feb. 16); Hall Cottage, Barlow, Selby (Feb. 19).

Swine Fever

Angus. Trottick Mains, Dundee (Feb. 15).

Ches. Baddington Farm, Baddington, Nantwich (Feb. 9).

Glam. Penhydd Fawr, Bryn, Port Talbot (Feb. 9); Nathin Farm, Blaengarw, Pontycymmer (Feb. 12); Ynysygwas Farm, Cwmavon, Port Talbot; Cadoxton Lodge Farm, Cadoxton, Neath (Feb. 13); Sunnyview, St. Brides Major, Bridgend (Feb. 15); The Hollies, Coity, Bridgend (Feb. 19).

Lancs. Briscoe Lane Piggeries, Newton Heath, Manchester 10 (Feb. 9); Harrison House, St. Michaels, Preston (Feb. 12); Mosley Acre Farm, Barfoot Bridge, Stretford (Feb. 19).

Lincs. Park Farm, Willingham-by-Stow, Gainsborough (Feb. 15); Station Road, Old Leake, Boston; Manor House Farm, South Hykeham, Lincoln (Feb. 19).

Northants. Poplars Farm, Holcot, Northampton (Feb. 14).

Notts. Boundary Farm, Claypole, Newark (Feb. 12); Chapel Place, South Leverton, Retford (Feb. 14).

Salop. Grindley Brook Farm, Whitchurch (Feb. 13); Tern, Wellington (Feb. 14); Hare and Hounds, Crackley Bank, Shifnal (Feb. 19).

Somerset. Joyces Farm, Preston Bowyer, Milverton, Taunton (Feb. 13); New Farm, Bowdens, Langport (Feb. 15).

Staffs. Church View, Goose Lane, Abbots Bromley, Rugeley; High House Farm, Great Wyrley, Walsall (Feb. 13); Bryn Lyn, Rugeley Road, Hednesford (Feb. 16).

Stirlingshire. Woodend Farm, Kilsyth (Feb. 12).

Suffolk. The Ivies, Worlingworth, Woodbridge; Manor Farm, Cratfield, Halesworth (Feb. 9); Green Farm, Badwell Ash (Feb. 13).

Surrey. Inwood Barn Farm, Hog's Back, Seale, Farnham (Feb. 15).

War. Fen Lane Farm, Upton, Nuneaton (Feb. 9); Kinwalsey Farm, Meriden, Coventry; Little Garth, Rowney Green, Alvechurch, Birmingham (Feb. 14); Rowleys Green Allotment, Longford, Coventry (Feb. 19).

Wilts. Park Farm, North Bradley, Trowbridge (Feb. 19).

Worcs. Yates Hay Farm, Yates Hay Road, Malvern Link, Malvern (Feb. 9); Greenhill Road Poultry Farm, Greenhill Road, Halesowen (Feb. 12); Cliffey, Hanley Castle, Worcester (Feb. 16); The Orchards, Hatfield, Norton, Worcester (Feb. 16); Big Winnall Farm, Stourport-on-Severn (Feb. 19).

Yorks. Skew Hill Farm, Grenoside, Sheffield (Feb. 9); South End, Seaton Ross, York; Victoria House Farm, Danby Wiske, Northallerton; Briarfield, 14, Lea Lane, Royston, Barnsley (Feb. 13); Low Field Piggeries and Oaktree Farm, Yatts, Pickering (Feb. 14); Whiston Hall, Whiston, Rotherham (Feb. 15); Upper Hirst Farm, Birley Carr, Sheffield 6 (Feb. 16); Paradise Farm, Woodmansey, Beverley (Feb. 19).

Letters to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

APPENDIX VI

Annexure (i)

Bone Marrow Biopsy in Goats

Sir,—The examination of the bone marrow in radiation studies is of fundamental importance. This is because the radiosensitivity of the bone marrow is very high and accounts for many of the long-term effects of irradiation.

It became evident early in our studies on goats that a satisfactory technique for obtaining bone marrow from the living animal would be essential. No references in the literature on the bone marrow biopsy of goats could be found. However, Grunsell (1951, 1955) described a technique in sheep by aspiration from the sternum using a Salah needle and this was used as a basis for our own studies.

Sternal puncture as a means of bone marrow biopsy in domestic animals is now more common (Hjarre, 1943; Grunsell, 1951, 1955; Köhler, 1957; Wilde, 1961). Some workers have used the iliac crest (Bloom & Meyer, 1944; Bloom, 1945; Duckworth, 1961) or the rib (Mulligan, 1941; Calhoun, 1946, 1954, 1955). Various sites were tried in the goat including the iliac crest, rib and long bones. All were found to be unsatisfactory. We found, however, that the taking of marrow from the sternum was both convenient and satisfactory. Some degree of dilution of the marrow with blood always occurred.

The sternum of the goat is similar to that of the sheep and consists of 6 or 7 sternebrae. The first sternebra (manubrium) is cylindrical, elongated, curved upwards with enlarged ends; the second, third and fourth are wider and flatter; the primitive division of the fifth or sixth into 2 lateral halves may persist into adult life. The last segment (xiphisternum) is long, flat and narrow and is extended posteriorly by a rounded xiphoid cartilage.

The cartilage and connective tissue uniting the sternebrae in adult goats is very thin making the whole sternum on sagittal section to appear as one structure. The marrow was aspirated from an area about 2 to 4 inches from the manubrium at about the 3rd or 4th sternebra. Often it was necessary to aspirate from the 2nd segment as only fatty marrow was obtainable from the later segments.

The goat was immobilised with phencyclidine (Cl-395) (Wilkins, 1961) and lifted on to a tubular metal cradle. It was placed on its side on a soft sorbo-rubber sheet with its head drooping over the end of the cradle to allow saliva to drip away. An assistant held fore and hind legs.

The hair was clipped from the sternal area and the skin cleaned with ether. A previously sterilised Salah needle, 50 mm. in length and of 16 B.W.G. with a bore of 1.5 mm., and a 20-ml. syringe were washed out with sterile 3.8 per cent. sodium citrate solution immediately before the aspiration to prevent clotting. The needle with closely-fitting stylet was pushed through the skin and subcutaneous tissue until the hard sternal bone could be felt. Then, by a gentle screwing motion penetration of bone was achieved to a depth of about ½ cm. The stylet was withdrawn and the syringe attached. The plunger was pulled out gently but firmly to about the 10-c.c. mark and suction thus applied. Sometimes bloody marrow fluid was seen to enter the syringe, but often nothing was visible. In the latter case it was rightly assumed that sufficient marrow material was in the lumen of the needle to enable smears to be made.

7th (Wed.). TECHNICAL DEVELOPMENT COMMITTEE OF THE B.V.A. Meeting at 7, Mansfield Street, 10.30 a.m.

8th (Thurs.). B.S.A.V.A. SUSSEX AREA. Meeting at the New Imperial Hotel, First Avenue, Hove, 7.30 p.m.

SOCIETY OF WOMEN VETERINARY SURGEONS. Spring Meeting at 27, Dover Street, London, W.1, 2.15 p.m.

9th (Fri.). NORTH OF ENGLAND VETERINARY MEDICAL ASSOCIATION. Annual General Meeting in the Agricultural Lecture Room, King's College, Newcastle upon Tyne, 2 p.m.

14th (Wed.). A.V.T. & R.W. NORTHERN REGION. Annual General Meeting at the Department of Veterinary Pathology, Liverpool, 2 p.m.

SOUTH WALES DIVISION. Annual General Meeting at the Golden Lion Hotel, Carmarthen, 2.15 p.m.

15th (Thurs.). NORTH WILTS. VETERINARY CLUB. Annual Dinner at the Goddard Arms Hotel, Swindon, 7.30 p.m.

AYRSHIRE VETERINARY ASSOCIATION. Meeting at the Ayr Station Hotel, 7.30 p.m.

28th (Wed.). NORTH WALES DIVISION. Meeting at Gwydyr Hotel, Bettws-y-Coed, 2 p.m.

LINCOLNSHIRE AND DISTRICT DIVISION. Meeting at the George Hotel, Grantham, 7.15 p.m.

27th, 28th and 29th (Tues., Wed. and Thurs.). A.V.T. & R.W. Annual Conference in the Peebles Hotel Hydro.

31st (Sat.). ASSOCIATION OF STATE VETERINARY OFFICERS. Annual General Meeting at the Wellcome Foundation, Euston Road, London, 4 p.m. Dinner at the Criterion, Piccadilly, 6.30 p.m.

April

7th (Sat.). BRX CLUB. 16th Annual Dinner at the Royal Veterinary College, Camden Town, 6 for 7 p.m.

ADDRESSES OF DISEASE-INFECTED PREMISES

The list below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and the date of outbreak.

Anthrax

Aberdeenshire. Wester Beltie, Torphins, Aberdeen (Feb. 19).

Cumb. Brunt House, Kirkoswald, Penrith (Feb. 3).

Derbyshire. Whitbank, Alton, Chesterfield (Feb. 9).

Lanarks. Lodgehill Farm, East Kilbride (Feb. 12).

Mon. Pentre Perfa, Maesbrook, Llanymynech (Feb. 15).

Salop. Holly House, Ossage Lane, Whixall, Whitchurch (Feb. 12); Home Farm, Shavington, Market Drayton (Feb. 16).

Stirlingshire. Westerton Farm, Kippen Stirling (Feb. 13).

Fowl Pest

Berks. Ockwells Farm, Bray, Maidenhead (Feb. 12); Stroud Farm, Holyport, Maidenhead (Feb. 13); Cox Green Lane, Maidenhead (Feb. 14); St. Francis, 100, Norven Road, Cox Green, Maidenhead (Feb. 15); 354, London Road, Newbury; Cox Green House, Maidenhead (Feb. 16); Poplar Cottage, Ockwells Road, Maidenhead (Feb. 19).

Bucks. High Clere Poultry Farm, Seer Green, Beaconsfield (Feb. 11).

Essex. Sprawls Farm, West Bergholt, Colchester; Mill Farm, 217, Mill Road, Colchester (Feb. 15); High Gates, Little Sampford, Saffron Walden (Feb. 18).

Glos. Stone Leaze Farm, Shipperdine, Thornbury (Feb. 16).

Hants. Pemba, Mockbeggar, Ibsley, Ringwood; The Convent, Andover Down; Cosy Retreat, Locks Road, Locks Heath, Southampton (Feb. 9); The Poultry Premises, Kings Farm, Stratford Saye, Reading (Feb. 10); Picket Piece Poultry, Ltd., 13a, Picket Piece, Andover; Woodington House Farm, East Wellow, Romsey (Feb. 11); Packington Estates, Ltd., Long Parish, Andover (Feb. 13); Harewood Farm, Andover Down, Andover (Feb. 14); White Cottage, Titchfield, Wickham (Feb. 15); Great Abshot Farm, Titchfield (Feb. 17); Woodington Farm, East Wellow, Romsey; Wonston Manor Farm, Winchester; Fleetlands, Warsash Road, Titchfield Common, Fareham; Manor Farm, East Wellow, Romsey; Wyke Down Farm, Picket Piece, Andover; Tia Maria, St. Johns Road, Locks Heath, Southampton (Feb. 19).

Herts. Falkenham, Wellpond Green, Standon, Ware (Feb. 11); Oakland, Wellpond Green, Standon, Ware; Taranvune, Wellpond Green, Standon, Ware (Feb. 19).

Kent. Chatterden Farm, Rochester (Feb. 13); Little Luckhurst, Headcorn, Ashford (Feb. 14).

Norfolk. Manor Farm, Houghton St. Giles, Walsingham; 2, Norfolk Square, Downham Market (Feb. 10); Lower Farm, Houghton St. Giles, Walsingham (Feb. 14).

Northants. 28, Great North Road, Water Newton, Peterborough (Feb. 9); 10, Great North Road, Water Newton, Peterborough (Feb. 13).

Suffolk. Red House Farm, Winston, Stowmarket; Redcroft, Snape Lane, Downham Market; The Caravan, Ashfield, Debenham, Stowmarket; Boundary Farm, Earl Soham, Woodbridge (Feb. 9); Collingwood Farm, Thwaite, Eye (Feb. 10); 8, Kentish Town, Earl Soham, Woodbridge (Feb. 14); Low Farm, Huntingfield, Halesworth; West Hill Farm, Brandeston, Woodbridge (Feb. 15); Cancey, Moor Hall Lane, The Causeway, Eye (Feb. 16); The Bungalow, Bedingfield, Eye (Feb. 19).

Surrey. 80, Little Woodcote Estate, Wallington; 79, Little Woodcote Estate, Wallington (Feb. 15).

Sussex. Southdown House, High Street, Amberley, Arundel (Feb. 13); Downs Farm, Mill Lane, Amberley, Arundel (Feb. 14); Drewitts Farm, Church Lane, Amberley (Feb. 16); South Stoke Farm, Arundel (Feb. 18); New House Farm, West Chiltington, Pulborough; Antye Farm, Theobalds Road, Burgess Hill (Feb. 19).

Wilts. Leech Pool Farm, Melksham; Whitley Brow, Whitley, Melksham; Old Hurst Farm, Keevil, Trowbridge; Clyffe Hall Estates, Ltd., Market Lavington, Devizes (Feb. 9); Manor Farm, Hilpaton, Trowbridge; The Elms, Nurstead, Devizes (Feb. 11); The Old Vicarage, Market Lavington; Wyke House, Wyke Road, Trowbridge; Redhouse Farm, Broughton Gifford, Melksham (Feb. 12); Caen Hill Chicken House, Devizes; Park Farm, Littleton Panell, Devizes; Frying Pan Farm, Broughton Gifford, Melksham; Marston Broilers, Marston, Devizes; Woodlands, Lodge Hill, Market Lavington, Devizes; Woodbridge Farm, West Lavington, Devizes; Hillside, White Street, Market Lavington, Devizes; Whitley Farm, Melksham (Feb. 15); Grove Farm, Market Lavington, Devizes; Griffin Farm, Lacock Chippenham; South Lawn Bungalow, The Sands, Market Lavington, Devizes; The Old Rectory, Beechingstoke, Pewsey; 1, Parsonage Cottage, Market Lavington, Devizes; Castle Farm, Devizes; Manor Farm, Southwick, Trowbridge (Feb. 16); Mutton Marsh Farm, Southwick, Trowbridge; West Park Farm, Market Lavington, Devizes; Flaxfield Farm, Southwick, Trowbridge; Littleton House, Littleton Panell, Devizes; Trowle Manor Farm, Trowle, Trowbridge; Rookery Farm, Hilperton, Trowbridge (Feb. 17); The Terrace, Market Lavington, Devizes; Kings Arms, Market Lavington, Devizes; Goose Hill Farm, White Street, Easterton, Devizes; High Street, Littleton Panell, Devizes (Feb. 18); Flowers Farm, West Ashton, Trowbridge;

with a fairly characteristic history and clinical picture, which has been well described in the literature, and one attempts to confirm the diagnosis by auscultation high on the left flank between the last 2 ribs where the characteristic tinkling sounds can be heard in positive cases. Mr. Jones is, I think, in error when he states that similar sounds are audible in a number of conditions in the bovine abdomen. What are these conditions? Mr. Jones cites only one—ruminal tympany—but this can easily be differentiated from "displaced abomasum," because in the latter condition one can still detect ruminal sounds in the posterior part of the left sub-lumbar fossa as, in my experience, the distended abomasum does not occupy the whole of the left flank.

I agree with Mr. Jones when he states that the pioneers who first described the condition of "displacement of the abomasum" were describing something other than a mere left of mid-line position. I have felt for some time that the term "displacement of the abomasum" is an unfortunate one in that it suggests we are dealing with a mechanical displacement, and for this reason I think the suggestions of Pinsent, Neal and Ritchie, and of Hutchins, Blood and Hyne, as cited by Mr. Jones, that we may be dealing with functional abnormalities of the abomasum, are valuable ones. In the cow suffering from "displacement of the abomasum" the main factor in producing the clinical signs seems to be the distension of the abomasum with gas, *i.e.* tympany of the abomasum. In the past it seems to have been assumed that the tympany has arisen secondarily to the displacement. Mr. Jones, however, has shown that in 30 per cent. of normal cases the abomasum lies to the left of the mid-line. Might it not be, therefore, that in such animals, if for any reason the abomasum becomes tympanitic, it tends to assume a position between the rumen and left costal arch and give rise to the condition which we call "displaced abomasum"?

Many practising veterinary surgeons, myself included, believe that this condition has become more common in recent years and that husbandry or nutritional factors, as yet not understood, are involved in its aetiology. It is easier to accept this hypothesis if we regard the condition as primarily a tympany of the abomasum rather than a mechanical displacement. This conception of the condition does not render surgical intervention for its correction invalid as it is reasonable to suppose that once the tympany has reached a certain stage it can only be rectified by surgical means.

Yours faithfully,
A. MARR.

Pennard House,
Sevenoaks.
February 15th, 1962.

Clinical Aspects of Equine Brucellosis

Sir,—On looking through your recent series of reports on contributions made by members of the British Veterinary Association at the various symposia of the Oxford Congress, the presentation of a Dublin practitioner, Mr. J. S. M. Cosgrove, entitled "Clinical Aspects of Equine Brucellosis," caught my eye and sent my mind back over the years to January, 1947. At that time I was able to publish a detailed account of a clinical case of brucellosis in a Thoroughbred gelding with references to the very relevant work of 17 different authors. A full report was given of the serological findings in the case, the effects of vaccine therapy and the significance of Brucella antibody titres in horses as discussed in the literature. The paper concluded with a brief account of serum antibody levels in a small random selection of horses from the College Clinic as illustrative of interpretations of serum antibody levels to Brucella antigens.

In so far as I am aware this 1947 paper was the first account in European literature of a possible association between intermittent lameness in the horse and significant antibody titres to Brucella antigen. The work referred to was published in *The Irish Veterinary Journal* (January, 1947), Vol. 1, No. 2, the official journal of the Veterinary Medical Association of Ireland of which Association Mr. Cosgrove is a member and, incidently, one of its past-Presidents.

In view of the undoubted effort expended by Mr. Cosgrove in making a contribution to the symposium in question many of us here were rather surprised that he should state: "We cannot recollect having come across serum examination records pertaining to equine *Brucella abortus*, but we qualify the latter by stating that the number of useful references to which we have had recourse has been limited," particularly when he frankly, if pointedly, admits that: "Our interest in the condition has slowly developed from as far back as *1947*." In the introduction to my paper of January of that year I stated: "It may well be that certain cases of obscure lameness met with here may be due to a similar cause (*Br. abortus*). To those veterinarians who have had an opportunity of studying *Br. abortus* in the human being especially the rheumatic complications such as synovitis and arthritis, affecting the neck, shoulder, elbow, back, hip, knee and intercostal regions, such a finding would cause little surprise."

That Mr. Cosgrove has chosen to ignore the writer's modest contribution to the literature in regard to the subject of his thesis is his own affair. That he should overlook the work of eminent scientists who have contributed to the international literature on this subject up to 1947, and others since that date, is understandable for a busy practitioner; but that he should attempt to seek support for his beliefs, however genuinely held, by quoting out-dated textbooks and drawing false analogies from a current one, is hardly excusable. In view of the fact that Congress contributions are being published in THE RECORD in the same way as formal scientific papers, surely, in the interests of readers, students and practitioners, all such contributions should be subjected, as are scientific papers, to a system of competent refereeing, particularly as the attendance at some symposia may be small (due perhaps to a clash of meetings) and the opportunity for constructive criticism not available. Mr. Cosgrove should

The syringe was detached and the needle contents were dropped directly on to clean slides and smears made immediately in the usual manner.

No after-treatment of the goat was necessary other than swabbing the puncture area with an antiseptic and painting with colloidion. No adverse sequelae were ever encountered.

The smears were stained and examined.

Yours faithfully,
J. H. WILKINS.

Medical Research Council Radiobiological Research Unit,
Harwell,
Didcot,
Berks.
February 19th, 1962.

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The Position of the Bovine Abomasum

Sir,—We read Mr. Jones's article (*Vet. Rec.* **74.** 159) on the position of the bovine abomasum with considerable interest. On the basis of his findings that in 30 per cent. of cows examined *post mortem* the abomasum was situated in a position to the left of the mid-line, Mr. Jones states "It may reasonably be assumed, therefore, that during clinical examination of cattle in the field evidence of the abomasum being on the left of mid-line may well be found in approximately 30 per cent. of cases." It is also implied that the condition commonly known as displacement of the abomasum is in the majority of cases of little clinical significance, and that cases in which it is diagnosed are, in fact, suffering from some other concomitant disease.

In our opinion these conclusions can be justified only if:—

(1) In the cases quoted, the presence of the abomasum to the left of the mid-line was demonstrated *ante mortem*, by simple clinical means.

(2) None of these cows showed clinical evidence of digestive disease.

On neither of these points is any information given. With regard to the first, it is unlikely that the abomasum would be detectable by clinical methods unless it were dilated with gas, and had moved upwards to occupy a position between the rumen and the left rib cage. Here again, Mr. Jones makes no comment concerning the degree of displacement of the abomasum in any of his cases, and in only 2 is reference made to the degree of dilatation (if any) of the organ. With regard to the second of the above points, we note that Mr. Jones appears to have selected dairy cattle for his investigation. It would be interesting to know for what reason these animals were disposed of—could it not be that some of them were, in fact, suffering from the very condition, the clinical significance of which Mr. Jones appears to hold in doubt? Since no *ante-mortem* clinical examination is reported, this possibility cannot be ruled out.

Mr. Jones asks, "Is it possible that operation for displacement of the abomasum is merely fashionable?" We do not think that this is the point at issue. Surely the query raised in his article is "Is displacement of the bovine abomasum a pathological condition?" In answer to this question we would say that the mere presence of the abomasum in a position to the left of the mid-line is not necessarily of any clinical significance, but when the organ becomes distended and moves upwards between the rumen and the left abdominal wall, then a pathological state of affairs is established. Further, we are convinced, on the experience of some 130 cases, that the rapid improvement following surgical correction which occurs in the overwhelming majority of cases, is the result of the operation, and is not some coincidental, non-specific response.

Yours faithfully,
P. A. NEAL,
H. E. RITCHIE,
P. J. N. PINSENT.

The University of Liverpool,
Faculty of Veterinary Science,
Field Station,
"Leahurst,"
Neston, Wirral.
February 17th, 1962.

Sir,—Mr. Ronald S. Jones is to be congratulated on his investigations of the position of the bovine abomasum as seen at autopsy in the abattoir.

It seems clear from his findings that it is wrong to regard the abomasum as an organ invariably situated to the right of the mid-line, and this observation is of great value. It is when Mr. Jones goes on to relate his *post-mortem* findings on normal animals to the consideration of the clinical condition of displaced abomasum as seen in the field, that his conclusions become somewhat controversial. From his findings he states it may reasonably be assumed that, during clinical examination in the field, evidence of the abomasum being in the left of the mid-line may well be found in 30 per cent. of cases, and later he asks whether it is possible that operation for displaced abomasum is merely fashionable. The inference to be drawn from this is that Mr. Jones considers that the condition is diagnosed erroneously and far too frequently in the field. A sceptical approach has much to recommend it but I think he has carried his scepticism a little too far. One does not diagnose "displacement of the abomasum" merely on evidence suggesting that the abomasum is to the left of the mid-line. One suspects that an animal is suffering from the condition when one is presented

Annexure (ii)

Bone Marrow Percentage Differential Cell Counts of 18 Normal Adult Goats (1000 Cells Counted)

No.	1 C298	2 F29	3 F40	4 F27	5 F30	6 W302	7 W316	8 F31	9 C175	10 C200	11 M3	12 M10	13 M1	14 M9	15 C315	16 C229	17 C118	17 C234
SEX	F	F	F	F	F	F	F	F	F	F	M	M	M	M	CM	CM	CM	CM
BREED	BT	BS	BS	BS	BS	BS	BA	AN	BA	BT	BS	BS	BT	BA	BS	BS	BS	BA
AGE	4	4	4½	6	5½	4½	4½	6½	4	4½	6½	4	4	3	4	4	4	4
WEIGHT (lbs)	112	122	108	111	165	153	141	134	119	120	190	190	174	174	142	210	235	164
Stem	0.1	1.4	0.5	0.6	0.6	1.0	0.8	0.5	0.2	1.0	0.6	0.3	0.1	0.2	0.0	0.2	0.2	2.0
Myeloblasts	1.5	1.7	0.6	0.8	0.8	1.0	1.2	0.8	1.5	1.2	1.0	1.0	0.6	1.4	1.4	1.6	0.6	1.5
Promyelocytes	0.2	0.4	0.6	0.6	0.8	0.8	0.4	0.4	0.6	0.3	0.6	0.6	0.3	0.3	1.1	0.5	0.4	0.4
Myelocytes N	1.5	1.8	1.2	0.5	1.1	0.8	0.9	0.9	1.0	1.2	1.6	1.2	1.2	1.0	0.4	1.6	1.3	1.7
" E	0.8	0.5	1.3	0.5	0.7	0.7	0.3	0.5	1.2	0.3	0.3	0.7	0.2	0.3	0.4	0.3	0.4	0.4
" B	0.3	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1
Metamyelo N	16.7	6.75	19.2	13.4	22.0	12.7	15.8	18.2	10.5	12.6	17.5	13.3	11.8	16.1	5.5	8.5	18.7	7.0
" E	1.8	1.05	2.8	1.8	2.4	1.3	0.8	0.8	2.6	0.4	0.6	1.1	0.6	1.2	1.1	0.6	0.4	0.6
" B	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
Polymorphs N	4.4	10.5	9.1	0.6	3.0	7.1	10.8	12.1	10.2	10.6	8.6	8.7	4.6	10.6	9.0	7.4	4.7	14.7
" E	0.1	1.35	0.3	0.1	0.2	0.6	0.5	0.8	0.6	0.3	0.3	0.2	0.5	0.6	0.0	0.4	0.0	0.3
" B	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Total Myeloid (M)	27.5	24.05	35.2	18.5	31.2	25.0	30.8	34.5	28.2	26.9	30.5	26.8	19.8	31.8	18.9	20.9	26.6	26.8
Pronormoblasts	2.9	0.75	1.4	1.2	1.1	2.2	1.9	1.6	2.0	1.8	3.2	2.5	2.8	1.0	5.0	2.6	1.6	2.0
Normoblast E	3.3	5.1	1.2	1.6	1.6	3.1	7.6	1.2	1.6	5.1	5.6	3.2	7.3	4.2	6.5	8.2	4.2	5.3
" I	10.8	26.75	1.5	1.8	2.1	4.1	19.8	10.8	4.2	13.2	16.4	16.6	14.2	18.1	25.0	16.2	19.9	19.7
" L	40.2	34.4	50.2	69.4	58.8	60.2	36.9	41.9	45.6	35.1	42.5	44.3	45.1	34.2	29.0	36.8	42.3	29.5
Total Erythroid (E)	57.2	67.0	54.3	74.0	63.6	69.6	66.2	55.5	53.4	55.2	67.7	66.6	69.4	57.5	65.5	63.8	68.0	56.5
Lymphocytes	2.7	3.2	2.4	1.3	0.8	1.0	1.1	1.9	5.5	4.8	2.2	1.0	1.8	2.1	2.5	5.2	1.8	5.5
Monocytes	2.2	0.0	0.9	0.8	0.7	0.7	0.1	1.6	2.3	2.9	1.5	0.8	0.9	1.6	6.0	3.2	0.5	0.1
Plasma Cells	0.4	0.3	0.9	1.2	0.4	0.7	0.1	0.2	0.2	0.3	0.1	0.2	0.3	0.1	0.0	0.4	0.5	0.0
Mast Cells	0.1	0.0	0.2	0.2	0.2	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Megakaryo	3.2	0.0	0.3	0.1	0.1	0.2	0.0	1.0	1.1	1.0	0.5	0.5	1.2	0.8	0.6	0.4	0.2	0.2
Necrobiotic	1.4	0.0	0.6	0.0	0.2	0.2	0.1	0.5	0.5	0.8	1.6	0.3	1.1	0.9	2.0	1.5	0.2	0.0
Smear Cells	4.7	3.55	2.1	2.9	2.2	2.1	0.7	4.0	8.1	6.3	5.8	3.4	4.8	4.7	4.3	4.2	1.5	6.6
Mitotic	0.5	0.5	0.6	0.4	0.1	0.2	0.1	0.2	0.5	0.8	0.5	0.4	0.6	0.3	0.2	0.2	0.4	1.9
M/E Ratio	1/2.1	1/2.8	1/1.5	1/4	1/2	1/2.8	1/2.1	1/1.6	1/1.9	1/2	1/2.2	1/2.5	1/3.5	1/1.8	1/3.5	1/3.1	1/2.6	1/2.2

APPENDIX VI

Annexure (iii)

Bone Marrow Percentage Differential Counts After Irradiation

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
TIME AFTER IRRADIATION	½ hr	½ hr	½ hr	½ hr	½ hr	½ hr	½ hr	2 days	3 days	4 days	4 days	4 days	5 days	6 days	80 days	100 days	118 days	162 days	238 days	Just prior to		Death
DOSE	2000	700	700	600	600	550	400	400	1000	600	650	2000	500	500	650	500	500	500	600	550	600	700
Stem	1.6	0.7	1.9	0.2	2.3	1.6	1.4	4.2	6.2	3.2	0.4	0.0	0.0	0.1	1.0	1.2	0.5	1.0	2.5	0.1	0.1	0.0
Myeloblasts	0.8	1.2	0.8	0.3	2.0	0.8	1.2	2.6	2.4	1.7	0.8	0.0	1.4	0.7	1.6	2.0	1.3	0.5	1.7	0.3	0.1	0.0
Promyelocytes	0.7	0.7	0.9	0.3	1.9	0.6	1.2	1.2	0.4	0.6	1.2	0.1	0.7	0.3	0.5	0.1	0.1	0.5	0.05	0.1	0.1	0.0
Myelocytes N	0.8	1.3	0.5	1.5	1.7	3.8	0.9	1.4	2.0	4.2	1.2	8.9	2.1	1.8	3.1	2.1	1.5	1.0	1.4	0.2	0.2	0.3
" E	0.2	0.6	0.2	0.4	1.0	0.1	0.6	0.5	0.0	0.0	0.5	0.0	0.1	0.1	0.6	0.3	0.0	2.0	0.3	0.1	0.0	0.1
" B	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Metamyel N	20.0	18.6	8.9	19.4	16.5	21.2	10.9	17.8	7.4	11.4	15.2	19.9	18.1	19.6	16.3	17.6	6.9	4.0	5.9	0.9	0.8	0.0
" E	0.1	0.8	1.6	1.4	2.0	0.2	1.2	1.8	0.2	0.2	0.8	0.1	0.2	0.2	0.8	0.3	0.5	3.0	0.6	0.1	0.1	0.0
" B	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0
Polymorphs N	15.3	8.6	2.0	3.5	2.0	5.6	5.5	4.1	4.5	4.6	8.3	2.6	8.8	5.2	14.2	12.3	10.3	6.0	12.6	1.5	0.9	0.0
" E	0.0	0.3	0.2	0.2	0.6	0.2	0.7	0.7	0.6	0.5	0.2	0.1	0.3	0.2	1.6	0.4	0.4	4.0	0.7	0.0	0.1	0.1
" B	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Total Myeloid (M)	38.0	32.1	16.3	27.1	27.8	33.5	22.2	30.1	17.5	23.2	28.3	31.7	31.8	28.1	38.7	35.1	21.1	21.1	21.1	3.2	2.3	0.5
Pronormoblasts	1.5	2.8	1.4	2.3	2.6	2.3	1.1	2.1	2.6	1.8	0.4	0.0	1.8	0.1	2.2	1.7	2.2	4.5	1.6	0.0	0.0	0.0
Normoblasts E	2.6	2.0	2.0	1.9	3.4	3.6	1.5	4.1	3.8	3.6	0.6	0.5	5.6	0.4	3.6	5.2	5.3	5.0	6.7	0.3	0.4	0.0
" I	5.0	15.1	1.8	9.2	4.6	15.3	1.9	11.3	10.2	10.8	1.2	7.5	15.8	4.8	18.3	16.1	13.4	14.0	20.3	2.6	1.7	0.9
" L	26.3	25.7	53.7	53.6	46.3	36.2	63.2	31.2	15.8	26.2	28.3	31.0	32.3	26.9	25.4	26.9	41.3	37.3	28.7	48.7	55.2	56.7
Total Erythroid (E)	35.4	45.6	58.9	67.0	56.9	57.4	67.7	48.7	32.4	42.4	30.5	39.0	55.5	32.2	49.5	49.9	62.2	59.8	57.3	51.6	57.3	57.6
Lymphocytes	9.5	5.1	18.3	1.2	3.9	3.3	1.3	8.3	11.4	6.3	13.6	10.0	2.1	11.8	2.2	2.6	2.8	2.5	1.9	8.4	6.3	4.0
Monocytes	2.5	1.6	0.9	1.9	1.3	0.8	0.9	0.9	1.6	1.6	4.2	1.1	0.2	2.3	2.6	0.8	0.3	2.0	0.3	1.2	0.1	0.1
Plasma Cells	3.0	2.6	1.1	0.2	0.7	0.7	2.0	1.8	1.6	1.3	2.6	2.2	0.4	2.1	2.1	2.1	2.1	1.5	0.3	25.7	27.8	31.8
Mast Cells	0.0	0.3	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Megakaryo	0.5	0.3	0.0	0.2	0.5	0.1	0.3	0.3	0.0	0.0	0.6	0.0	0.0	1.8	0.8	1.6	1.6	2.0	0.1	0.0	0.0	0.0
Necrobiotic	0.7	3.4	0.8	0.2	1.0	0.3	0.8	1.1	3.2	9.6	7.2	0.4	2.1	4.2	0.4	1.8	1.6	0.5	0.2	4.1	2.2	1.4
Smear	8.3	7.9	2.6	1.6	5.1	2.0	3.4	4.1	25.6	12.3	12.6	15.6	7.9	17.4	2.2	4.1	8.2	9.0	13.6	9.4	3.8	4.5
Mitotic	0.5	0.4	0.0	0.4	0.3	0.3	0.0	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.5	0.8	0.4	0.6	0.4	0.2	0.1	0.1
M/E Ratio	1/0.9	1/1.4	1/3.6	1/2.5	1/2.1	1/1.7	1/3.1	1/1.6	1/1.8	1/1.8	1/1.1	1/1.2	1/1.7	1/1.1	1/1.3	1/1.4	1/3	1/2.8	1/2.7	1/1.6	1/2.5	1/115

APPENDIX VII

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PRELIMINARY OBSERVATIONS ON THE SEMEN OF GOATS

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Little information has been published on the characteristics of goat semen. aton and Simmons (1952) carried out an extensive study on Toggenburg nd Common American goats, some of which were on observation for three ears. Rollinson (1950) presented data on one British Saanen male goat and illips et al. (1943) gave the results of two male goats which were observed r a year.

The following observations were made in order to obtain data on a few men characteristics of goats which were considered to be important in certain der studies on the effects of ionizing radiations.

METHODS AND MATERIALS

llection of Semen

It was originally planned to collect semen in an artificial vagina but this as abandoned early due to the varying libido of normal goats, which was pected to vary even more after irradiation when apathy is a common nical sign. Amongst other difficulties was the necessity to have a female oat on heat to tease the male goat. As female goats are usually on heat ly from August to December, operations would therefore have been limited. onsequently, the electro-ejaculator was used throughout the study. Gunn 936) was the first to make extensive use of an electrical stimulus as a means collecting semen from rams. This method is now used extensively to otain semen samples for experimental work and in the practical application artificial insemination in animals. The ram responds well to the electrical ethod of collection but the cumbersome method of Gunn using two ectrodes has been replaced nowadays by use of the single bipolar rectal ectrode which decreases the muscular reactions produced by Gunn's technique aplaud and Cassou, 1945 ; Blackshaw, 1954 ; Dziuk et al, 1954). A bipolar ectrode made in this unit and used with two kinds of electrical stimulator s given good results on goats. The apparatus consisted briefly of the llowing :—

1. Mains Stimulator (*Fig.* 1)

This consisted of a variac transformer to control the voltage from 240 to zero.

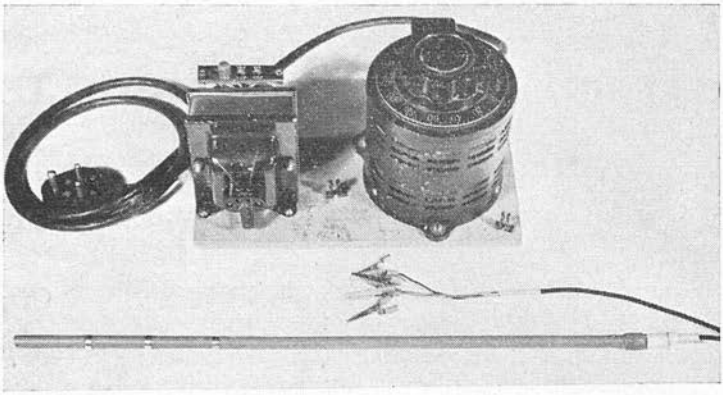

FIG. 1.—*Mains Stimulator and Copper Tube Electrode*

2. Battery Stimulator (*Fig.* 2)

This was used almost exclusively as it was so much more successful. It consisted of a common "shocking" galvanic coil powered by a $4\frac{1}{2}$ volt torch battery and costing not more than a few shillings.

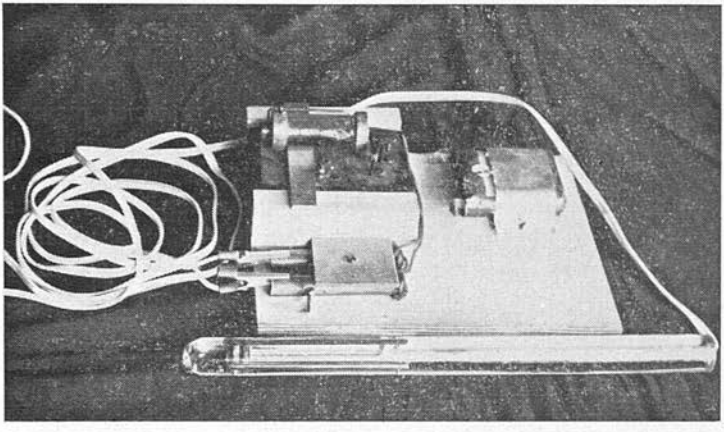

FIG. 2.—*Battery Stimulator and Perspex Electrode*

The rectal electrode was a 15in. perspex rod of $\frac{7}{8}$in. diameter into the ends of which were embedded four strips of brass 4in. long and $\frac{1}{2}$in. apart capable of being connected to either stimulator by means of wires and clips. A second type of rectal electrode was also used (Blackshaw, 1954). This consisted of a 15in. length of copper tube of diameter $\frac{1}{4}$in. A smooth brass cap, the terminal pole, was soldered to one end. Three holes were drilled in the tube at 2in., 4in. and 6in. from the the brass cap. Rubber tubing was slipped over the tube and insulated wires passed up the tube through the holes. A brass ring was soldered to the end of each wire and clamped tightly over the rubber insulation.

The electrode was dipped in liquid paraffin and inserted into the rectum to a depth of 4-8 inches. The stimulator was switched on and the semen was collected in a plastic funnel to the end of which was attached a conical graduated tube.

The volume of semen was immediately measured and the appearance noted. There were three main characteristics, watery, creamy or stringy (mucinous) or any combination of them. Viscosity and opacity were arbitrarily rated on a scale of 0-4 as maximum.

Immediately after collection an estimate of sperm motility was recorded. The procedure was to place a drop of semen on a warm slide, cover with a cover slip and examine. The degree of overall motility was rated on a scale of 0 to 4 : 0 was equivalent to no motility and 4 to maximum motility. An estimate of the per cent of motile (live) sperm was also made but this was very empirical and served as a cross check for the next procedure.

Immediately after the examination for motility a determination of the live sperm was done by placing at one end of a warmed glass slide a drop or two of semen and mixing this with two to five drops of Nigrosin—Eosin stain (Nigrosin 30G, Eosin 5G, distilled water 300 ml). After allowing to stain for 15-30 seconds drops were transferred to other warmed slides and smears made in the usual manner. After drying the smears were microscopically examined under the 2/3 and 1/6 objectives. Two to five hundred sperms were counted and the clear, unstained sperms recorded as the per cent live sperm. Similarly, the stained sperm, pink in colour, were recorded as the dead sperm. These figures were cross checked with the previous rough estimation of motile (live) sperm from immotile (dead) sperm. (*Fig.* 3).

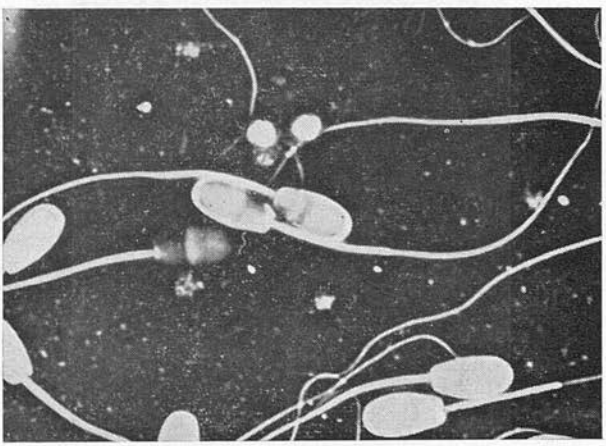

FIG. 3.—*Live sperm* (*clear*) ; *Dead sperm* (*dark*)

An estimate of abnormal sperm was made from the same slides and cross checked with further smears stained as follows :—

A drop of semen was placed on a warm slide and, depending on its appearance and estimated sperm concentration, either smeared directly or diluted

with a drop of saline and then spread evenly and thinly in the usual manner. After drying it was stained by the following procedure :

1. Smears were cleared in 1% chloramine for 3 minutes.
2. Washed in water.
3. Washed in 95% alcohol.
4. Dried.
5. Stain I : stained for 2 minutes

in	Ziehl-Neelsen Carbol Fuchsin	2 parts
	Conc. Alcohol soln of blue Eosin	1 part
	95% Alcohol	1 part

6. Washed in water.
7. Stain II : counter stained for 10 seconds

in	Loeffler's Methylene Blue	1 part
	Distilled water	4 parts

8. Washed in distilled water.
9. Dried and examined.

Abnormal spermatozoa were divided into two main categories viz : (*Fig.* 4) :

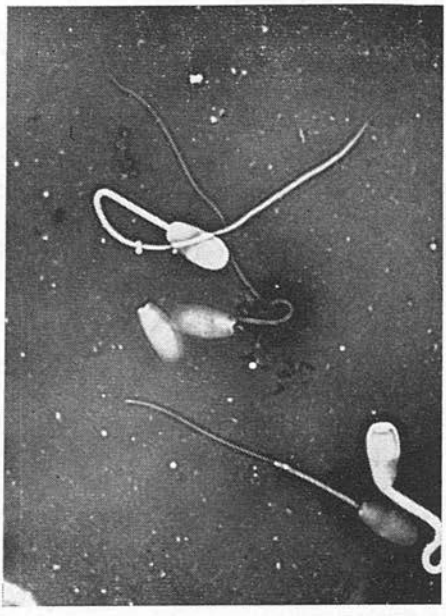

FIG. 4.—*Head and Tail Abnormalities*

1. Head and Neck Abnormalities, which included separation of head from body, heads with protoplasmic extrusions, odd-shaped heads, big heads and pin heads.
2. Tail abnormalities included coiled tails, bent tails, broken tails or tails with protoplasmic beads.

The next procedure carried out was the estimation of sperm concentration as follows :—

The semen was thoroughly mixed and with a Thoma-Zeiss pipette semen was drawn up to the 0.5 mark. As the background of the pipette is white it was difficult to observe the semen. This was overcome by viewing it from a little to one side. The end of the pipette was then introduced into buffered formol saline and suction applied until the diluting fluid was drawn up to the 101 mark. The mixture was then shaken well in the bulb. A little of the fluid was discarded to ensure that the drop used for the count came from the bulb of the pipette. The number of spermatozoa in 80 small, i.e. 5 large, squares of a Neubauer haemocytometer was counted. The figure thus obtained was multiplied by 10,000 to give the number of spermatozoa in one cubic millimetre.

A rough cross check of the number of abnormal spermatozoa was also estimated at the same time.

Finally, the pH of the semen was roughly determined using BDH indicator paper.

All apparatus was kept in an incubator at a constant temperature of 37° C. All collections and determinations took place in a warmed laboratory at a temperature not less than 30° C.

RESULTS AND DISCUSSION

Table I gives the semen characteristics of twelve male goats of different breeds and ages chosen at random from a colony of over sixty male goats. The mean values are given in table 2 :—

Age

Three of the five goatlings examined shewed very low sperm counts and two of these also had mostly dead, with high percentages of abnormal, spermatozoa.

Quantity

The average volume given by Eaton and Simmons (1952) for their study was 0.65 ml and Rollinson (1950) gave 0.5 ml. In both cases an artificial vagina was used.

In this study using an electro-ejaculator the average quantity of semen collected was 2 ml. The actual collection was very easy to effect and could be done at any time. The volume on other goats not included in this series was often as much as 4 ml.

Concentration

Perhaps the most significant finding was the great variation in the concentration of spermatozoa in individual goats. This may be a reflection of the fact that pseudohermaphroditism is common in the British domestic goat. Testicular hypoplasia may be another related factor (Rollinson, 1950). The average concentration of sperms per ml. in this study was lower than that given by other authors, 2,724 millions by Eaton and Simmons (1952) and 4,200 millions by Rollinson (1950). However, the latter figure referred to only one goat and the former to a mixed American stock. There was a fair correlation between concentration and opacity, but not all opacity was sperm.

Motility

This also varied a great deal from goat to goat and probably indicated some fertility defect. It was found generally that the sperms in watery or mucinous semen were less motile than those in creamy semen. They usually also contained a larger percentage of epithelial cells and other debris. The technique of handling the semen obviously affects the motility : every effort was made in this study to standardise the procedures especially to prevent any sudden changes in temperature. The resistance of semen to temperature shock is related to the percentage of living spermatozoa present (Bishop et al, 1954) and possibly to motility as well.

Living, Dead and Abnormal Spermatozoa

The differential staining used could not differentiate motile from immotile sperm but only living from dead. It was considered important to keep the slides, stains and all apparatus continuously in an incubator at 37 degrees Centigrade. In fact, the whole procedure of smear making and staining was actually carried out within the incubator itself. The percentage of living sperms was greatest in creamy semen and least in watery specimens. The percentage varied greatly between individual goats but the two types of abnormalities recorded were on the whole equally represented although in individual goats approximately the same percentage of abnormalities occurred in successive samples from the same goat.

THE pH was mostly on the alkaline side and no relationship was evident between pH and any other parameter investigated.

SUMMARY

Some observations are presented on the characteristics of the semen from twelve British goats obtained by electroejaculation.

ACKNOWLEDGEMENTS

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The work reported here is a section of a wider research programme on the effects of radiation on large animals upon which the writer is engaged under the direction of Dr. J. F. Loutit (Director, Radio-biological Research Unit, M.R.C., Harwell) and Dr. J. R. Greening, (Director, Medical Physics Unit, University of Edinburgh).

TABLE I

SEMEN CHARACTERISTICS OF 12 MALE GOATS PICKED AT RANDOM FROM A COLONY OF OVER 60

Goat	*Breed*	*Age*	*Quantity*	*Opacity**	*Viscosity**	*pH*	*Motility**	*Conc* $10^6/ml$	*Total* (*Mils*)	*% Alive*	*Abnormal*		
											% Total	*% Head & Neck*	*% Tails*
1	BT	4	2.0	4.0	0.5	6.7	1.5	650	1300	94	3	33	67
2	BS	5½	2.4	4.0	0	8.2	2.0	520	1250	25	73	40	60
3	BS	6½	2.5	4.0	0.5	8.2	2.0	1920	4800	82	9	100	0
4	BA	3	1.4	0	3.0	8.5	1.5	100	140	35	50	50	50
5	BS	4	2.0	4.0	0	7.0	4.0	430	860	86	6	67	33
6	BS	2½	1.9	2.0	0.5	8.2	3.0	160	304	70	20	95	5
7	BT	1¼	1.0	4.0	0	8.2	4.0	3500	3500	94	4	25	75
8	BS	1¼	1.8	0	4.0	8.5	0.5	17	30.6	73	19	68	32
9	BS	1¼	1.9	4.0	0	8.5	4.0	850	1615	98	3	67	33
10	BS	1¼	2.0	0	0	8.5	0.5	2.5	5	12	35	43	57
11	BA	1¼	3.0	1.5	4.0	8.5	0.5	2.0	6	5	75	93	7
12	BS	5½	2.0	4.0	0.5	8.2	2.0	1800	3600	86	8	50	50

BS—British Saanen. BT—British Toggenburg. BA—British Alpine

Results are the mean of two or more examinations

**See text for rating*

TABLE 2

Characteristic	*Mean*	*Standard Error*
Age	3 years	0.56
Quantity	2 ml	0.14
Viscosity	1.1	0.46
Opacity	2.6	0.51
Motility	2.1	0.39
pH	8.1	0.17
Concentration (10^6/ml) ...	829	308
Total Sperms (Millions) ...	1451	474
% Alive	63.3	9.9
% Total Abnormal	25.4	7.7
% Head and Neck Abnormalities	6.1	7.2
% Tail Abnormalities ...	3.9	7.2