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EOSINOPHILS AND STRESS

Field Studies in Antarctica.

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I N T R O D U C T I O N.

The Falkland Islands Dependencies are the part of Antarctica which is claimed and governed by Great Britain. On these ice-covered lands, bases are kept permanently occupied for the purposes of research. It was while I was a medical officer on one of these bases that the present investigations were carried out.

In October 1955, I went South in the Royal Research Ship "John Biscoe" which makes annual visits to each of the Dependency bases in the short open season for shipping. The ship left me at Hope Bay, which is 600 miles South of Cape Horn. More than two years were spent on this base with eleven other men.

During the first winter, much time was spent learning how to live and travel in a cold environment. Little time was actually given to practising medicine and there was ample opportunity to observe the effects of physical and mental stress on the party. One was in no doubt that some mental change took place in each man.

It occurred to me that it would be of interest to measure some effects of the stresses to which all were subjected. The conditions in an Antarctic base are peculiarly suited to long term observations. In the winter, with the sea frozen and the weather uncertain, there are no ships or aircraft for eight months. There is also no

chance for the men to change base.

It is well known that stresses may be associated with activation of the adrenal cortex and medulla. It was thought that an assessment of adrenal function might give an objective indication of the impact of the stresses to which the members of the party were subjected and also might add to our understanding of the physiological control of the adrenal cortex. It was not possible with the limited resources available in Antarctica to make a direct measurement of catechols and corticoids in the blood or urine; of the indirect methods for estimating adrenal function, one of the simplest and most sensitive is the circulating eosinophil polymorphonuclear leucocyte level*

Accordingly in October 1956 I telegraphed the Falkland Islands Dependencies Scientific Bureau for some necessary apparatus, and eosinophil counts were begun in January 1957. In the first stage I established an eosinophil base line for each man living in the most equable conditions, i.e. base hut in the summer. Stress situations could then be studied to see if they were associated with a lowering of the count. The "t" test of significance between means could then be employed to evaluate the possibility of any difference being due to chance alone. (Chambers 1946)

*The eosinophil polymorphonuclear leucocyte is subsequently called "the eosinophil" in this thesis.

The counts in Antarctica were completed with the arrival of the relief ship on Boxing Day, 1957. Other counts were observed later in the Andes and in the physiology department of the University of Edinburgh.

A. REVIEW OF THE EOSINOPHIL LITERATURE.

A(a) INTRODUCTION to the LITERATURE.

In 1845, Wharton-Jones wrote a paper entitled "The blood corpuscle in its different phases of development in the animal series". The coarsely granular cells he describes were undoubtedly eosinophils. Ehrlich (1879) demonstrated the great affinity of these coarse granules for acid dyes and he called the cells "eosinophils". Eosinophils were recognised as a separate entity from the other cells of the white cell series and an increasing number of other special properties became evident.

Ehrlich believed that the eosinophils originated extra-vascularly in the bone marrow and that mature cells, on gaining the ability to migrate through vessel walls, passed into the blood stream. He described the size of the adult eosinophil as exceeding that of the neutrophils and thought that the normal number of circulating eosinophils was 2 - 4% of the total white cell count. Tissue eosinophilia (e.g. aggregations of eosinophils around an encysted *Trichinella* parasite) was explained by specific eosinophil chemotaxis. Systemic eosinophilia, he thought to be the result of large quantities of the chemotactic agent reaching the blood stream. Much support for these ideas came from the fact

that eosinophilia was proportional to the area of skin involved in some diseases (e.g. Psoriasis) and from the demonstration of a relative eosinophilia in the fluid of pemphigus bullae (Myer's translation of Ehrlich 1898).

In the century which followed these first observations, very many papers were published noting alterations in the numbers of circulating blood eosinophils in a wide variety of conditions. Zappert (1893), for example, found an eosinopenia in some infectious diseases but no change in pregnancy or during menstruation. Opie (1904) made an experimental study of trichinosis infestation and came to the conclusion that the parasite made some product which had a specific influence of increasing the circulating eosinophils. No eosinophils could be found in racing skiers (Weindrach 1929) or in soccer match players (Wood & Edwards 1932) after athletic activities.

The great diversity of conditions in which alteration in the number of eosinophils was found merely emphasised the ignorance of the underlying mechanism of the change. This first century of knowledge has been ably reviewed by Rud (1947) and it is not proposed to consider in detail this early literature in this thesis. As a general conclusion, however, eosinophilias appeared to be associated with an antigenic stimulation of the bone marrow or with neoplasias whilst eosinopenias were associated with stresses whether

physical, chemical or bacterial. Rud (1947) points out that, in view of the inaccurate methods usually employed, only gross changes could come to light. Direct methods of counting had been described (Mayet 1888; Dunger 1910) but in general the tedious and inaccurate white count and differential ratios had been used to arrive at the eosinophil count. Rud (1947) re-emphasised the importance of thinking in terms of eosinophils per cu.mm. rather than in percentages of eosinophil cells. He also showed that each individual had his own normal eosinophil level and that this level could not be predicted on the basis of body shape, sex or age. He gave the normal range of variation between individuals as 50 - 250 eosinophils/cu.mm. of blood.

In 1939 Dalton and Selye demonstrated an eosinopenia in rats following forced exercise or the intraperitoneal injection of turpentine. The eosinopenia was aggravated if the rats were starved and in this case, the eosinophils might drop from 1000/cu.mm. blood to zero. On the basis of these and other observations Selye postulated that the eosinophil level fell as a result of non-specific stress but there was no mention of the adrenal cortex in this paper.

However, he had shown in 1936 (see also Harlow and Selye 1937) that in his "alarm reaction", the involution of lymphatic tissue could be prevented by adrenalectomy and that in the intact animal there was a diminution of circulating lymphocytes.

A(b) The ADRENAL CORTEX and EOSINOPHILS.

Prior to 1943 only minute amounts of (A.C.T.H.)*¹ were available for experimental use. Methods were then published for obtaining highly active extracts from the pituitaries of sheep (Li, Evans, Simpson, 1943) and from hogs (Sayers, White, Long 1943). Using the new extract, Dougherty and White (1943) were able to demonstrate that on injecting it into rats there was a marked lymphocytopenia maximal in three hours. Adrenocortical extract itself induced the same response more rapidly. After the rats had been adrenalectomised, there was no response to ACTH. The same workers later (1944) made observations in rats and rabbits and found that corticosterone or cortisol*² produced the same effect in both normal and adrenalectomised animals as their cruder cortical extracts used in 1943. Desoxycorticosterone, on the other hand, had no effect on the number of circulating lymphocytes. Reinhardt^{et al.} (1944) confirmed that ACTH caused leucopenia in the rat and dog.

In 1947, Nordensen investigated the effect of ACTH and desoxycorticosterone in man but he found no consistent

*¹ ACTH will be used to designate the hormone of the anterior pituitary called variously adrenocorticotrophic hormone, corticotrophin and adrenotrophin.

*² Cortisol will be used to designate hydrocortisone, compound 'F' or 17-hydroxycorticosterone.

haematological change; in view of later findings this may have been due to inactive extracts.

In 1948, three classic papers were published from Harvard University concerning the blood changes after the injection of ACTH in man. (Thorn, Forsham, Prunty, Berner and Hills 1948; Forsham, Thorn, Prunty and Hills 1948; Hills, Forsham and Finch 1948). They found that similar effects to those found in the small mammals also exist in man but that the eosinophils are affected to a greater extent than the lymphocytes. After the injection of 25 mgm. of ACTH intramuscularly, they concluded that the following changes took place:-

1. There was an eosinopenia, which on a percentage basis was twice the magnitude of the lymphocytopenia. With a long infusion of ACTH there was a steady fall of the eosinophils but the lymphocytes tended to increase after the initial depression. The eosinophil was therefore established as a more sensitive haematological index of adrenocortical function than the lymphocyte.

2. The eosinopenia was always present after two hours, maximal at four hours and the count usually returned to normal by eight hours.

3. There was no similar haematological change in patients with Addison's disease. Both patients with Addison's disease and normal individuals showed lymphocytopenia

and eosinopenia after injection of 11-17-oxysteroids. This was good evidence that ACTH was not effecting the blood changes directly but only through activation of the adrenal cortex.

4. In standard conditions the eosinophil counts varied widely between individuals (60 - 200 per cu.mm. blood). The magnitude of the eosinopenic response between different individuals was of the same order when compared on a percentage basis.

5. There was a similar haematological response in thymectomised and splenectomised patients indicating that a shunt of eosinophil cells into these organs was an unlikely explanation for the observed eosinopenia.

Apart from these studies on the effect of ACTH they showed:-

1. Desoxycorticosterone induced no haematological change.

2. Patients with Addison's disease do not have a statistically significant difference in the circulating eosinophil level from the normal population. It therefore appeared that factors other than corticoids were maintaining the basal level.

3. In patients with eosinophilia, the ACTH response was diminished.

The numerical relationship between a cortisone dose and

the percent of eosinophil depression has been worked out by Miller (1955). Giving increasing doses of oral cortisone to twenty fit males he established that the eosinophil percent drop has a linear relationship with cortisone up to 50 mg. Thereafter increasing dosage of cortisone, induced relatively less eosinopenia, i.e. the curve was asymptotic. He also found that with increasing ACTH, changes in the urinary sodium, potassium, 17-ketosteroids or uric acid were all less predictable than the eosinophil response.

Cortisol is thought to be one of the main glucocorticoids in man (see Grant, Forrest and Symington 1957) and it is a potent inducer of eosinopenia (Recant, Hume, Forsham and Thorn 1950). It is consistent, therefore, that the diurnal variation of eosinophils strongly resembles the diurnal trends of the plasma cortisol. The evidence for this will be found later (section A(c)).

The eosinophil count was at first thought to be a good measure of increased circulating corticoids in stress (Sayers 1950) but it should be noted that adrenaline may induce an eosinopenia and is also released in stress situations (Cannon 1915). Stress eosinopenia may not therefore measure cortical activation alone. This subject is discussed later (section A(c)).

The fate of eosinophils in the eosinopenia induced by adrenal cortical activation is still undecided. Esselier,

Jeanneret and Kopp (1954) reviewing the subject favoured the possibility of increased activity of the reticulo-endothelial system coupled with an inhibition of release of marrow eosinophils. They carried out extensive experiments in vitro which appeared to make a direct cytolytic action of glucocorticoids unlikely. Muehrcke, Lewis, Kark (1952) on the other hand, demonstrated in vitro lysis of eosinophils with cortisone when defibrinated blood was used instead of heparinised blood.

Esselier et al's (1954) evidence for increased reticuloendothelial system activity comes from observations that trypan blue, which is taken up by the system, greatly diminishes the eosinopenic effect of glucocorticoids.

A(c) The ADRENAL MEDULLA and EOSINOPHILS.

Bertelli, Falta and Schweeger showed as long ago as 1910 that the intravenous injection of adrenaline in the dog was associated with an eosinopenia. Later, this was found to be true in man (Godlowski 1948; Laragh and Almy 1948).

The observation of Vogt (1944) that adrenaline injected into the cat or dog caused an increased output of cortical hormones was of great interest. The plasma of the adrenal venous effluent was bioassayed by the method of Selye and Shenker (1938) in which the corticoids are estimated by an increased survival of adrenalectomised mice.

Later Vogt (1945) had confirmation of this observation by demonstrating an increased adrenal weight in the rat following a chronic infusion of adrenaline. This effect was abolished if the rats had been previously hypophysectomised. It appears, therefore, that adrenaline activates the pituitary to produce an increased output of ACTH. In 1945, Long and Fry showed that, in the rat, the adrenal ascorbic acid and cholesterol fell after adrenaline injection and that the effect was not present without an intact pituitary. Since the depletion of adrenal ascorbic acid and cholesterol are well established indices of cortical stimulation (Sayers 1950), Vogt's observations seem amply confirmed.

There was thus evidence from work on three mammals, the

cat, dog and rat, that the sympathetic and adrenocortical systems were inter-related by way of the pituitary.

In 1948, the remarkable effect of the cortical hormones on the eosinophil cells of man was demonstrated conclusively for the first time (see section A(b)). Both ACTH and cortisone, when given in known dosage, resulted in a reasonably predictable fall of circulating eosinophils from the pre-test level.

In 1950, the effect of adrenaline on the eosinophils of man was investigated by Recant, et al. Although these workers failed to show an increase of urinary corticoid output they concluded on the basis of the available evidence that adrenaline-eosinopenia was mediated by the pituitary. Fisher and Fisher (1951) concluded that the reaction might involve the hypothalamus as well as the anterior pituitary. On the basis of this sequence a number of clinical tests were evolved (see e.g. Recant et al. 1950) to localise the lesion in patients exhibiting signs of a deficiency of circulating corticoids. It was thought that the integrity of the adrenal cortex could be determined by showing whether the injection of ACTH was followed by an eosinopenia or not. If the adrenal was not implicated then the pituitary link could be tested by determining the effect of adrenaline on the eosinophils. The rationale of this concept was further supported by the diminished adrenaline-eosinopenia that

occurred in patients with Addison's disease, (Recant et al. 1950).

There appears to be little doubt that the injection of adrenaline does cause an eosinopenia in man, though the effect is less certain than that following ACTH (Godlowski 1948; Laragh and Almy, 1948; Fisher and Fisher, 1951; Hunter, Bayliss and Steinbeck, 1955). During a continuous infusion of adrenaline there may be an eosinophilia which does not change into the characteristic eosinopenia until after the infusion has stopped (Hortling and Pekkarinen 1949). This will have to be confirmed. Nor-adrenaline has little effect on the eosinophils (Humphreys and Raab, 1950), and it is interesting to note, at this juncture, that in man nor-adrenaline is produced mainly at extra-adrenal sites whilst adrenaline is produced in the adrenal medulla (see Moran, 1957).

In 1952 the mechanism of adrenaline eosinopenia in man was re-examined by Muehrcke, Eckert and Kark and they showed clearly that an injection of adrenaline could cause an eosinopenia even in patients who had been adrenalectomised. Consistent with this finding Nelson, Sandberg, Palmer and Glean (1952) could find no increase in the urinary cortisol after the injection of adrenaline. Moreover the first authors demonstrated that the adrenal cortex had been removed effectively by showing that the injection

of ACTH was not followed by an eosinopenia. Subsequently these findings have been amply confirmed by Bayliss (1955); Muehrcke, Staple and Kark (1952) and Hunter et al. (1955). Guillemin and Rosenberg (1955) showed that the hormonal link between the hypothalamus and the anterior pituitary (postulated by Harris, 1955) was unlikely to be adrenaline or noradrenaline. In stress situations there may be an eosinopenia without an alteration of urinary corticoids; endogenous adrenaline is thought to be a likely factor (Thorn 1953).

The response of the eosinophils to corticoids and adrenaline has been considered and we must now deal with the release of these hormones in adverse and stressed conditions. In tense, anxious, mental states both the urinary adrenaline (Elmadjian, Hope and Lamson 1957) and the plasma cortisol (Persky 1957) appear to be increased. In traumatic and in planned surgery, there is an increase of both urinary adrenaline and plasma cortisol (Franksson, Gemzell and Euler 1954). In exercise there may be an increase in urinary adrenaline (Euler and Hellner 1952) and in Channel Swimmers the urinary corticoids increased four fold, (Pace, Walker, Pugh, Thomas, Timiras, Siri, Young, Kellog, Karler, Hencken, Haertle and Fava 1956).

The diurnal rhythms of both corticoid and adrenaline excretion also appear to run parallel for the highest resting urinary adrenaline output is about the awakening time

in the morning (Euler, Luft and Sundin 1955) and this coincides with the diurnal maximum of the plasma corticosteroids (Bliss et al. 1953; Doe et al. 1954; 1956; Peterson 1957). (Note should, however, be made of the fact that Euler et al. (1955) think that the high morning adrenaline values are purely related to the assumption of the upright posture.) It can thus be concluded that in many human situations the adrenal medulla and the cortex are activated together.

Recant et al. (1950) showed that commercial extract of the adrenal cortex which had no eosinopenic action on injection per se could induce an eosinopenia when it was combined with an otherwise ineffective dose of adrenaline. Speirs and Meyer (1951) showed in adrenalectomised mice that the eosinopenic effect of cortisone was greatly enhanced by pre-treatment with adrenaline. Adrenaline can, it appears, produce an eosinopenia in adrenalectomised animals sustained by cortisone (Thorn and Laidlaw 1954). Hunter et al. (1955) concluded that the presence rather than an increase of corticoid must be circulating for an adrenaline eosinopenia to exceed 50%. From all of these observations and the diminished adrenaline eosinopenia of hypo-adrenal patients already referred to it would appear that in man whilst adrenaline injection is not associated with adrenal cortical stimulation it may join with the circulating corticoids in

some way as yet unknown in the mechanism which results in an eosinopenia.

The absence of an eosinopenia in a situation thought to involve stress would appear to be good evidence that no adrenal cortical stimulation has taken place. Since, however, the eosinophils do not necessarily fall after the injection of adrenaline in man (Djavid 1935) similar arguments cannot rule out activation of the adrenal medulla. If the supposed stress does on the other hand induce a significant eosinopenia then the apportionment of this to the activity of the adrenal cortex or medulla is problematical. From the available evidence it would appear that both layers of the gland will often be implicated. The work of Canon (1925) suggests the importance of adrenaline in the immediate "flight" or "fight" and Selye (1946) expands this concept by suggesting the importance of corticoid in chronic stress. A similar, tentative conclusion was reached by Hill, Goetz, Fox, Murawski, Krakauer, Reifenstein, Gray, Reddy, Hedberg, St. Marc and Thorn (1956). Their opinion was based on studies of examinees, marathon runners and Harvard boat race crews and their measurements included urinary 17 hydroxycorticoids, circulating eosinophils and standard psychological tests. Thus, in the interpretation of an eosinopenia in a chronic stress situation glucocorticoids rather than adrenaline are probably

involved.

In conclusion, if an eosinopenia is noted in a situation involving mental or physical strains and stresses then there is little doubt that the adrenal gland has been stimulated. The mechanisms causing release of these two substances are not well understood and the way in which they give rise to an eosinopenia is largely unknown. It is, moreover, usually difficult to say with certainty whether the eosinopenia is caused by the medullary or the cortical hormones. The use of this eosinopenic response to measure the impact of the stress on an individual is discussed later (section A(e)).

A(d) The BLOOD GLUCOSE and EOSINOPHILS.

In 1924 Canon, Maciver and Bliss demonstrated in the cat that an insulin induced hypoglycaemia was associated with a rise in circulating adrenaline. This observation was confirmed in the human by Heilbrunn and Leibert (1939) and is consistent with the observation of Riddle (1924) that the pigeons suprarenal enlarges after repeated insulin injections.

Godlowski (1946 and 1948) showed that the injection of insulin was associated with an eosinopenia similar to that produced by adrenaline. Laragh and Almy (1948) also demonstrated a post-insulin eosinopenia in eleven healthy subjects.

In 1947, Vogt showed that the injection of insulin was associated with an activation of the adrenal cortex and medulla in the rat. Moreover the cortical action was not necessarily due to release of adrenaline since the same effect was present when the adrenals were bilaterally denervated. In 1950, she showed that this cortical activation after insulin was due in part to the low blood sugar and in part to adrenaline produced secondarily.

In the interpretation of stress eosinopenias in man the possibility of hypoglycaemia being a factor must be considered. However, any marked hypoglycaemia should be clinically evident.

A(e) STRESS SITUATIONS and EOSINOPHILS.

In the study of stress situations the eosinopenic response may be conveniently divided into three categories. First, the stress may be so slight that any eosinophil response is masked by such factors as diurnal changes or counting errors; secondly, with intermediate stresses, there will be a widely variable response depending on the individual's age, physical ability etc. (e.g. the stress of a ten mile walk.) On the other hand, extreme stresses e.g. bad burns, major surgery or a myocardial infarction will evoke a maximum response in which the individual variation is masked. Some of the most interesting responses to study, therefore, are the intermediate group since it is in them that the individual impact of any given stress can be seen. This concept of three levels is expanded from Gofton, Graham, McGrath and Cleghorn (1953).

The literature relating to eosinopenias apparently induced by various types of stresses may, I think, be clarified by a Table such as Table 1. The type of stress, the magnitude, and time of the response are noted. In some cases no consideration was given to diurnal variation, but it may be seen from Table 2 in section A(f) that a maximum error of 50-60% might be incurred in experiments which neglected it. On the other hand most experiments take place during one working day when the resting eosinophil

TABLE 1.

Author	Stress	Any Note of Diurnal Trend	Blood	Time Relative to Stress	No. of Subjects	Order of Fall and Comments
SWEIN	HEAT daily	Same time	Venous	Day after	1	mean -14% (24 obs. of control + stress)
BADIER	(dry bulb 107° F., wet 86° F.)	each day.				
ELLIOT	Wind 3 m.p.h. 5 hrs.				1	mean -48% (2 observations)
BASS	1½ hrs. 3.5 m.p.h. on treadmill. Light clothes)				1	mean -32% (19 observations)
	COLD daily	"	"	"	1	mean -19% (20 obs.)
	-20° F. 4 m.p.h. wind.				1	mean -16% (23 obs.)
	Arctic clothing.				1	mean -39% (25 obs.)
	3 m.p.h. on treadmill if cold. ? 5 hrs.					
ALTSCHULE	Therapeutic electrically induced convulsions.	No.	"	Blood taken before and 4 hrs. after.	21	0-100% fall.
PARKHURST						
TILLOTSON						
1949						
ROCHE, THORN and HILLS	Major Surgery	No.	?	Immed. Post Op.	22	-86% to -100%
1950						
GABRILOVE	Minor Surgery	No.	Finger	Day after	9	-40% to -100%
1950	General Anaesthesia		"	"		Down to -100%. No controls
	Myocardial Infarction					

Contd.

TABLE 1 (Contd.)

Author	Stress	Any Note of Diurnal Trend	Blood	Time Relative to Stress	No. of Subjects	Order of Fall and Comments
FROST DRYER 1951	Racing Drivers in 500 mile Indianapolis Speedway	No	Venous	1 hr. after	9	-100%: -97%: -96%: -94%: -93%: -65%: -63%
REYNOLD QUIGLEY THORN HARRISON 1951	Harvard Boat Races	Controls taken	Ear	2 - 4 hrs. after	9	Practice means -57% -30% Time trials -35% -48% -69% Races -78% -70%
DOMANSKI T.J. SWAN A.C. WELLS J.C. HUGHES B.A. 1951	Graded walking on the treadmill	Controls taken for all times	Finger	1, 2 & 3 hrs. after	9	When exercised to exhaustion, One subject showed no change; others had eosinopenias up to -75%. Less severe exercise often had no effect. The exercise was always short term.
CUKLER 1952	Anoxia: Subjects raised from sea level to 14750' in few hours by car	Controls taken	Venous	1 hr. after arriving	21	+12%: -8%: -22%: -29%: -30%: -31%: -35%: -38%: -41%: -45%: -56%: -62%: -81%: -81%: -85%: MEAN -43%

Contd.

TABLE I (Contd.)

Author	Stress	Any Note of Diurnal Trend	Blood	Time Relative to Stress	No. of Subjects	Order of Fall and Comments
THORN 1953	Major Surgery	No	?	Immediate Post Op.	1	-100%
	Cold - 4 hrs. 4 C. No supp. Clothing	Late a.m. Early p.m.	?	Immediately after	6	mean -23% (statistically signif)
	Anoxia - Artificially induced over 30 min. to 27,000'	"	?	"	7	mean -39%
WAKE GRAHAM McGRATH 1953	Emotional Film	"	?	4 hrs. after	20	mean -30%
	Day of exercise e.g. Badminton	Controls taken	Ear	8 p.m. after exercise	18	mean -43%
FACE et al. 1956	Channel Swimming	No		Venous 1 - 3 hrs. after	12	-97% to -100%
	L.D.S. Examinees	Done in P.M.	Finger	During and immediately after	4	mean -90% (one no change) Controls: Maximum -25%

Contd.

TABLE 1 (Contd.)

Author	Stress	Any Note of Diurnal Trend	Blood	Time Relative to Stress	No. of Subjects	Order of Fall and Comments
RINDANI T.H. PONEKAR B.D. KOTHARI N.J. 1956	E.C.T. adequate to elicit petit and grand mal	No	Venous	Pre E.C.T. 3 hrs. post E.C.T.	10	Grand Mal -53 -67 -71 -79 -83 Petit Mal -39 -40 -55 -63 -68
						Percent fall of eosinophils
FUCH 1959	4 hrs. skiing in resort	Early evening	Finger	Soon after	2	When unfit -73% -63% -92% When fit -61% -67%

count would be expected to rise; in this instance, a fall at the end of the day would carry more weight. The observation of Miller (1953) that the injection of ACTH abolishes the diurnal trend and does not sum with it might suggest that in the study of stress situations note does not have to be taken of the trend; however this observation would have to be confirmed.

In the Table, note is also made of the origin of the blood used to make the eosinophil count. Rud (1947) observed spontaneous fluctuations in the count but these have not been confirmed. It is possible that some of these are due to the use of arteriolar rather than venous blood (see Lucey 1950). It is thought therefore that more weight may be put on estimations using venous blood.

Since every individual has his own normal mean eosinophil count (Baagoe 1958) it is usually essential to have control counts in non-stress situations in order to evaluate any change. However, in the event of eosinophil counts of zero (e.g. after myocardial infarction Gabilove 1950) it is obvious that a profound fall has taken place since the lower limit of normality is 30-40/cu.mm. (See Baagoe 1958).

Since an oral dose of cortisone produces a similar percentage fall of eosinophils in different individuals (Thorn et al. 1948; Miller 1953) it is probably fair to compare similar percent falls between individuals as

eliciting the same quantitative response.

This statement is made with the possible reservation that high counts may not fall so much on a percent basis as low counts (see Esselier et al. 1954)

The following conclusions may be reached from these studies of the eosinopenic response to stress:

1. Major stress is invariably associated with an eosinopenia approaching 100%.
2. Mental stress alone can account for eosinopenias of this order.
3. Intermediate stresses cause a wide range of eosinopenic response which cannot entirely be predicted by the age, physical state and so on of the subject, although these are obviously important.
4. Minor stresses must be considered statistically in order to gain definite information about the eosinophil change. Many readings in the same situation will have to be made and compared with a large control series.

A(f) The DIURNAL VARIATION of EOSINOPHILS.

Domarus (1931), Djavid (1935) and Appel (1939) made investigations into the variation of the eosinophil cells during the day and they found a definite rhythm. This rhythm was characterised by constant low levels of eosinophils at 10 a.m. and higher levels at the beginning and at the end of the day (7 a.m. and 7 p.m.). This rhythm appeared unaffected by illness or food. Appel (1939) found a similar rhythm in dogs which disappeared when the dogs were put in continuous darkness. On the basis of his observations and the fact that he could find no fall of eosinophils in night nurses waking for duty in the evening, he concluded that the variations were associated with daylight.

Since these preliminary investigations many workers have investigated this rhythm and their work has been summarised in a Table. In some instances the figures included in the Table have been estimated from graphs. Since individuals may have widely different eosinophil counts (see Baagoe 1958) most authors reduce readings to a percentage of a reference point. Rud (1947), for example, uses 8 a.m. Table 2 has been constructed using 9 a.m. as a reference point since this is near the daily nadir.

The Table shows that there is close agreement between these different workers about the existence and timing of

TABLE 2.

THE DIURNAL VARIATION OF EOSINOPHILS

Authors	Time								No. of Subjects
	9 a.m.	12 m.d.	3 p.m.	6 p.m.	9 p.m.	12 m.n.	3 a.m.	6 a.m.	
RUD (1947)	100%	120%	108%	116%	133%	150%	138%	133%	6
HALBERG (1953)	100%	136%	123%	191%	199%	209%	232%	223%	17 fit males
DOE FLINK GARY FLINT (1954)	100%	-	108%	-	133%	-	-	149%	4
KAINE HOLBROKE SELPZER CONN (1955)	100%	89%	100%	102%	122%	128%	128%	128%	6
DOE FLINK GOODSELL (1956)	100%	-	119%	-	140%	-	-	135%	17
UHRBRAND (1957)	100%	94%	108%	127%	144%	161%	155%	133%	15
ARNOLDSSON HELANDER (1958)	100%	101%	118%	117%	118%	142%	156%	133%	16
Means	100%	108%	112%	131%	141%	158%	162%	147%	81

the rhythm. The daily nadir occurs at approximately 8 a.m. to mid-day and the zenith about midnight. Further support for this concept comes from the observations of:- Halberg, Cohen and Flink (1951)(1); Halberg and Visscher (1951)(2); Halberg, Visscher, Flink, Berge and Bock (1951)(3); Swanson, Bauer and Ropes (1952); Donato and Strumia (1952); Halberg, Engel, Treloar and Gully (1953); Thorn (1953); Wake, Graham and McGrath (1953). The diurnal variation of eosinophils is independent of the other leucocytes (Uhrbrand 1958).

Diurnal rhythms of eosinophils have also been observed in mice (Halberg and Visscher (1950); Halberg (1951)(4); Speirs (1953); Brown and Dougherty (1956); Halberg, Peterson and Silber (1959).

In support of Appels (1939) observations on the possible importance of light, Halberg and his colleagues (quoted by Webb and Brown 1959) have found that the diurnal rhythm in mice may be reversed by reversed illumination but in continuous darkness the initial rhythm is maintained.

Halberg et al. (1951)(2) found that the characteristic morning nadir of the eosinophils was absent in patients with panhypopituitarism, Addison's disease or with bilateral adrenalectomy. Consistent with this finding the diurnal variation of plasma corticosteroids as investigated by Bliss et al. (1953); Tyler et al. (1954); Doe et al. (1954)

(1956); and Peterson (1957) closely resembles the described diurnal variation of the eosinophils. The diurnal rhythm of urinary cortisol (Thorn 1953) and 17-ketosteroids (Pincus 1947) are also in agreement.

In asthmatics the daily nadir and zenith of eosinophils may be reversed (Arnoldsson and Helander (1958). This is of great interest in view of possible disorder of the adrenal cortex, but will have to be confirmed.

To conclude it would appear that there is a diurnal variation of eosinophils which is closely associated with the daily variation of plasma corticosteroids.

B. STRESSES IN ANTARCTICA.

B(a) DISCUSSION of the Word STRESS.

As early as 1843 the word "stress" was used to denote "strain upon a bodily organ or mental power".* Also Locke** said "Though the faculties of the mind are improved by exercise, yet they must not be put to a stress beyond their strength." Thus the word "stress" has been used in relation to health from at least these early dates.

Since 1936 the word has appeared frequently in the medical literature mainly as a result of the work of Selye. He uses it to describe all those non-specific stimuli (e.g. cold, anxiety, injection of morphine, infection) which when applied to the mammalian organism are liable to evoke the same response (in which there is pituitary-adrenal activation).

It is interesting that in view of the difficulty of finding a suitable translation for other languages the terms "le stress"; "der stress"; "lo stress"; "el stress" are in use in the appropriate countries.

* Shorter Oxford Dictionary 1956.

** 1632 - 1704.

DEFINITION of the word ANTARCTICA.

The word "Antarctic" implies all that area within the Antarctic Circle, S. Lat. $66^{\circ} 32'S$. This does not include however much of the fringes of the great continent and islands. The term "Antarctica" was therefore introduced by Bartholomew to include all that area falling South of the Antarctic convergence - the great junction line of warm and cold water which occurs between $50 - 60^{\circ}$ S. Latitude.

MAP SHOWING REGION OF THE EXPERIMENTS.



The Falkland Island Dependencies are at the top right hand corner. Graham Land is the great peninsula and Hope Bay, where the present experiments were carried out, is on its Northern extremity.

B(b) ANTARCTIC STRESS.

The word "Antarctic" has always been associated with cold, death marches, crushed ships, crevasses and so on. It is this very "stress" which has provided such a sustained thrill to the explorer whether active or passive. One has only to read a few pages about Shackleton's "Endurance" being crushed in the Wedell Sea to realise the challenge.

The polar pioneers spent much time fending off the stress of the environment. Although valuable scientific work was done it was an achievement merely to stay alive. When Captain Scott was on his death march back from the pole he had high friction sledge runners, insufficient paraffin (therefore none to dry clothes, the tent, and the sleeping bags whose condensation water would increase all the time), and scurvy-producing rations.

The experience of the pioneers and the scientific development of equipment has greatly diminished the stresses. Such advances as the two-walled tent, the "Primus" stove, adequate rations, the double-down sleeping bag, the twelve foot Nansen sledge and the motorised "sno-cat" have contributed to this. With the experience, equipment and methods, maximum attention can now be given to the glaciology, geology meteorology and physiology of cold regions. Amundsen did brilliantly in merely reaching the pole, whilst Sir Vivian Fuchs' great success was the completion of a scientific

programme.

Stress, then, may not always be with the modern polar explorer. On the other hand some new stresses have appeared as the classical ones have diminished. The leader is constantly harassed with radio signals which disturb his equanimity; domestic crises intrude and interrupt the peace and teamwork of the isolated group of men. Many explorers might be in favour of scrapping radio completely, the safety of SOS signals notwithstanding. Air travel is a hazard and so is the mental strain of completing the scientific programme. For the man who stays in the wooden base hut the mental strain of a completely isolated male community may be considerable. This is especially so if he is not suited to his job and there is no hope of change. Moreover if he is a skilled technician (e.g. a wireless operator) no one else can do his job and there is no hope of a holiday. For the traveller the difficulties can be quickly summarised: thin sea-ice, poorly bridged crevasses, hurricane force winds and bad sledging surfaces. Any of the above conditions, especially in combination, can rapidly defeat the most skilled, competent party with the best equipment.

While stress is now a mere fraction of what it was fifty years ago it is still the main concern of the explorer and it is the evaluation of this stress that this thesis is concerned.

LIVINGSTON ISLAND.



Representative of the mountainous scenery of Graham Land. The crevasses on the glacier (left) would be very difficult to cross. Note small ice-fall in picture centre. Mountains about 5,000 ft. high.

GRAHAM LAND.



Typical blizzard scene. This untravellable day was used to make eosinophil counts. Note dogs lying back into the wind. The two men have just fed them.

B(c) THE NEED for EOSINOPHIL EVALUATION of STRESS
in ANTARCTICA.

Canada and Russia are rapidly expanding their civilisations into the Arctic and there has been more activity in the Antarctic than ever before. The recent voyage of the Nautilus under the Arctic pack has demonstrated the economic possibility of shipping oil from the Arctic throughout the year. On the basis of this voyage an intensive search for oil and other natural wealth has been stimulated. Consequently investigations into physiological changes in these Polar regions is a matter of increasing interest. In the past comparatively little physiology has been completed whilst dog-sledging and manhauling and the demonstration that en route haematological work was possible, using venous blood, might enable the sledging physiologist to become more ambitious than he has been.

One of the problems of the human physiologist is that of obtaining experimental subjects who can remain under observation. In the U.S.A. prisoners are often used. The Antarctic base is peculiarly suitable for some long term experiments. With the right approach, in which the whole investigation is explained individually, the personnel will nearly always co-operate. It is possibly an important piece of strategy not to ask all the base members to co-operate, but only the ones you are "interested" in whilst

the remainder will stay on your side in any threatened revolt in case they are asked to co-operate. It is possible that some general physiological problems may be attempted by the future Antarctic Medical Officer as well as those of polar interest. Clearly the completion of any long termed planned investigation on a base may stimulate others to take advantage of this unusual opportunity for experiment, e.g. for the study of lengthy dietary balance experiments.

Subjective accounts of exploration give some general indication of the stresses and strains in which the party were involved. Usually there is only one author and another would have written a different account. Often the response of the individual is masked in such accounts partly because of etiquette and partly because in another's interpretation there was no stress. Thus the initiation of objective experiments into Antarctic stress seemed of importance and the establishment of a new base line from which the trials of any expedition might be judged.

An understanding of the magnitude of the affects of different Antarctic stresses would guide organisers, leaders and men and give new insight into conditions of stress that might be ameliorated. For example, the demonstration that the week's cooking duty, done by all, on a roster caused a stress eosinopenia might indicate that a professional cook was required.

The importance of stress in the aetiology of disease is undecided. Whether it is implicated or not a first investigation should be one to determine the type of "stress" which is associated with maximum adrenal response. Many workers have emphasised the importance of purely mental stress in the formation of eosinopenias (e.g. Thorn 1953; Kerr 1956; Hill et al. 1956) and this is of great interest since mental stress is probably more characteristic of a normal life than other stresses (e.g. surgery or burns). Clearly the positioning of mental stress in comparison with other stresses (e.g. physical stress) is of interest. From a study of the result it is hoped that a greater understanding of the general human and individual response to stress would be obtained. Moreover, the results would further the understanding of the long term control of the adrenal gland and give some information to other workers in Antarctica whose measurements might be affected by concurrent adrenal activity.

C. PRESENT WORK.C(a) DESCRIPTION of LIFE DURING YEAR.

In order to appreciate the stresses which the subjects of the experiments underwent, it is necessary to build up a picture of the Hope Bay life as it was experienced during 1957.

Ten men were left in the hut after the departure of the last ship in March. Apart from radio there was no contact with the outside world for eight months. Much of the responsibility of the base and its work programme fell to the base leader who was also a topographical surveyor. There were also men engaged in making routine meteorological observations, two wireless operators, a diesel mechanic, a surveyor and a doctor (myself).

The hut was large and the main outer wall consisted of two layers of planking sandwiching the frame and straw insulation. Only the base leader, the surveyors and the doctor had private offices. In the centre of the hut was the main living room (see photograph next page). Bunks for all men were fitted around the walls and at a central table everyone met for meals. In spite of the two anthracite stoves the shoulder height temperature sometimes registered -5.5 C. at night. With proper stoking it was

HOPE BAY HUT - GRAHAM LAND.



Winter scene outside hut. Surgery - 3rd window from left. Subject RW and a husky puppy, too young to be tied up.

HOPE BAY HUT, GRAHAM LAND, ANTARCTICA.



Main room. Note bunks around walls, and hob fire. Typical evening scene.

usually possible to keep the living room and offices at a comfortable temperature.

Every effort was made to keep a daily routine with fixed meal times. Thus the duty cook (done by all on a roster for a week at a time) brought morning tea to all bunks at nine, lunch was served at one and so on. The man on night meteorological duties usually had difficulty in making up his sleep during the day.

Most of the activity of hut life in Winter was sedentary but with the following important exceptions. The feeding of the fifty dogs was done on alternate days by cutting frozen seal meat with an axe (about 4 lbs./dog). The pieces had then to be dragged round on a sledge to each dog. This took 2 men $\frac{1}{2}$ -2 hrs. depending on how hard the meat was frozen. One man bathed each night and the following morning had to refill the tank with sawn snow blocks. This was one hour's hard work depending on snow conditions. In the Summer bringing up annual stores from the beach was very hard work. Voluntary exercise, skiing and husky training also contributed to exercise.

Evidence of mental stress was of frequent occurrence on the base and apart from very occasional medical and surgical emergencies mental health is usually the largest problem for the Antarctic M.O. In any isolated community there is usually a clash of personalities. Probably we did not have

more than our share but the problem was always there. Outside influences, e.g. telegrams from girl friends or from authority tend to become out of proportion since there is so little to distract one's attention from them. Apart from these personal problems there were three instances of stress, during the period of test, which might have affected the whole base. These are probably representative of any Antarctic base.

1. One man lost his way back to the hut after feeding the dogs in a blizzard in a temperature of -16°C . Apart from the physical effort of the search there was considerable anxiety felt by all before the man found his own way back to the hut eight hours later. Probably most men in the hut would have considered him dead in this period.

2. An Argentinian drifted out to sea in a small boat when his outboard ran out of petrol. A desperate effort was made to try and reach him in another boat that was almost disastrous. The man was saved by radio SOS and ship's helicopter 24 hrs. later when 25 ml. out to sea.

3. The anticipation and arrival of the relief ship which was holed by ice, sending out SOS signals and reported to be in a "sinking condition". After initial worry and the later "all clear" there was still uncertainty whether the year's mail was under water. These are the three main instances of mental stress on the base in the year of

observations. There was one major illness with eosinophilia which is described.

There were also more obvious stresses whilst sledging in the field. In general, sledging was looked forward to with excitement and was seldom dangerous. One could sledge for two months without contact with the base and still be in excellent physical and mental condition. This is attributed to the sledging technique and equipment as evolved by members of the survey and the tonic effect of change from base routine. It contrasts strongly with the early sledging days when men arrived back on their last biscuit (see Scott).

However, the sledging in 1957 was more hazardous than usual and since the subjects for the experiments spent more than half of the year sledging, it is very significant. Eosinophil counts were made during the first and fourth journeys from the base and a description of the particular stresses on these journeys will be found in RESULTS, section 3 (a) and (b).

C(b) METHODS.The Technique of Counting Eosinophils:

After Ehrlich's (1879) demonstration of the differential staining of the white cell series, the eosinophils could be estimated by making an absolute white count and then from a stained film the ratios and absolute number of the different types could be worked out. Ehrlich gave the normal eosinophil count to be 2 - 4% of the total white cell count.

This indirect method is only a rough guide, Rud (1947); Speirs (1952). In 1888 Mayet described the first method of counting eosinophils directly. In this method the cells were stained with eosin and suspended in water and glycerine. Dunger (1910) described another technique using a diluent of eosin, acetone and water whereby cells other than eosinophils are invisible or appear merely as ghosts. With these direct methods available, Rud (1947) laments the amount of "futile biological research" of workers using the indirect methods. Rud (1947) used a direct technique with magda red, acetone and sodium carbonate solution. The blood was diluted 1:20 in a white blood cell pipette and the mixture capillated into two Fuchs-Rosenthal counting chambers. These cerebro-spinal fluid counting chambers are twice the depth of ordinary white cell counting chambers and as Speirs (1952) points out the error of the count is proportional to

the square root of the number of cells counted.

Thorn et al. (1948) modified the Dunger (1910) technique with the simple 5 ml. 2% Aq. Eosin, 5 ml. acetone, 90 ml. aq. dist. (i.e. acetone concentration halved). The eosinophils show up brilliantly with other white blood cells and red blood cells as ghosts or lysed. Again, 2 Fuchs-Rosenthal counting chambers were used. This is the staining and chamber technique used in the present study.

Acetone-eosin techniques have been criticised by Henneman, Wexler and Westhaver (1949) who demonstrated the "eosinolytic" action of acetone and that errors on the low side resulted with this method. But this is negligible if the count is made within 5 mins. and they raise no criticism to using this technique in a laboratory where the count may be done at once.

Speirs (1952) showed that acetone was not in fact eosinolytic but that increasing concentrations of it in the stain caused proportionately less lysis of cells. In view of the fact that the most fragile cells are the red cells, then the white cells and finally the eosinophils, suitable fractionations of water to acetone can be made to leave only the eosinophils and some white cells. In 50% water:acetone no cells are lysed. In distilled water all the cells are ruptured by the hypotonicity. In the present study the concentrations are such that all the red cells are lysed and

some of the fragile white cells, leaving all the eosinophils and some tougher white cells as ghosts.

The majority of workers use a standard white blood cell pipette in which the blood and stain are diluted and mixed in the proportions 1:20. The same dilution is used in the present study but without using the usual special mixing pipette. .38 mls. of stain are measured by a 1 ml. pipette into a small tube. .02 mls. of blood is drawn up into a .02 ml. (British standards) pipette and then ejected into the centre of the stain, and the pipette washed back and forwards twice to help removal of any cells sticking to the glass. The small tube was then shaken horizontally with the index finger on top and thumb on the bottom for about 15 secs. Using the same blood pipette four samples (sometimes six in low counts) were run into the 4 sides of 2 Fuchs-Rosenthal counting chambers, (the first two samples having been rejected in case small quantities of blood were still adherent to the glass.) The counting was begun, using the 1/6 th. objective, and a x 6 eyepiece, within five minutes of mixing the stain and blood. All the ruled areas were counted. The final calculation to find the number of eosinophils/cu.mm. were as follows:-

Depth of counting chamber	= 1/5 th.mm.
Area of one "smallest" square	= 1/16 th.sq.mm.

Number of these squares in
4 chabs. = 16 x 16 x 4 squares.

Therefore total volume of
counted area = 4 16 x 16 x 4 x 1/16 x 1/5 cu.mm.

But blood diluted .02; .38 i.e. 1:20 dilution.

Let y = no. of eosinophils counted.

Therefore number of eosin-
:ophils/cu.mm. = $y \times 16 \times 5 \times 20 \div 16 \times 16 \times 4$
= $y \times 100/64$

or if as in the case of low (under 50/cu.mm.) counts 3
double chambers were used the final multiplication of number
counted is 25/24, i.e. almost the number in a whole cu.mm.
of blood.

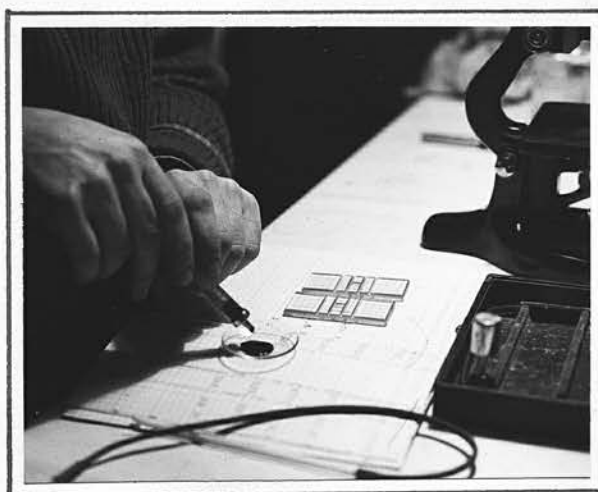
The following points were thought to be of importance
in contributing to the accuracy of the counts:-

1. Use of two or three double sided deep counting
chambers.
2. Use of antecubital venous blood. A generous 3-3 ml.
sample was taken for the .02 ml. needed. Lucey (1950) draws
attention to errors which may arise from ear blood. In view
of the cold environment finger hacks would have healed slowly.
3. Doing all the counts personally. Since fluctuation
rather than absolute concentration is important any consis-
:tent error of technique will not matter.
4. In many instances the counts were done at the same
time of day. The difficulty of diurnal variation, Rud
(1947) is thus obviated.

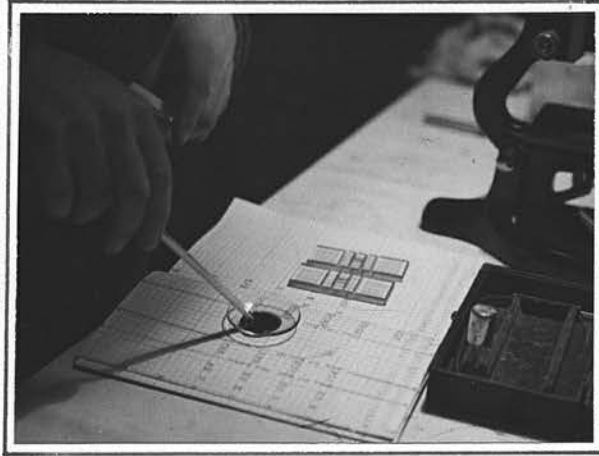
THE STAGES OF AN EOSINOPHIL COUNT:
PHOTOGRAPHS TAKEN IN THE BASE HUT.



1. The Venē-Puncture. No Anticoagulant.



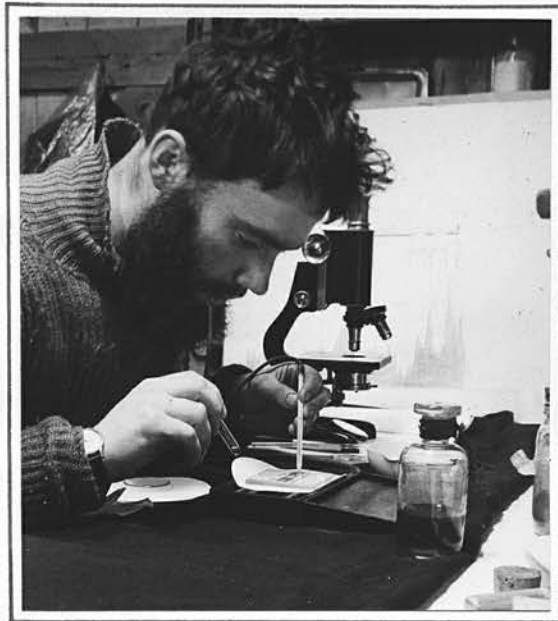
2. Blood is ejected into a Watch Glass.



3. .02 ml. Blood is aspirated into a straight pipette.



4. Blood is ejected into a small tube containing .38 ml. Stain.



5. The contents of the small tube are shaken for 15 secs. and then 4 samples are put in both sides of 2 Fuchs-Rosenthal counting chambers.



6. All the ruled areas are counted.

5. No anticoagulant was used with the venous blood. (There is some evidence that oxalate may increase the fragility of the cells - Henneman et al. (1949)).

There are various special points of particular importance to haematological work on sledging journeys.

1. A nylon double walled pyramid tent with dural poles was used. This was more or less essential because of the great increase of transmitted light as compared with cotton. Double walls are essential to minimise condensation. Thus dust on the apparatus can be seen and venipunctures made easier. Articles are less likely to be lost and the counting chambers can be kept spotless - the slightest contamination of paraffin from the primus on the cloth used to clean the counting chambers will cause bubbles (tent made by Camptors-London).

2. A well maintained primus running on top grade paraffin is essential. We used two pints of paraffin daily. More accurate work is done when the tent can be kept about 60°F. without fumes. The tent door is shut but the ventilation in the apex open; there is a constant circulating current of air. A badly maintained primus or blocked ventilator will cause headaches and streaming eyes in a few minutes. With the tent any colder the microscope tends to condense up - water dripping on the slide down the main tube - and it is necessary to be muffled up in sweaters

which makes veins inaccessible. (Primus by Hgorth-Sweden).

3. A strongly built microscope was used, well packed in a box to resist breakage when the sledge overturns. Prior to the morning investigations the microscope was hung up in the apex of the tent to warm up. The heat from the primus in the middle of the floor collects in the apex with an estimated temperature of 100°F. At first hoar frost forms on the metalwork, then water pours off as it heats up; this probably does the lens no good but there was no obvious loss of resolution in a hundred days of such use. A holed filter paper, impaled by the lens tube, is a useful way of preventing condensation water dropping onto the slide.

4. Stains must be unaffected by freezing or carried in a thermos and frequently warmed. Thorn's stain appeared unaffected by freezing. At night the only place where the temperature is above freezing is inside the sleeping bags - in an emergency one could keep the bottle inside. The stain, and fresh ingredients, were all kept in 1 lb. kilner jars which are strong and have a rubber washer to prevent the acetone evaporating or contents escaping. They do not break when the stain freezes owing to the straight sides.

5. All breakable equipment must be heavily duplicated especially the counting chamber coverslips which are inevitably broken sooner or later. Long pipettes can be well protected in wooden thermometer cases (the same shape as a

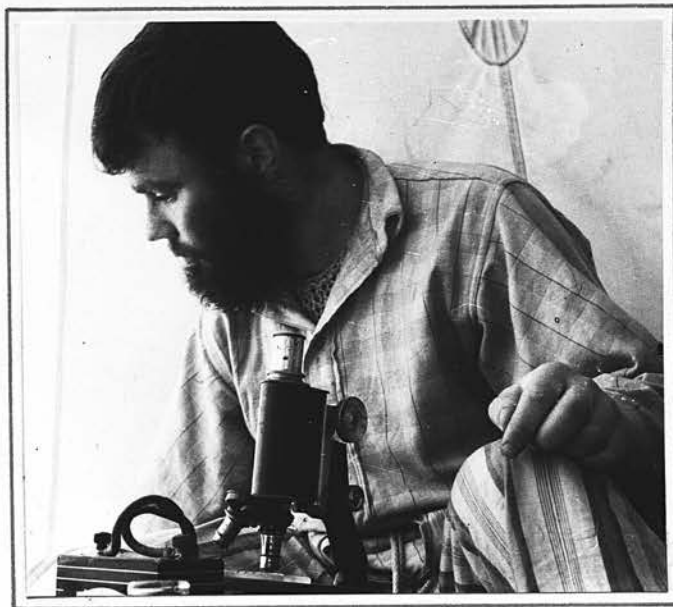
clinical thermometer case.)

6. The light source used was a 4.5 volt battery dry (Ever-ready) with stiff leads to a bicycle lamp reflector and socket. The battery being square and heavy is stable and the stiff leads allow one to manipulate the bulb and reflector in any desired direction. In an emergency a candle is surprisingly good.

7. When man-hauling the sledge the reduction of weight became even more important. The base was taken off the microscope at the inclination hinge, then the remaining tube fixed into a standard food box. The final arrangement was such that the microscope slide platform rested on the top of the corner of the open box and the remainder of the sub-platform stage fitted in a sawn out slot in the plywood wall. The microscope was thus very stable, and the candle that was then used for a light source, was inside the box and protected from draught. The latter is important in windy weather. Thus the total weight of the research equipment could be reduced to under ten pounds.

8. It is very important to duplicate all results and keep one set of results inside the sleeping bag. On the first journey in 1957 after we had been away from base for 70 days both tents were blown down in a hurricane in the middle of the night and a large quantity of equipment was blown away - including one duplicated set of the previous

TECHNIQUE OF FIELD EOSINOPHIL COUNTS.



Photograph taken inside nylon walled tent. Note dry battery for light source, the amount of light inside, and the warmth from the primus (as evidenced by quantity of clothing worn).

2 months' work!

9. Considerable sledging experience is required in order to have enough energy and inclination in order to do any experiments. Over 800 miles experience had been obtained before these experiments were started.

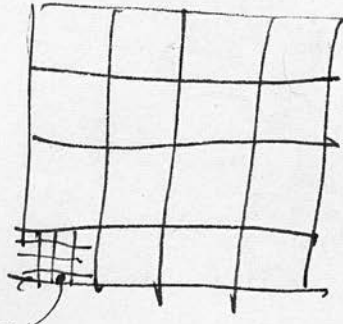
R E S U L T S

In all, 715 eosinophil counts have been made on 12 subjects. The vast majority of these counts were made in Antarctica; the remainder were made in Peru and in the United Kingdom.

The Results are indexed as follows:-

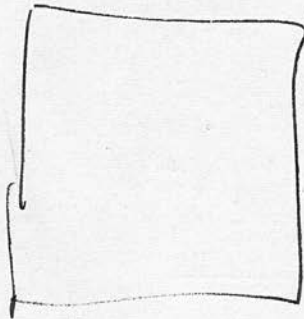
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$\frac{1}{16}$ sq mm . i.e. $\frac{1}{4}$ mm \times $\frac{1}{4}$ mm .

\therefore 1 small square = 1 mm²
4 4 mm²



1. OBSERVATIONS ON THE ACCURACY OF THE EOSINOPHIL
COUNTS USED IN THIS THESIS.

During the compilation of this thesis the eosinophil counts were recorded in the log book in the following manner:-

SUBJECT: L.R.

DATE: 31/7/57

TIME: 9.30 a.m. After breakfast.

					<u>TOTALS</u>
CHAMBER ONE	3103	3200	0141	1221	22
CHAMBER TWO	1002	1302	3320	1002	20
CHAMBER THREE	0210	0111	1302	3001	16
CHAMBER FOUR	3113	0101	2120	0410	<u>20</u>
				Sum	<u>78</u>

Each individual number refers to the number of eosinophils seen in four small Fuchs Rosenthal squares; therefore the number of eosinophils = $78 \times 100 \div 64 = 122/\text{cu. mm.}$

It was shown theoretically ("Student" quoted by Berkson et al. 1940) and later in rigid laboratory conditions, (Berkson et al. 1940) that blood cells are scattered in a haemocytometer chamber according to Poisson distribution. Therefore in equal areas of the haemocytometer there is the following relationship between the mean population and its standard deviation:-

STANDARD DEVIATION

$\sqrt{\text{MEAN}}$

= 1

Large numbers of instances have to be averaged before a mean value of one can be expected owing to random variation. Bad mixing, agglutination etc. would make the average result greater than one.

As a test of the technique used in this thesis the value for $S.D. \div \sqrt{\text{mean}}$ has been calculated for 130 haemocytometer chambers filled with the blood stain mixture in the course of the work. The 32 chambers analysed for subject L.R. are given in the accompanying Table to show the computation employed. The chambers are taken consecutively from the log book to avoid bias. It is seen that the grand mean of 32 chambers is very close to one (.971) and it is therefore concluded that the technique is satisfactory.

This computation has been extended to 3 other individuals whose eosinophil counts varied in magnitude. The results are given in the next Table:-

Subject	Average No. Eosinophils per Four Small Squares	Mean Value for Standard Deviation $\div \sqrt{\text{mean}}$
M.E.	.25	mean = .943 for 34 chambers
L.R.	1.4	mean = .971 for 32 chambers
W.W.	2.2	mean = .943 for 32 chambers
H.S.	4.1	mean = 1.086 for 32 chambers

Grand mean = .986 for 130 chambers

Col. I <u>Raw Data</u> <u>Individuals</u>	Col. II Sum of Squares of 16 individuals	Col. III Sum of Squares - 16	Col. IV Sum of 16 individuals	Col. V Mean of 16 individuals	Col. VI Mean Squared	Col. VII Variance i.e. Column III Minus Column VI	Col. VIII Variance Divided by Mean	Col. IX Value of Standard Deviation divided by Square Root of Mean, i.e. Square Root of Column VIII
3101320001411221	52	3.250	22	1.375	1.890	1.360	.989	.995
1002130233201002	46	2.875	20	1.250	1.563	1.312	1.049	1.024
0210011113023001	32	2.000	16	1.000	1.000	1.000	1.000	1.000
3113010121200410	48	3.000	20	1.250	1.563	1.437	1.1496	1.072
3102023110311332	62	3.875	26	1.625	2.641	1.234	.759	.871
3021100224311211	56	3.500	24	1.500	2.250	1.250	.833	.913
2200205311004111	67	4.188	23	1.438	2.068	2.120	1.474	1.214
2110113300124311	58	3.625	24	1.500	2.250	1.375	.917	.958
0110011011122011	17	1.063	13	.813	.661	.402	.494	.703
1333122213044423	112	7.000	38	2.375	5.641	1.359	.572	.756
2310212101422210	54	3.375	24	1.500	2.250	1.125	.750	.866
0311320021000122	38	2.375	18	1.125	1.265	1.110	.987	.994
1110023012101001	24	1.500	14	.875	.766	.734	.839	.916
0110211121112111	23	1.438	17	1.063	1.130	.308	.290	.539
1102202021101110	23	1.438	15	.938	.880	.558	.595	.771
2331135321101020	78	4.875	28	1.750	3.063	1.812	1.035	1.017
1331224231001020	63	3.938	25	1.563	2.443	1.495	.956	.978
2100202020101111	22	1.375	14	.875	.766	.609	.696	.834
1101050000100110	31	1.938	11	.688	.473	1.465	2.129	1.459
1000111011230201	24	1.500	14	.875	.766	.734	.838	.915
2131122011311222	49	3.063	25	1.563	2.443	.620	.397	.630
1210211010113042	44	2.750	20	1.250	1.563	1.187	.950	.975
1112032131022010	40	2.500	20	1.250	1.563	.937	.750	.866
1302074201300230	106	6.625	28	1.750	3.063	3.562	2.035	1.427
1122220102110210	30	1.875	18	1.125	1.265	.610	.542	.736
6241011320121010	79	4.938	25	1.563	2.443	2.495	1.596	1.263
0122211202001112	30	1.875	18	1.125	1.265	.610	.542	.736
0100013111210162	60	3.750	20	1.250	1.563	2.187	1.750	1.323
3231061111112143	95	5.938	31	1.938	3.756	2.182	1.125	1.060
0110231103123040	56	3.500	22	1.375	1.890	1.610	1.170	1.082
0130046130231111	89	5.563	27	1.688	2.849	2.714	1.608	1.268
1411010230210121	44	2.750	20	1.250	1.563	1.187	.950	.975

31.094

Grand Mean of 32 Chambers ÷ 32 = .971

The values of $S.D. \div \sqrt{\text{mean}}$ are thus very close to unity and it is thought that this indicates that the eosinophil count technique used in this thesis gives results close to the maximum theoretical possible accuracy of the haemocytometer method.

Since the within chamber distribution is Poisson then so also is the sum of eosinophils in one or more counting chambers, i.e.

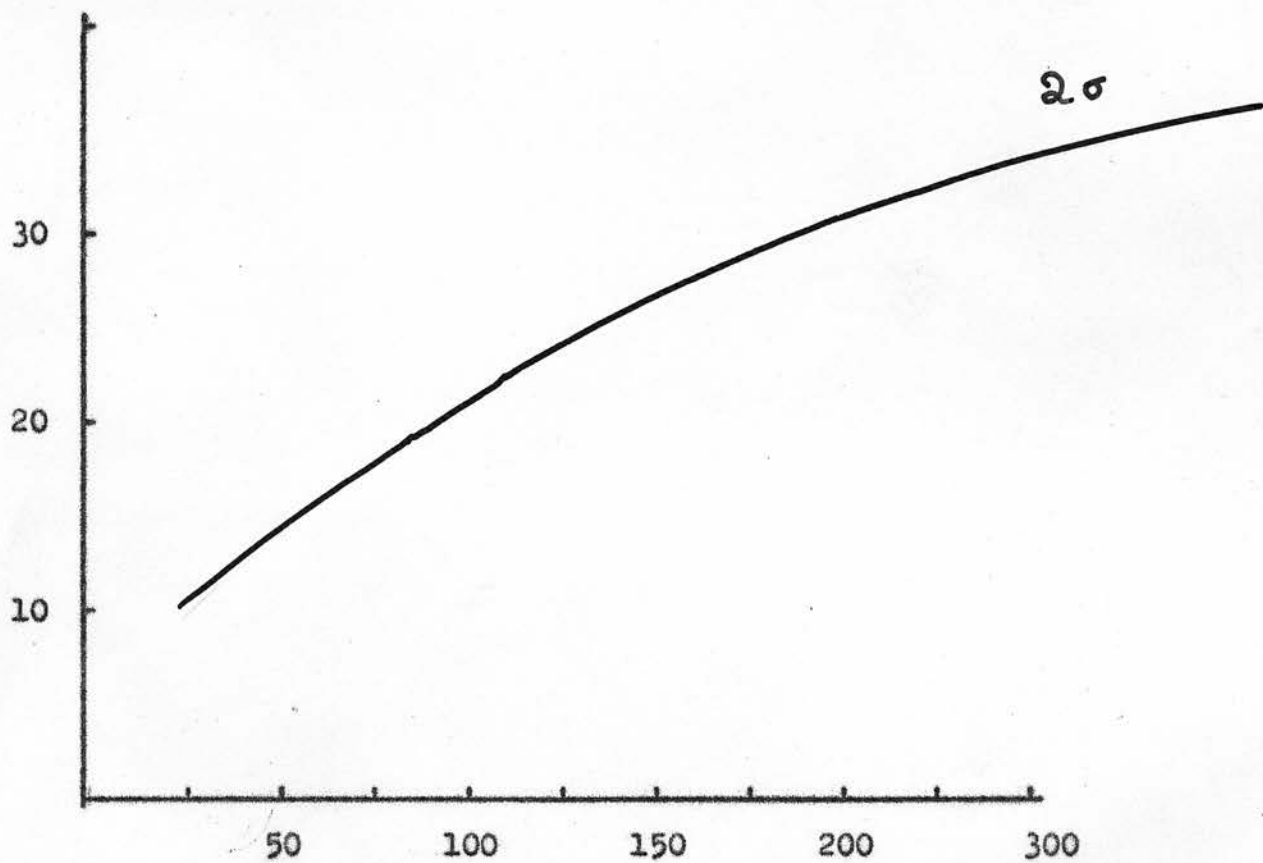
$$\text{Standard Deviation} = \sqrt{\frac{\text{Number of Cells Counted on one Sample of Blood}}{\text{Number of Samples}}}$$

Since the standard deviation only increases as the square route of the number of cells counted then it follows that the relative accuracy of the count increases with large counts.

From the graph overleaf one may obtain, immediately, the range within which there is a 95% chance that the true eosinophil count will be for various numbers observed in the counting chamber.

For example, if 100 cells are counted then $2\sigma = 20$ eosinophils and there is a 95% probability that the real value will lie between 80 and 120 eosinophils. If it is desired to convert the chamber value to the actual value the standard deviation may be multiplied by the same factor (in this case x 1.56).

± VALUE FOR OBSERVED NUMBER OF EOSINOPHILS



NUMBER OF EOSINOPHILS COUNTED IN COUNTING CHAMBER(S)

The accuracy of eosinophil count as arrived at in these Tables does not take into account any errors in the pipettage of stain and blood. The same 1.0 ml. British Standards stain pipette was used throughout the experiments and the British Standards blood pipette was only changed on three occasions. The same counting chambers were also used throughout the experiments. By and large the experiments were concerned with fluctuations rather than absolute values and small errors due to manufacture are not important. Similarly small numbers of eosinophils will stick to the glass-ware during the count. There is also the possibility of unconscious bias when making counts. Thus a doubtful eosinophil in a chamber which had appeared unusually full might be disregarded. To lessen this possibility a clerk was often used and in practice one is almost oblivious of what the total is after five or more of the groups have been recorded.

Further evidence that this technique will give a satisfactory base line was obtained in the following manner. Two subjects, L.R. and R.T., had their eosinophil level measured daily, at the same time, in constant conditions for the same ten consecutive days. The subjects were living in a tent, in complete isolation, but with adequate food and equipment.

SUBJECTS:/

SUBJECTS:

<u>DAY</u>	<u>L.R. (9 a.m.)</u> <u>Eosinophils/cu.mm. blood</u>	<u>R.T. (10 a.m.)</u> <u>Eosinophils/cu.mm. blood</u>
1	84	59
2	75	44
3	87	56
4	75	53
5	70	63
6	78	44
7	73	46
8	78	46
9	78	48
10	<u>81</u>	<u>46</u>
Mean =	77.9	Mean = 50.5

The differences between these figures represent the sum of the experimental accuracy and the biological variation. Since this compound variation is small then both the factors comprising it are likely to be small.

In a similar way subject R.P. was observed in laboratory conditions in Edinburgh on two different days in which there was an identical routine. The subject was in the post-absorptive state. The routine was first lying on a couch, then walking in a treadmill, then lying on the couch again.



<u>TIME</u>	<u>DAY 1</u>	<u>DAY 2</u>
	<u>Eosinophils/cu.mm.</u>	<u>Eosinophils/cu.mm.</u>
9 a.m.	60	68
9.30	69	64
10.10	70)	61)
10.45	48) Exercise	54) Exercise
11.15	51)	49)
11.45	57)	66)
12.15 p.m.	60	67
1.15	49	53

In view of the diurnal variation and the routine one can only consider these results in pairs; within pairs it can be seen that there is close agreement (not exceeding 9 eosinophils).

The main criticism brought against the acetone-eosin diluents is that the eosinophils tend to be lysed with time (Henneman, Wexler and Westhaven 1949). Counts therefore not completed at once would tend to be too low.

In the present method counting was begun in 4 - 5 minutes after mixing the blood and stain and would normally be complete in 15 minutes. Four separate chambers were counted and if there was a significant lysis in this method then the first chamber count must be greater than the last if sufficient cells are counted.

200 consecutive eosinophil counts were analysed and the

total number of the eosinophils seen in the first and last chambers summed:-

<u>FIRST CHAMBER</u>	<u>FOURTH CHAMBER</u>
8738 eosinophils	9002 eosinophils

Since, in fact, more eosinophils appeared in the last chamber (3%) eosinophil lysis is thought to be unimportant in count error.

2. VARIATIONS(a) At the same time each day.

A table has been constructed of the mean eosinophil count and the number of readings from which this mean count was derived. All individuals studied in the thesis are included and all readings were made within the time limit 9 - 11 a.m. on different days in apparently unstressed conditions (i.e. normal daily duties)

Subject	Age	Mean Eosinophil Level and the Standard Deviation	No. of Readings
M.E.	27	34/cu.mm. \pm 12	14
R.T.	25	54/cu.mm. \pm 18	17
R.P.	49	66/cu.mm. \pm 13	12
R.W.	24	87/cu.mm. \pm 27	8
L.R.	32	119/cu.mm. \pm 22	22
S.B.	34	216/cu.mm. \pm 23	3
A.B.	34	223/cu.mm. \pm 16	4
W.W.	27	229/cu.mm. \pm 41	15
M.R.	22	234/cu.mm. \pm 45	16
K.B.	34	254/cu.mm. \pm 60	7
H.S.	26	363/cu.mm. \pm 30	7
H.D.	24	427/cu.mm. \pm 47	8

From these results it may be seen that there is a remarkable range of variation in mean normal level in different individuals at the same time on each day. (i.e. from 34/cu.mm. in M.E. to 427/cu.mm. in H.D.)

An analysis of variance has been carried out on the mean normal eosinophil counts cited in the previous table. The method used is found on page 51 of "Industrial Experimentation". The variance of each mean individual eosinophil count/cu.mm. has two components:-

1. A between day effect (σ^2_A)
2. A between chamber effect (σ^2_B)

Normally four chambers are used in determining one eosinophil count. Between chambers there will only be the second effect whilst between all chambers there will also be the day to day effect. The analysis * separates out these two variances and the individual results are as follows:-

* One analysis will be found in detail in the Appendix.

Subject	σ^2_A	σ^2_B	Significance Level
	Between Day Variance for One Chamber Reading	Between Chamber Variance for One Chamber Reading	
M.E.	1.2	2.6	.01
R.T.	6.1	9.8	.01
R.P.	1.8	9.9	Not significant
R.W.	15.4	13.9	.01
L.R.	9.1	14.2	.01
W.W.	33.1	40.1	.001
M.R.	39.1	46.7	.001
K.B.	88.3	99.0	.05
H.S.	1.0	90.0	Not significant
H.D.	53.5	60.8	.05

The variance ratio test has been applied to see if the between day mean square was significantly greater than the between chamber mean square and the appropriate level of significance has been recorded.

In order to convert these one chamber readings to the reading/cu.mm. the following calculation is necessary:-

$$16 K^2 \sigma^2_A \quad \text{i.e.} \quad 39.06 \sigma^2_A$$

$$4 K^2 \sigma^2_B \quad \text{i.e.} \quad 9.7 \sigma^2_B$$

where $K = 1.56 =$ Conversion factor from sum of eosinophils in 4 chamber to eosinophils/cubic mm.

$$\begin{array}{l} \sigma^2_A = \text{Between day variance} \\ \sigma^2_B = \text{Between chamber variance} \end{array} \left. \vphantom{\begin{array}{l} \sigma^2_A \\ \sigma^2_B \end{array}} \right\} \text{for a single chamber count}$$

Therefore the total variances of the individual mean eosinophil counts are as follows:-

Subject	Mean Individual Eosinophil Count	Between Day Variance	Between Chamber Variance	Total Variance	S.D.
M.E.	28/cu.mm.	105	38	143	12
R.T.	54/cu.mm.	238	96	334	18
R.P.	66/cu.mm.	71	97	168	13
R.W.	87/cu.mm.	602	136	738	27
L.R.	119/cu.mm.	355	139	494	22
W.W.	229/cu.mm.	1291	391	1682	41
M.R.	234/cu.mm.	1527	455	1982	45
K.B.	254/cu.mm.	2586	970	3556	60
H.S.	363/cu.mm.	39	873	912	30
H.D.	427/cu.mm.	1465	697	2162	47

It may be seen from the results that the between chamber variance increases (as expected, from Poisson distribution) with the increasing mean count. However the increase is

relatively small and the coefficient of variation due to experimental limitations $\frac{(S.D. \times 100)}{\text{mean}}$ actually decreases with increasing mean count.

The day to day variance also tends to increase with increasing mean count. This biological variation has, however, large individual differences in counts of similar order. R.P. has a remarkable biological stability; this might be anticipated since his readings were made in laboratory conditions and not like the others made whilst the individuals were completing normal expedition duties. The low reading for H.S. is probably not representative since at other times of the day (see next table) he has standard deviations as high as ± 85 eosinophils. In normal daily duties it did, however, seem that subjects such as K.B. were very unstable and L.R. very stable from day to day at the same time each day and completing similar tasks.

As a general conclusion it would appear that if the eosinophil level in a number of individuals is determined in apparently normal working conditions, at the same time of day, for a number of days, each individual emerges with a characteristic mean level and zone of fluctuation. The size of this zone depends mainly on the magnitude of the mean level but also, intrinsically, on the individual.

It was a clinical impression that phlegmatic individuals possessed more stable counts but of course in this small

sample, and with such subjective evaluation, little weight can be given to this conclusion.

In standard conditions, the individual mean levels did not appear to vary much over long periods. Some examples of this are included in the following table:-

Subject	Time Interval	Reading at Beginning	Reading at End
R.T.	8 months	45/cu.mm. \bar{x} S.D. of 13 (4 readings)	40/cu.mm. \bar{x} S.D. of 3* (4 readings)
L.R.	7 months	129/cu.mm. \bar{x} S.D. of 23 (5 readings)	112/cu.mm. \bar{x} S.D. of 18 (5 readings)
H.S.	2 years	402/cu.mm. \bar{x} S.D. of 73 (11 readings)	370/cu.mm. \bar{x} S.D. of 36 (6 readings)

* All four readings at different times of day. The same times have been used in comparison readings.

DIURNAL VARIATIONS: The mean number of eosinophils per cubic millimetre and its standard deviation is given for each reading.

Subject	9-11 a.m.	11-1 p.m.	2 - 3 p.m.	4 - 6 p.m.	8-10 p.m.
R.T.	54 ±18	38 ±8	47 ±21	--	65 ±17
R.W.	87 ±27	--	91 ±21	65 ±14	--
L.R.	119 ±22	--	127 ±28	--	118 ±13
M.R.	234 ±45	218 ±29	194 ±39	--	--
H.S.	363 ±30	402 ±73	397 ±58	380 ±45	453 ±85
H.D.	427 ±47	409 ±61	403 ±142	412 ±91	412 ±87

DIURNAL VARIATIONS: Percent change from 9 - 11 a.m. readings.

R.T.	100%	70%	87%	--	120%
R.W.	100%	--	105%	77%	--
L.R.	100%	--	106%	--	99%
M.R.	100%	93%	83%	--	--
H.S.	100%	110%	109%	104%	125%
H.D.	100%	96%	94%	96%	96%
Mean	100%	92%	97%	92%	110%

2(b) Diurnal.

The main object of the work in Antarctica was to study long term changes in the eosinophil counts and consequently diurnal variations were not considered in detail. Multiple daily venipunctures would have been required and this would have strained the cooperation of the subjects for the long term experiments.

However a table has been constructed with readings taken over the year at different times of the day with no stress apparent. Each reading represents a mean of at least five readings and the standard deviation is given. 203 counts were used in the compilation of the table.

It can be seen from the table that the different time readings are remarkably similar to one another. A possible reason for the absence of a well marked diurnal variation in these results will be found in the discussion.

2(c) With weather and season.

At one period of the year the sledging party was marooned on a peninsula by lack of sea ice and dry glaciers inland which for technical reasons were almost impossible to sledge on. The party was completely isolated but apprehension was minimal since there were large quantities of food and fuel and ample tents to live in. Days were spent feeding the 18 dogs, making weather observations and hunting seal. Since it was the Autumn violent weather fluctuations took place. The temperature varied from -23°C . to $+7^{\circ}\text{C}$. and the wind from a calm to 40 kts. In winds over 30 kts. one has to shout to one's companion in the tent because of the roaring canvas and sleep is difficult in these conditions. During the period eosinophil counts were made daily on the four men for four weeks (Sundays excepted). The counts were made in the morning after breakfast and in the same order of persons to eliminate diurnal differences. The minimum temperature was recorded each day and the weather factor was calculated. The weather factor is the sum of the number of degrees of frost $^{\circ}\text{F}$. and the maximum wind speed in knots. This enables the combined effect of temperature and wind to be studied; it is well known that a calm frosty morning can be quite bearable as long as there is no wind.

It was thought that correlations between the eosinophil count and the weather on the same day as the count, and also

EOSINOPHILS AND THE WEATHER

	Min. Temp. Same Day	Min. Temp. Previous Day	Weather Same Day	Weather Previous Day
Degrees of Freedom	23	23	23	23
R.W.	+.19	+.33	+.003	+.17
Degrees of Freedom	23	23	23	23
L.R.	-.02	+.21	+.31	+.19
Degrees of Freedom	23	23	23	23
R.T.	-.32	+.13	-.19	-.33
Degrees of Freedom	17	17		
H.S.	-.11	+.07		

+1 or -1 indicates complete dependence; values approach zero with complete independence.

the weather on the previous day to the count, would show up any affect of the meteorological conditions on the level of circulating eosinophils. Accordingly the following correlation coefficients were computed:-

1. The minimum temperature the same day as the eosinophil count.
2. The minimum temperature the previous day to the eosinophil count.
3. The weather factor the same day as the eosinophil count.
4. The weather factor the previous day to the eosinophil count.

Consideration of the correlations coefficients computed in this way shows clearly that the weather does not appear to affect the count.

2(c) With the weather and season.

It was also thought possible that readings taken at the same time of day in summer and winter might have a different level.

SUBJECT	BASE HUT SUMMER (Mean)	No. of Readings	BASE HUT WINTER (Mean)	No. of Readings	DIFFERENCE (By 't' test)
L.R.	129/cu.mm.	10	116/cu.mm.	14	Not Significant
H.S.	386/cu.mm.	12	416/cu.mm.	12	"
R.T.	43/cu.mm.	12	50/cu.mm.	12	"
R.W.	97/cu.mm.	10	65/cu.mm.	10	P = .01
	OUTSIDE TEMP. ABOUT FREEZING		OUTSIDE TEMP. ABOUT -17° C.		17° C.

In order to achieve a reasonable number of readings for the seasons at the same time of day paired readings one from each season at the same time of day were used in this table.

Consideration of these readings shows that there was little difference between the Summer and Winter readings except in the case of R.W. The possibility that his lower readings in the Winter are due to cumulative mental stress associated with a second Winter in Antarctica must be considered; many of these base hut Winter readings were lower than sledging readings when the temperature was much lower.

2(d) In different Antarctic environments.

(Subject L.R.)

In one of the three subjects, who had more than 100 different day eosinophil counts observed in 1947, different eosinophil levels emerged for different environments. In the 3 other subjects for whom similar readings are available (H.S., R.T., R.W.) no such differences appeared.

Environment	Mean Eosinophil Count	Mean Outside Temperature	Number of Readings
Base Hut Summer	129/cu.mm.	-1° C.	10
Base Hut Winter	116/cu.mm.	-16° C.	14
Living in Tent	80/cu.mm.	--	21
Morning after Sledging	54/cu.mm.	--	10
Same day as Sledging	46/cu.mm.	--	2

All readings have been made between 10 - 11.30 a.m. with the exception of those made the same day as sledging. The latter were made about 5 p.m. when one would expect the level to be higher than morning values.

The base hut Summer and Winter means are not significantly different from one another. The living in the tent level is significantly lower than the base hut Winter level ($P = .01$ by the 't' test). The day after sledging level is

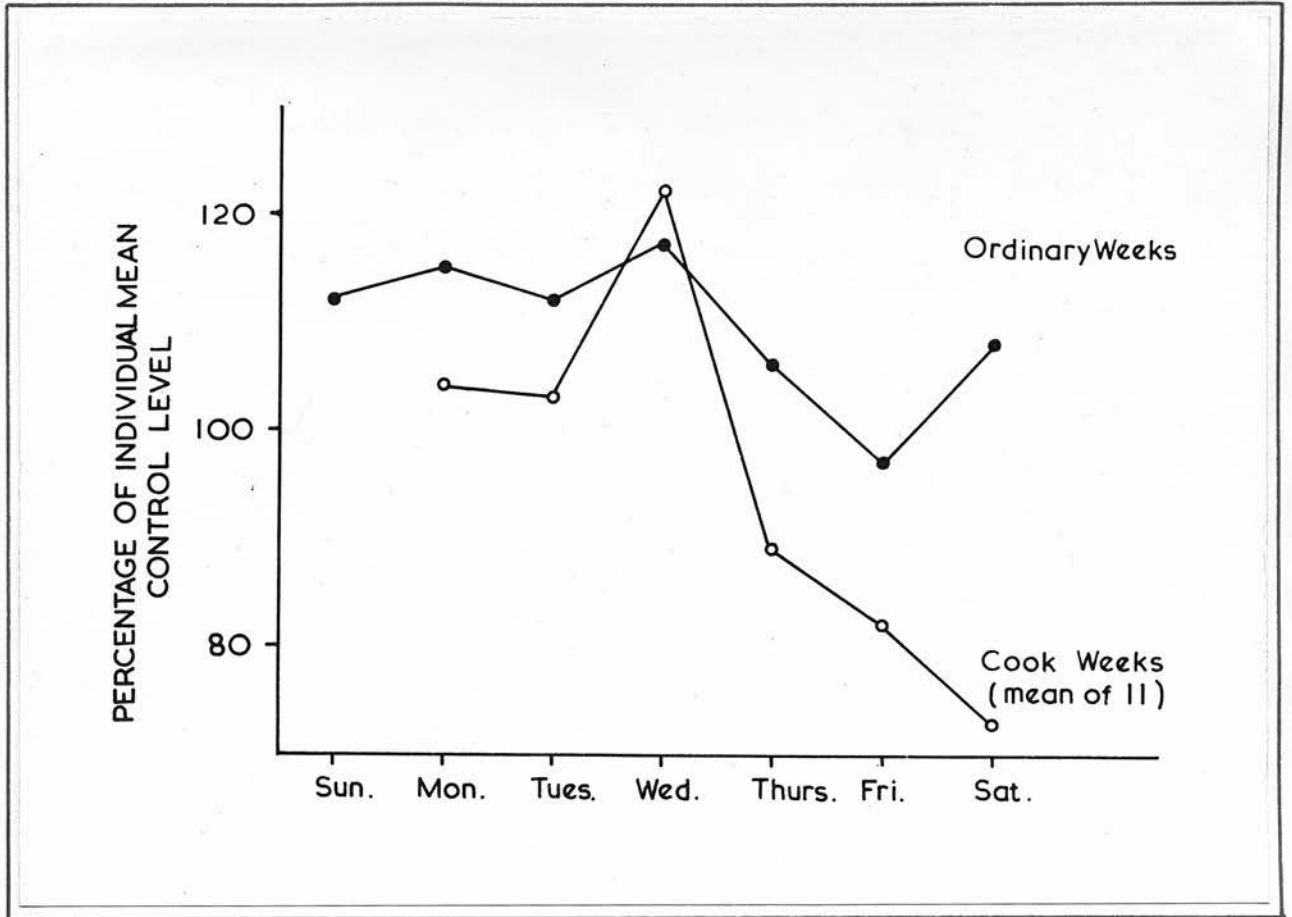
significantly lower than the living in the tent level
($P = .001$ by the 't' test). No calculation has been carried
out for the last reading.

2(e) During cooking duty on the base.

It is the tradition for all members of the survey to be the weekly cook on a roster system. It will be readily understood that to be cook for 8 - 10 men and do one's main job can be a considerable strain. Not only must the cook bake bread but he must also help with the washing up and at the end of his week scrub out the kitchen. One man could not sell his cook week for £15. As a result of the strain involved a professional cook has now been installed at the main scientific base.

There was difficulty in analysing the affect of the cook week on the count because over the year the base counts could not be controlled for time of day. All observations made on the base in what were considered to be standard conditions were used in the computation. First each observation was converted to a percentage of the individual's mean control level. This level was considered to be the mean of the first twelve eosinophil counts made in non-stress conditions in the base hut. Secondly each percentage had a small correction made from the diurnal variation as found for that individual. (See the diurnal rhythm table). In this context it should be noted little diurnal rhythm was found during the base day life. Thirdly all percentages for all men for each day of cook weeks and normal weeks were summed and meaned for each day.

THE STRESS OF COOKING.



This table is based on 31 cook week eosinophil counts and 101 ordinary week eosinophil counts from seven men. A total of 11 cook weeks have been included since some men had more than one of their duty weeks observed.

Consideration of the ordinary week and cook week graph shows that there is a steady fall of the cook's eosinophil count towards the end of the week. The normal weeks act as a control for the method. The moderate fall at the end of the ordinary weeks might be a reflection of the fact that many other base activities are on a weekly basis, e.g. "gash hand" duty. The gash hand collects water, washes up, disposes of rubbish, etc.

2(f) In a subject who was isolated.

Subject H.D., who was a wireless operator, and whose duties were therefore largely static made a dangerous one day sledging journey in June 1957. Previous to this he had been marooned in a small hut with one other man for three months. The journey was dangerous since 8 miles of bay ice had to be crossed for the first time in the Winter season. The ice had been only formed a week and was therefore only just thick enough.

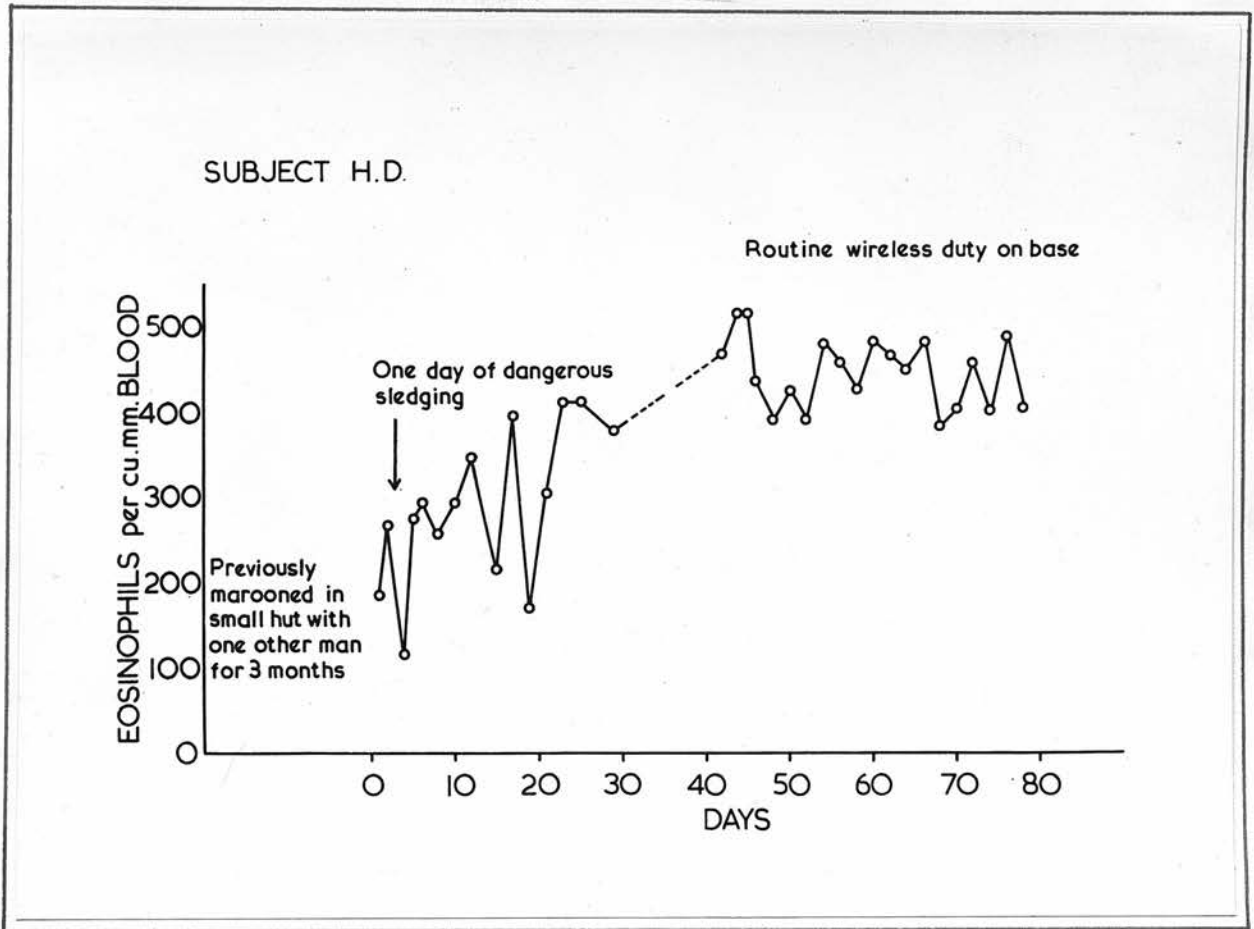
Consideration of his diurnal fluctuations shows that it is very small (see first section of results) and therefore although not all readings in the following graph are at the same time of day the serial comparison is thought to be valid.

It can be seen from the graph that there were very low readings before and after the sledge journey and on arriving back at the main base 3 weeks elapsed before a stable (?normal) count was achieved.

It is thought that the low initial levels were associated with:-

1. The mental stress of being isolated in the satellite hut for three months where he was cut off by untravellable conditions.
2. The anticipation of dangerous sledging.

EOSINOPHIL VARIATIONS IN A SUBJECT
WHO WAS ISOLATED.



All the readings observed in the Antarctica for subject H.D. are included in this graph.

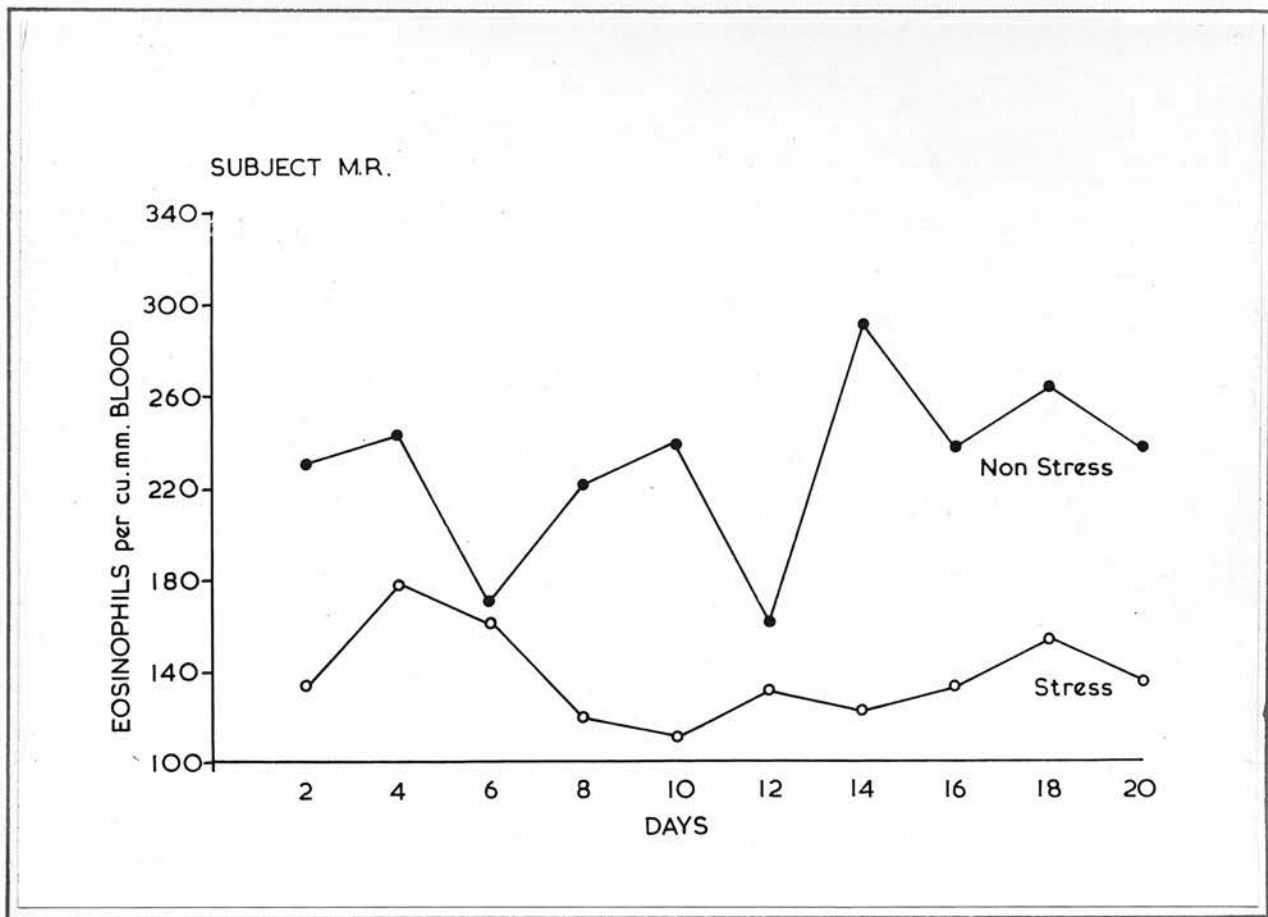
3. The days sledging with mental and physical stress in a man who had never done any before.

However the possibility that the whole effect was not due to stress but to the acquisition of a parasite or a latent illness cannot be ruled out. There were no symptoms or signs of illness and the timing of the lowest reading the day after the sledging would be a remarkable coincidence. The stability of the readings for 40 days on base would also seem to be against this.

2(g) Chronic Stress.

In some instances without apparent stress, the day to day count stability appeared to be less than when the same individual was in stress situations (sledging). The actual stress involved will be found in section 3.

As an example, two periods for subject M.R. are contrasted with one another. It is seen that the stress period fluctuates less than the control. More evidence for this possibility may be seen by comparing the stress and non stress graphs in section 6(d) "The effect of treadmill walking and starvation on the eosinophil count".



Mean change/reading Non stress = 51 Eosinophils.
stress = 22 Eosinophils.

No.	Time	No. of Readings	Eosinophil Level	Stand. Devt'n	% of Normal	
CONTROL						
DAYS: NO STRESS APPARENT	11-12 a.m.	14	mean 237 per cu.mm.	$\bar{\pm}30$ per cu.mm.	100%	
1	*Received news that 1st ship in 'sinking condition'	11.30 a.m.	1	165 per cu.mm.	-2.4	69%
2	1st actual ship due in 4 hrs.	11.30 a.m.	1	165 per cu.mm.	-2.4	69%
3	Due to leave on sledging journey in 4 hrs.	12 m.d.	1	135 per cu.mm.	-3.4	54%
4	Nearly killed in small boat rescue the previous day	11 a.m.	1	128 per cu.mm.	-3.6	54%
5	Morning after manhauling 21 miles**	10 a.m.	1	110 per cu.mm.	-4	46%

* Ship later managed to reach harbour although holed.

** Not in the present period.

2(h) Over a prolonged period on base (one man).

It was decided to make a prolonged study of one man (M.R.) over a two month period since on a base there is a probability of some accidental stress occurring in such a long period and in which case there would be a complete record of the event. The period also included the arrival of the first relief ship which the base had had for eight months. Readings were made on alternate days (see next table).

Since a fall of more than two standard deviations is normally considered an adequate level of significance all these stresses would appear to be associated with a genuine eosinopenia.

Only 21 readings out of 29 made are included in this table; the remainder were not made within the specified time period (11 a.m. to 12 m.d.) and have been omitted because of diurnal variation.

In stress number 4 an Argentine NCO was seen drifting helplessly out to sea in the prevailing current; his outboard motor had run out of petrol. He had lost an oar and was unable to row. Already one Argentinian had been killed in an identical manner the previous year and the situation appeared desperate. A disused and leaky British rowing boat put out to sea and after an unsuccessful attempt to contact the man because of large waves turned back. This latter

boat was out for 4 hrs. and very nearly did not get back because the wind was gusting seawards at up to 40 kts. The man was later rescued by Argentine helicopter 25 miles out.

Night meteorological duty nearly always resulted in a net loss of sleep whatever the attempt to make it up during the day. After two occasions there was no significant change in the count as compared with control days.

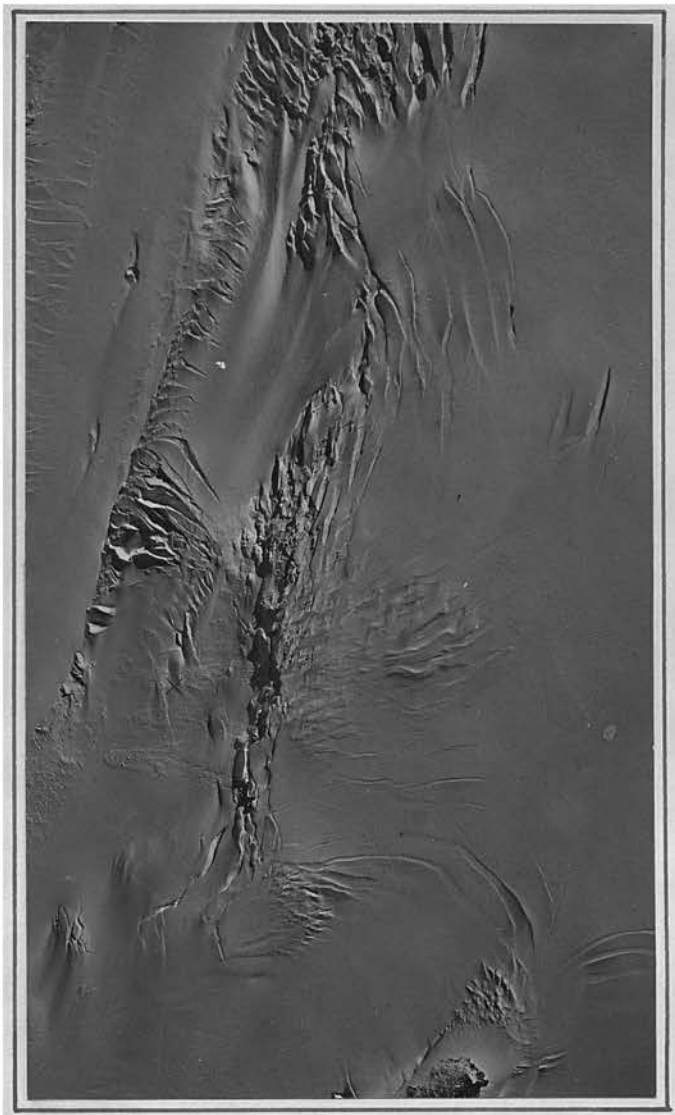
3. SLEDGING(a) Results from the first sledging journey 1957.

Before the Winter of 1957 closed in the final Summer ship left a party of four of us on a rocky peninsula 50 miles from the main base. The object of this was to speed up the Winter mapping programme by finding a route from the coast to the unmapped Detroit Plateau. The plateau is a gentle undulating ice cap running for hundreds of miles at 6 - 8000'. The edge of this plateau is marked by an abrupt escarpment almost all along its length. The main difficulties encountered by a party endeavouring to sledge onto it are crevasses, steepness, ice-falls, and bad weather. 85 days elapsed between the time of disembarking from the ship and finally reaching the base with the work programme completed. During the field period 192 eosinophil counts at the same time of day were made on the four men, (48 each). Counts were begun after breakfast and completed in the same order. Clearly they were a reflection of the previous day's activity or an anticipation of the present day's programme. No readings during this period were made the same day as sledging, though many were made the morning after. On the following pages a series of photographs will be found to give an idea of the conditions on this journey.

The results will first be presented individually then individuals will be compared on a percentage basis.

FIRST SLEDGE JOURNEY 1957

DETROIT PLATEAU ESCARPMENT.



Aerial photograph from 13,000 ft. From this height only vast crevasses are shown. The photograph is intended to give some idea of the difficulties of a party attempting to reach the plateau.

THE FIRST SLEDGE JOURNEY 1957.



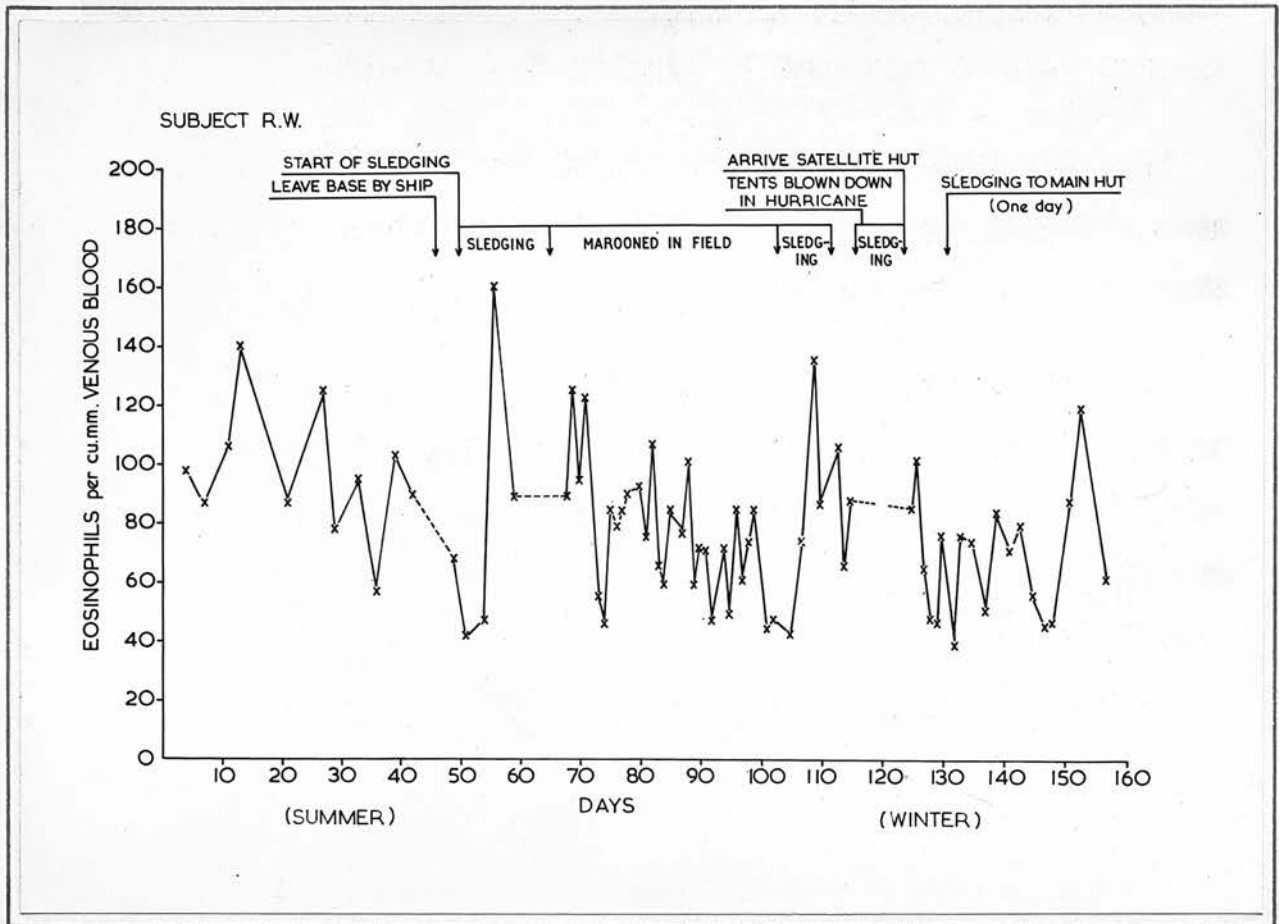
NOTE: Outline of route crossing crevasse systems.
Medical research box on top of sledge.
Subject R.T. doing a meteorological observation.

PITT POINT, GRAHAM LAND.



Taken during the 84 day dog sledging journey. This was the camp where the party was marooned by bad conditions. Note almost dry glacier above aerial pole (left centre).

EOSINOPHIL CHANGES BEFORE, DURING AND AFTER
THE FIRST SLEDGE JOURNEY 1957.



All field readings at the same time of day. Hut readings at different times but these are scattered so that a constant error is unlikely.

R.W., aet 24.

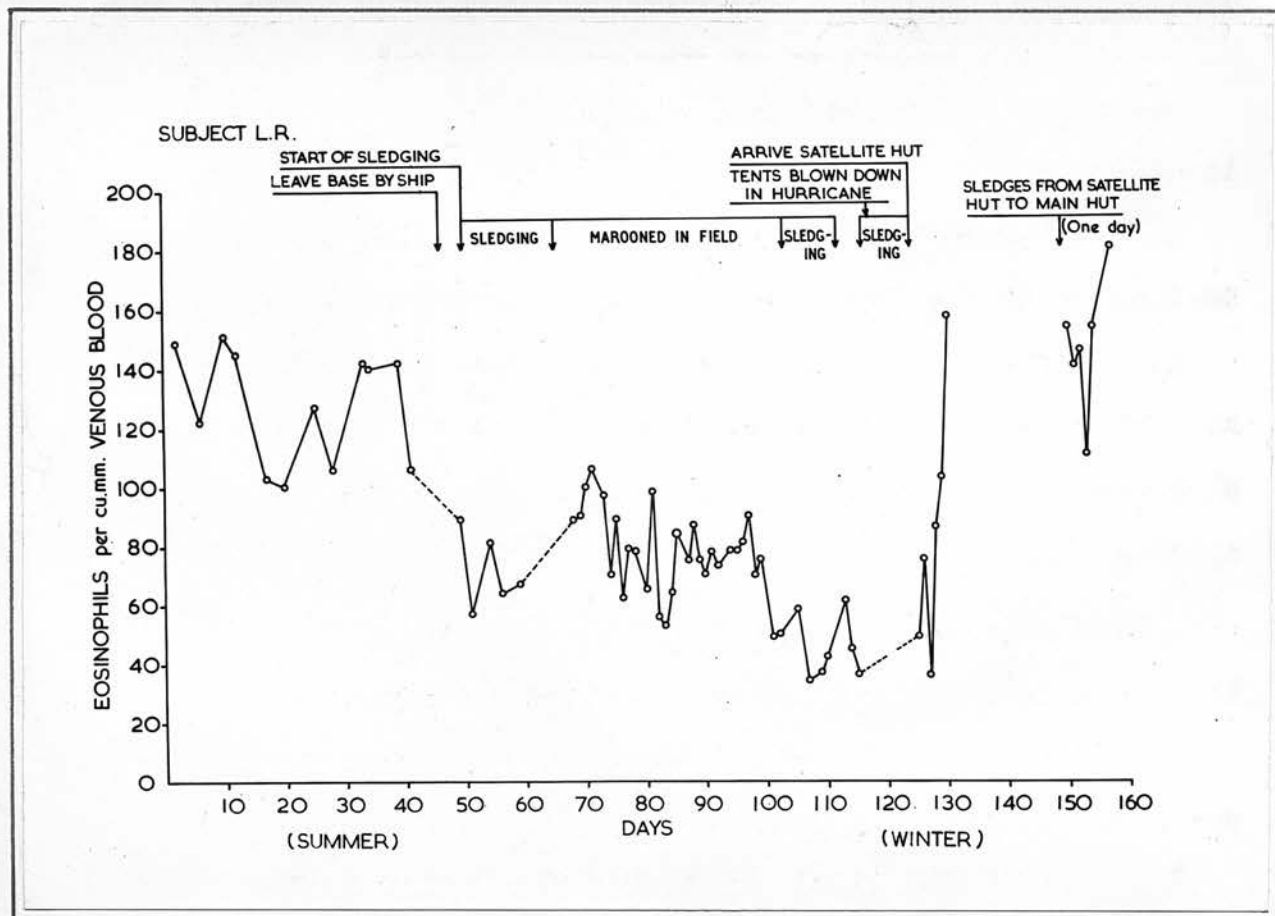
1. There is no general fall in the eosinophil level whilst in the field*. The count is relatively unstable cf. Subject L.R. whose count is similar in magnitude.

2. Low readings were registered the morning after some sledging had been completed but these were not lower than some control readings.

3. These low sledging readings were followed by a brisk rebound whilst he was still sledging. The low and high readings corresponded to difficult and easy sledging respectively.

* "The field" in this thesis indicates all life away from the base hut whether sledging or static.

EOSINOPHIL CHANGES BEFORE, DURING AND AFTER
THE FIRST SLEDGE JOURNEY 1957



Up to day 130 all readings were made between 9.30 - 11.30 a.m. (except readings at days 12 and 17 made at 2 p.m.) Readings at days 150 - 160 were made 3 - 5 p.m. but L.R. had an almost negligible diurnal variation during the working part of the day (see Section 2 (b)).

Subject L.R.: Eosinophil changes during the first sledge journey, 1957.

1. There was an immediate persistent fall in the count for the entire period of 85 days in the field.
2. The longer the field period the lower the eosinophil level.
3. Three days after reaching the satellite hut* at the conclusion of the journey the non-stress level was passed.
4. Since all readings were made after breakfast and not later in the day when some activity might have taken place the low levels appear to represent a chronic eosinopenia.

L.R. was thought to have been under more stress than the other members of the party for the following reasons:-

1. He was the leader of the party but had had no previous sledging experience.
2. He was the least physically fit member of the party.
3. The journey turned out to be particularly hazardous because:-
 - (a) Dogs fell into crevasses on four occasions;
 - (b) Tents were blown down in a 100 kt. hurricane in the middle of the night and a large quantity of equipment was blown away. A 1 cwt. sledge was blown over the top of the tent.
 - (c) The party was nearly caught out on thin breaking up sea ice.

* Photo of Satellite Hut - see over.

- (d) Twice when traversing steep glacier slopes the dogs charged downhill in the middle of a large crevasse system with L.R. roped up to the main trace.

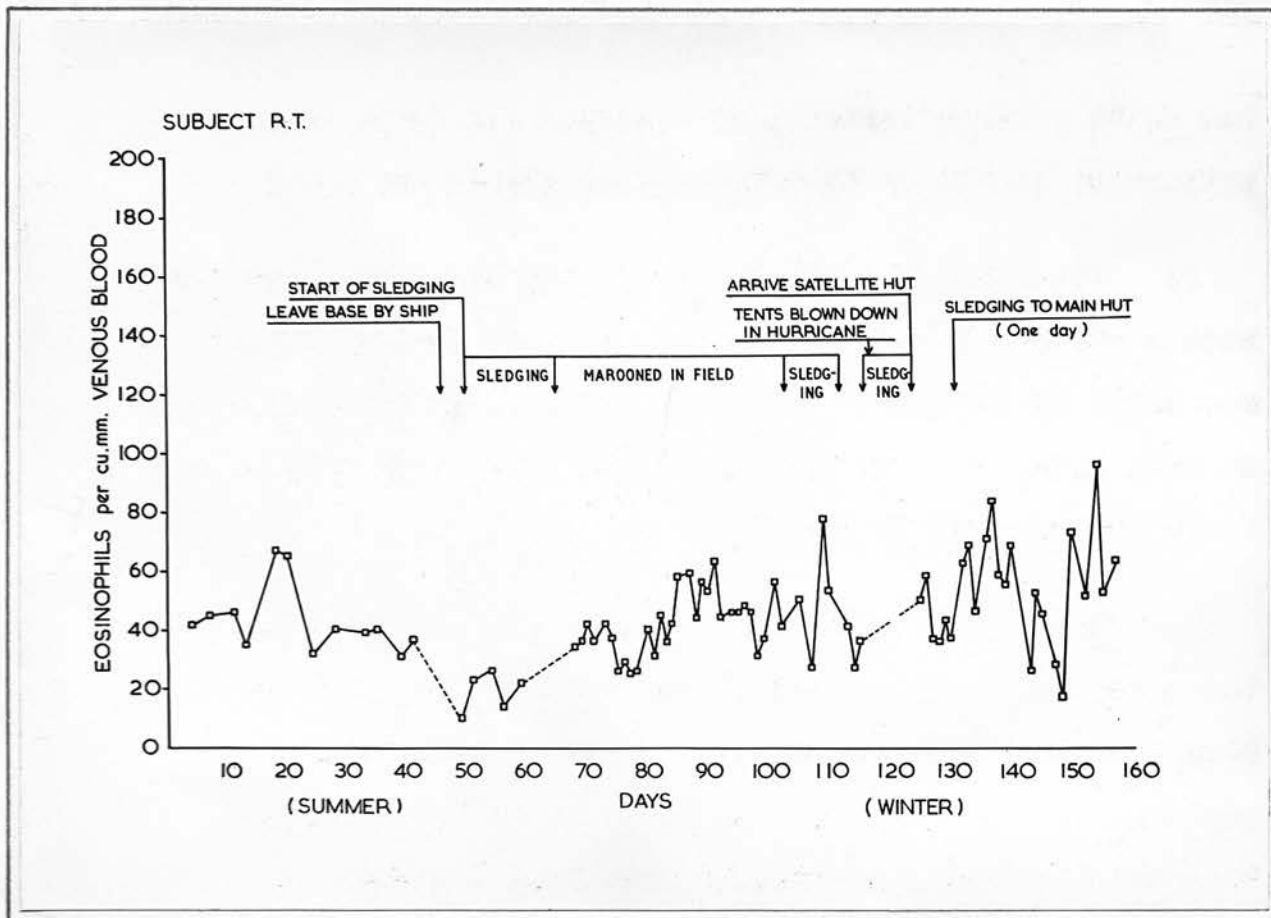
On subsequent sledging journeys his count was low on every occasion.

HOPE BAY SATELLITE HUT.



This photograph is taken along the full length of the hut. Note clothing including snowboots. Two bunks extreme right.

EOSINOPHIL CHANGES BEFORE, DURING AND AFTER
THE FIRST SLEDGE JOURNEY 1957.



All field readings about 11 a.m. Most hut readings about this time \pm 1 hr., but some in afternoon (days 24 - 35, 137, 139).

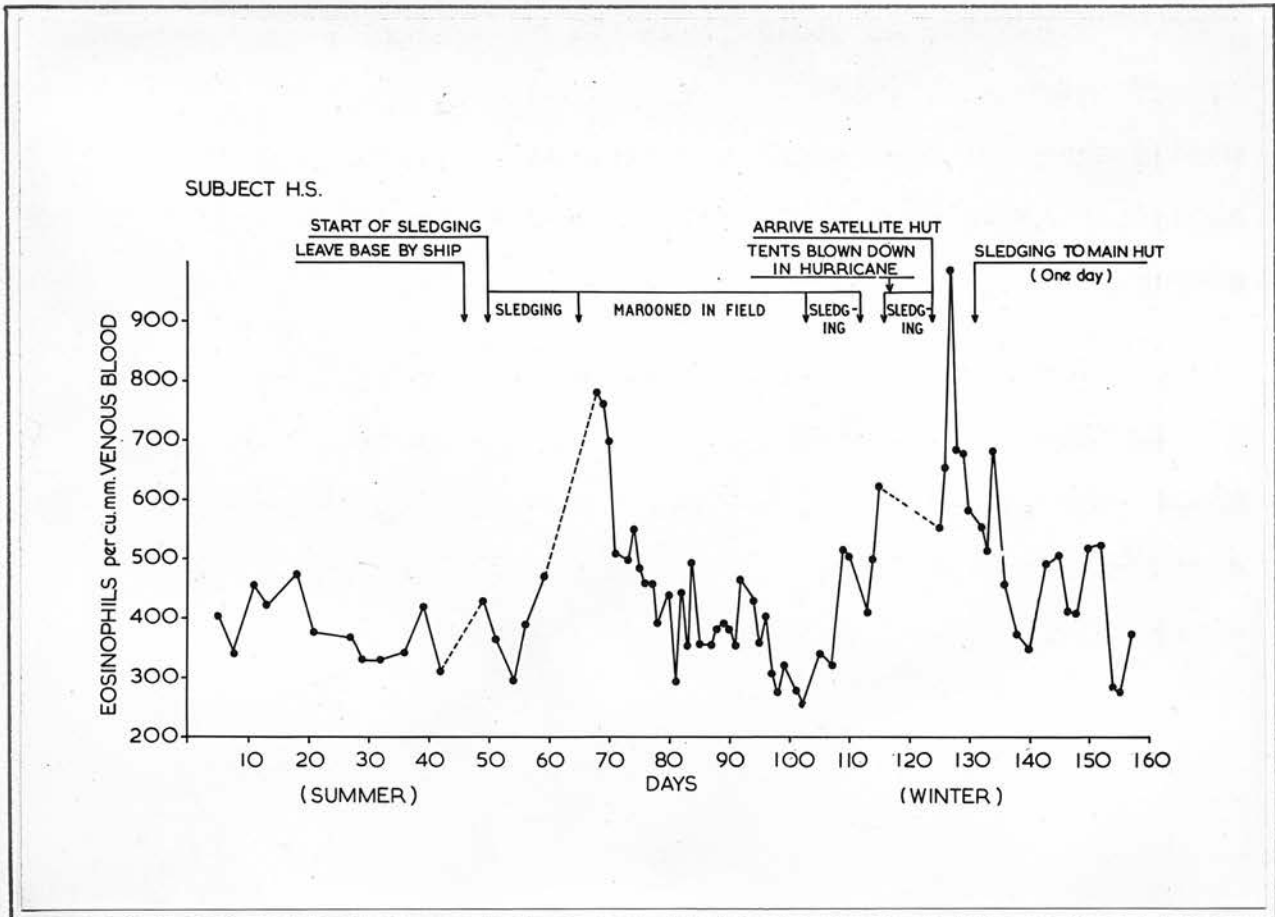
R.T., set 26.

1. The first sledging period was associated with some of the lowest readings of the whole 160 day period. This was R.T.'s first experience of sledging and it is therefore reasonable to assume that the mental stress was high.

2. The subsequent sledging journey was only associated with a transient fall followed by a sharp rebound whilst he was still on the move. This is interpreted as adaptation. As with R.W., this low and high were associated with difficult and easy sledging.

3. Subsequent to the sledging period the readings on the base tended to be much higher than in the pre-sledging base period. A possible explanation is that this is a prolonged rebound in a man who was initially new to the base and to sledging. Consideration of the sledging readings show that they rise all the time with increased experience, yet all these journeys had a similar amount of danger. It is of note that this man applied for and was accepted for a third Winter South, indicating satisfactory work.

EOSINOPHIL CHANGES BEFORE, DURING AND AFTER
THE FIRST SLEDGE JOURNEY 1957



H.S., set 27.

1. There was no eosinopenia whilst sledging; the lowest readings occurred during a period of high mental stress when the departure on a dangerous journey was impending (days 98 - 102), but a similar low was recorded at day 155 during a cook week on base).

2. After four different periods of sledging there was a remarkable rise of the level reaching a zenith on the third post sledging day. These zeniths may be seen at days 68, 115, 127, 134. This rebound will be considered separately.

The first sledge journey, 1957.

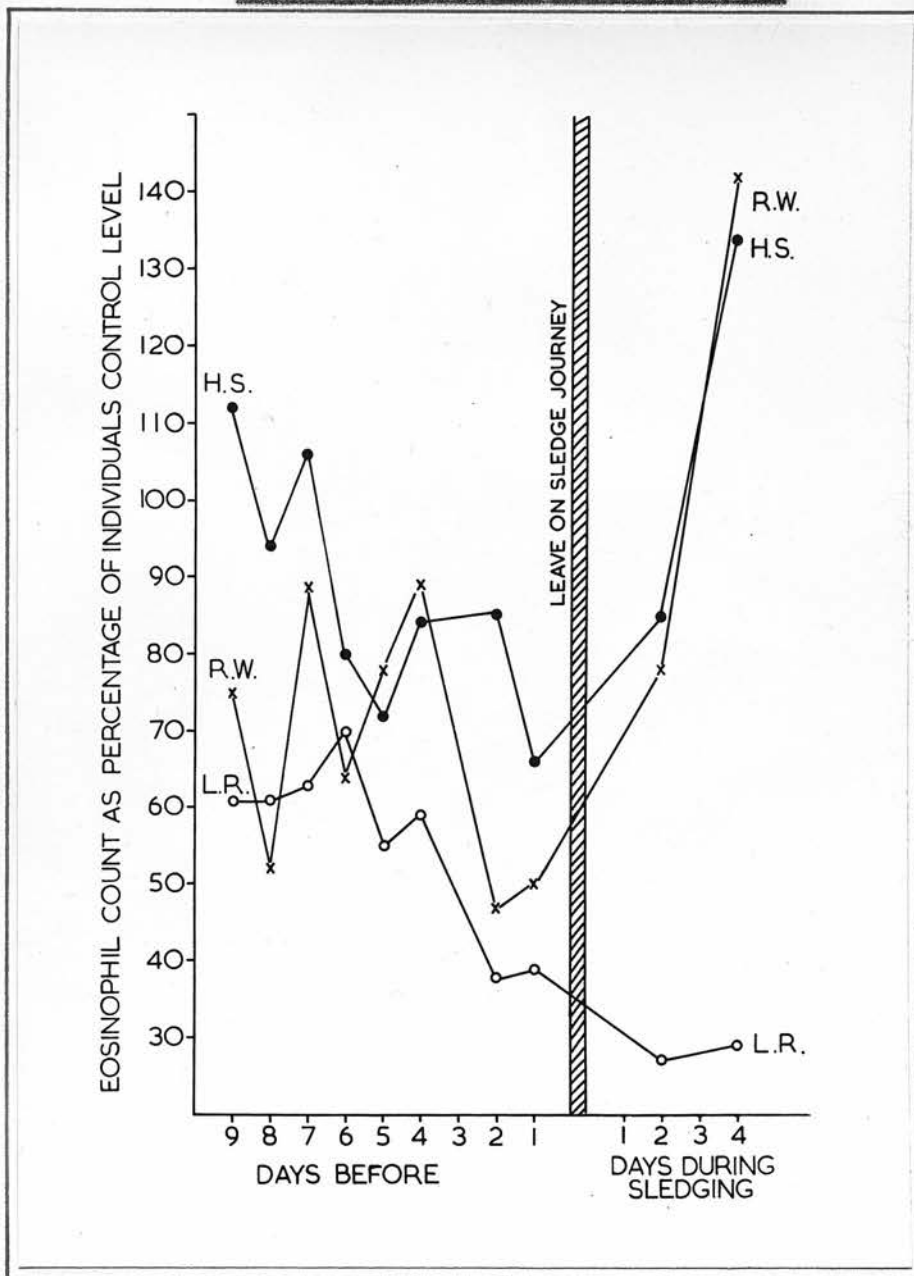
During the first journey, 1957, two subsidiary journeys were made over the same ground by the same subjects; these journeys have been superimposed. The ground was initially dangerous and later easy.

In order to compare the four individuals who had different basal eosinophil levels each reading during these two subsidiary journeys has been reduced to a percentage of the "individual's control level". In each case this was considered to be the mean eosinophil count of the first 12 made for the individual in the base hut in Summer.

The first graph is for three of the four subjects only. It covers the period preceding the departure to the plateau for the second time. The mental stress was high since our first experience of the route had shown it to be hazardous. There was, however, no alternative. In these three subjects there is a tendency to an increasing eosinopenia as the departure date is approached.

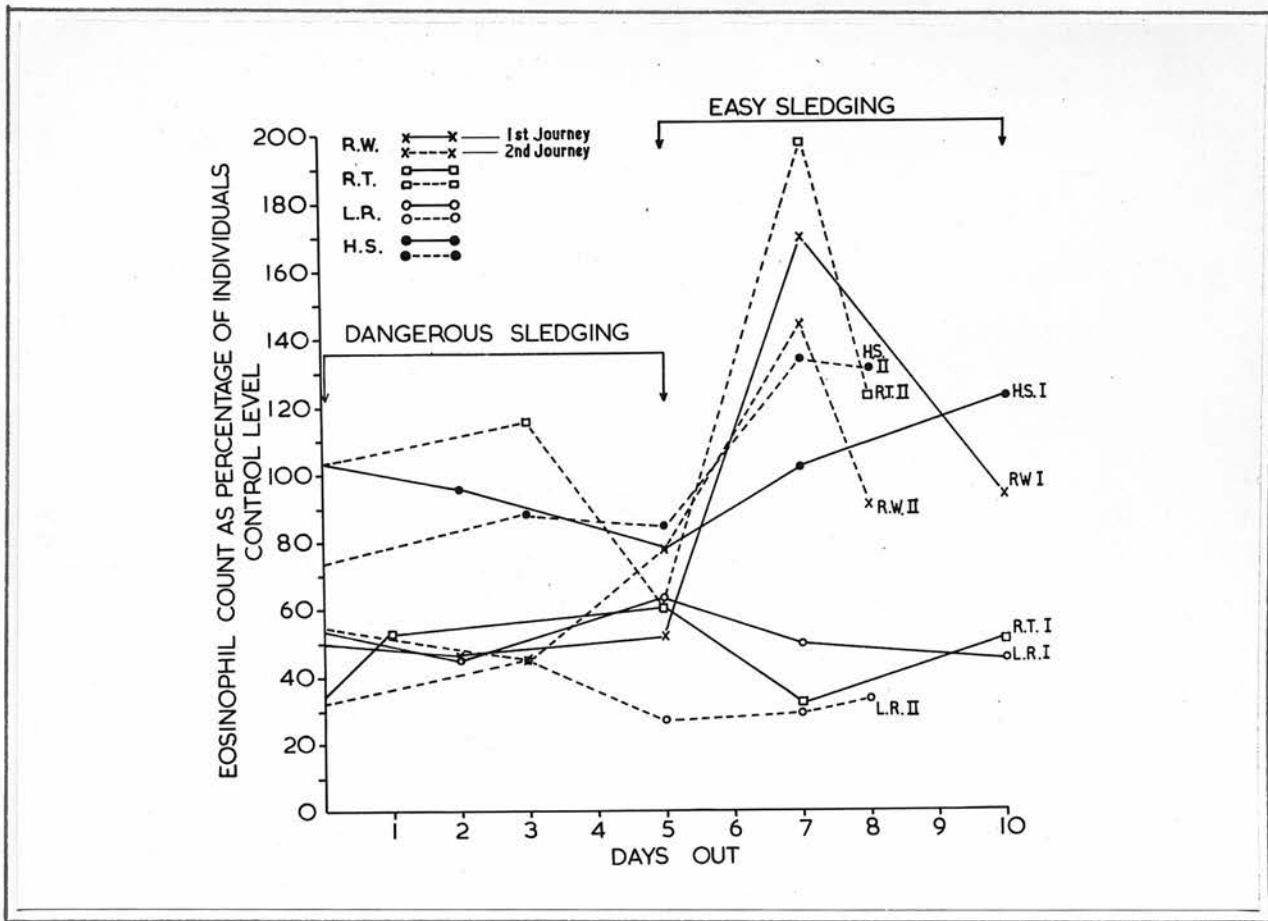
The second graph contains all readings for the two sledging periods.

THE FIRST SLEDGE JOURNEY 1957



Subject R.T. has been omitted. There is a tendency for the eosinophil counts to fall towards the departure date of a dangerous journey. In fact the difficulties were less than anticipated. See next graph for continuation.

THE FIRST SLEDGE JOURNEY 1957.



Different vertical scale to previous graph. The interrupted line for R.W., H.S. and L.R. is the continuation of the previous graph.

The physical output of work on the first five days (dangerous sledging) was similar to that of the second five days (easy sledging). There was no consistent alteration of temperature during these periods.

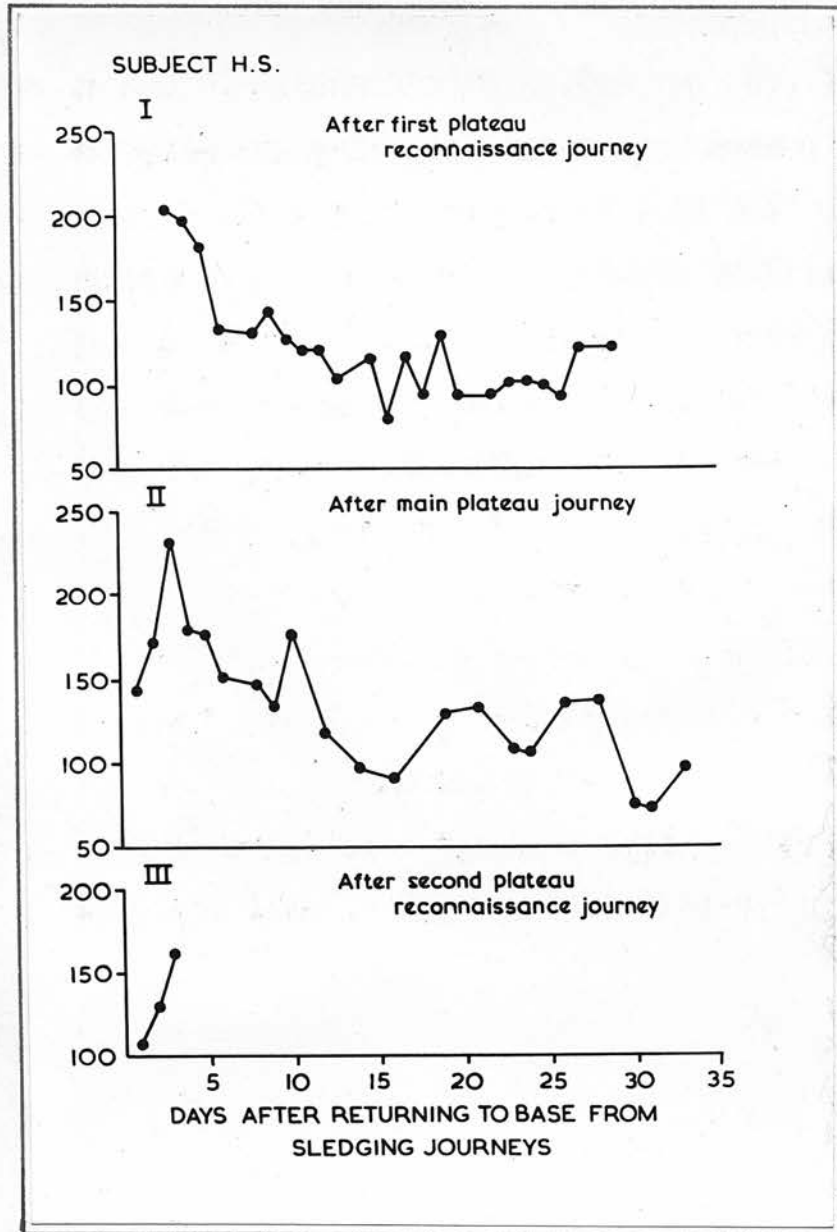
	Mean Min. Temp. 1st 5 Days	Mean Min. Temp. 2nd 5 Days
First Journey	-8° C.	-18° C.
Second Journey	-28° C.	-22° C.

and therefore change of atmospheric temperature would not appear to explain the eosinophil rise in the second five days. The possibility of continued output of endogenous adrenaline being responsible for the eosinophilia is at variance with the results of the fourth sledge journey in which R.T. also participated.

It is thought that the rise on the second five days is most easily explained by the relaxation of considerable accumulated mental stress. The fact that R.T. and L.R. on the first journey and L.R. on the second are exceptions seems to substantiate this for the following reasons. Both were novices and could reasonably be expected to incur more mental stress; moreover, L.R. was leader and there were many difficult decisions to make regarding route, etc.

REBOUND OF THE EOSINOPHIL COUNT
AFTER SLEDGING, IN SUBJECT H.S.

EOSINOPHIL COUNT AS PERCENT OF INDIVIDUALS CONTROL LEVEL.



3(b) Rebound of the eosinophil count after sledging.

After the completion of three different sledging journeys, subject H.S. showed a marked rise of the eosinophil level, (see graphs). Consideration of the graphs for these three periods shows the following characteristics:-

1. The rise is maximal on the third post-sledging day. A subsidiary zenith may be seen on the decline slope of the main plateau journey; this occurred three days after a one day journey from the satellite hut to the main hut.

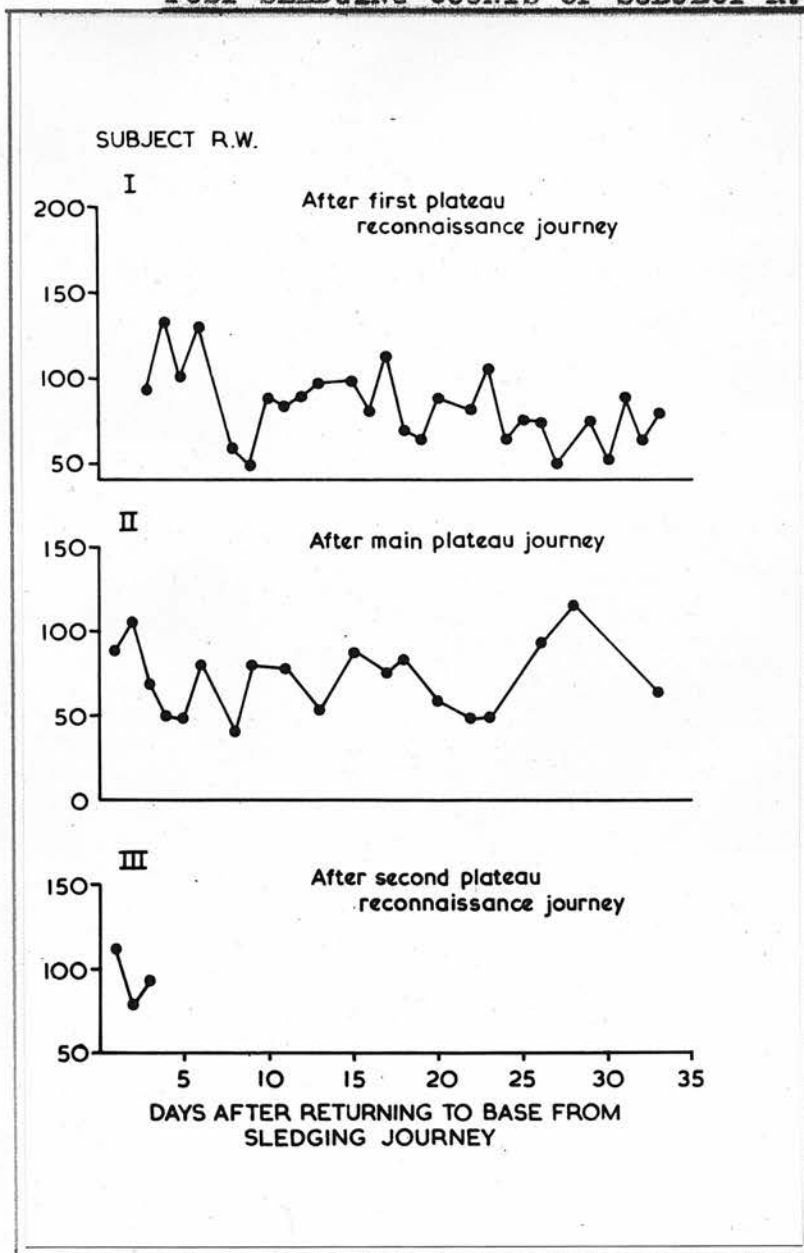
2. The rise and fall are more or less steady reaching a zenith on day three at over 200% of the base normal count (about 800/cu.mm.) and taking two weeks to subside.

3. Similar readings are available for three other subjects; none show similar results. Subject R.W's results are given as an example on the next page, but irregularly raised readings were found in R.T. after the first and fourth journeys and in M.R. after the fourth journey.

The possibility that the effect is due to a change of diet and food allergy is thought to be unlikely since there was, in fact, little change.

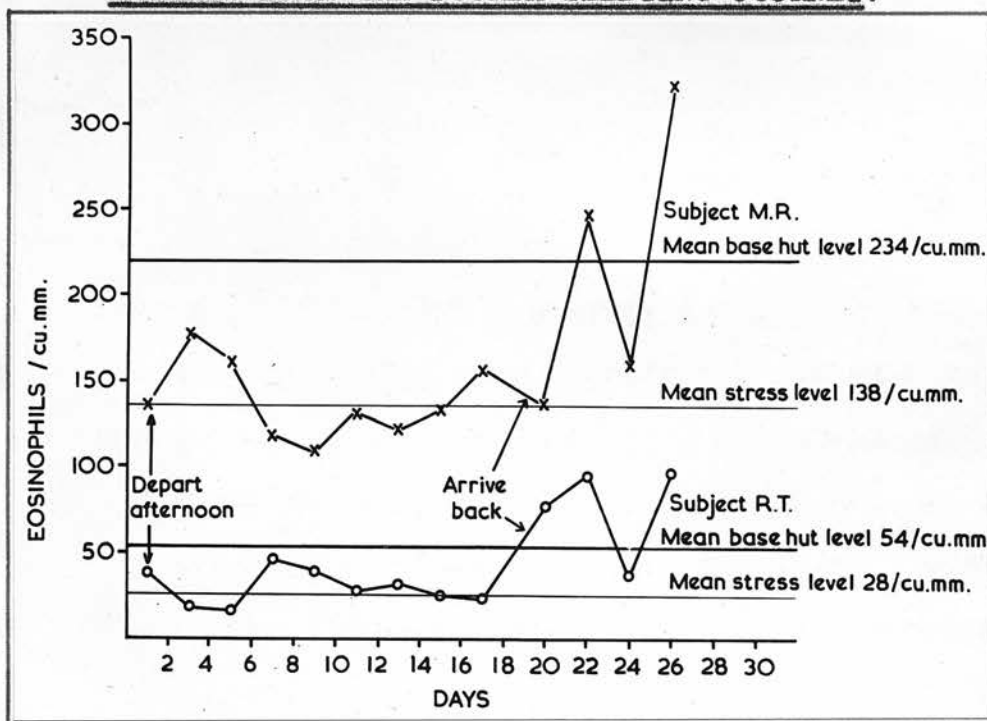
POST SLEDGING COUNTS OF SUBJECT R.W.

EOSINOPHIL COUNT AS PERCENT OF INDIVIDUALS CONTROL LEVEL



For comparison with the Eosinophil counts of subject H.S. in the same situations.

RESULTS FROM THE FOURTH SLEDGING JOURNEY.



During the 18 day period 3 men manhauled a 300 - 400 lb. sledge 237 miles mainly over sea-ice (i.e. a circumnavigation of James Ross Island). The "mean base hut level" for R.T. was based on 17 readings made in the base hut at the same time of day in control conditions; similarly M.R.'s "base hut level" was based on 17 readings. During the journey readings were made at the same time each day.

18 DAY MANHAULING JOURNEY.



Rohss Bay: M.R. and R.T. manhauling on skis. The medical research box is on the front of the sledge. The

3(c) Results from the fourth sledging journey, 1957.

The first sledge journey being hazardous and of long duration had an unusual amount of mental stress and relatively little physical stress. Under 200 miles were covered in 85 days and the sledges were pulled by huskies.

Accordingly, later in the year, a journey was made with the purpose of finding out the effect of predominately physical stress on the eosinophil count. During this journey 3 men manhauled* a 3-400 lb. sledge 237 miles mainly over the sea ice. (See photograph of the technique). This is the classical British method of Polar travel. Unlike the first journey the eosinophil counts were the main object. Alternate mornings were left free to determine the level in the venous blood such that on the day prior to the count an average distance of 18 (15 - 22) miles had been sledged. By good fortune the progress of the journey was never interrupted by bad weather. Body weights were taken before the journey, immediately after and then at daily intervals until the initial weight was regained. There was a standard 4200 Calorie ration/day/man.

R.T. had a mean eosinopenia of 28 eosinophils/cu.mm. on the day after manhauling as compared with a mean of 54/cu.mm. whilst living in the base hut in standard conditions. This

*In polar literature the term "Manhauling" is used when sledges are pulled purely by man with no assistance from dogs.

represents an eosinopenia of 52% and is significant. (P = .01). However, several of the readings taken the morning after manhauling overlap the base hut Summer readings and it could not be said that the post manhauling readings were invariably low. After R.T. arrived back at the base there was an enormous rise of circulating eosinophils with two readings over 90/cu.mm. which were the highest ever recorded for this subject. (It is worth noting that he had 104 different day counts made over a period of nine months.)

M.R., who was less experienced than R.T., also younger and persistently hungry, showed a continuous eosinopenia 138/cu.mm. compared with his base normal of 234/cu.mm. (59%). None of the stress readings in fact came within one standard deviation of the base normal count and the difference between the means is highly significant (P = .001). Also 8 days after the stress had finished he had the highest reading of his entire series (320/cu.mm.)

It is emphasised that on this journey both men appeared happy and in harmony with their environment. This was why it was possible to manhaul such large mileages each day. Although it was thought that mental stress was of minor import on this journey it was interesting that R.T. showed a very high eosinophil count the first day after returning which is really a normal post sledging day and if physical stress was

of prime import in the production of these day-after-sledging eosinopenias it is difficult to see why this day should be an exception. In any case there was some mental stress on the journey. First, we were unable to find paraffin at a depot; shortage of fuel and therefore of water is more serious than food. On the shelf ice large rifts* were encountered but this was for a short period. On the last day the weather broke and we only reached base with a tremendous effort before the hurricane broke; another party 15 mins. behind was delayed for 4 days.

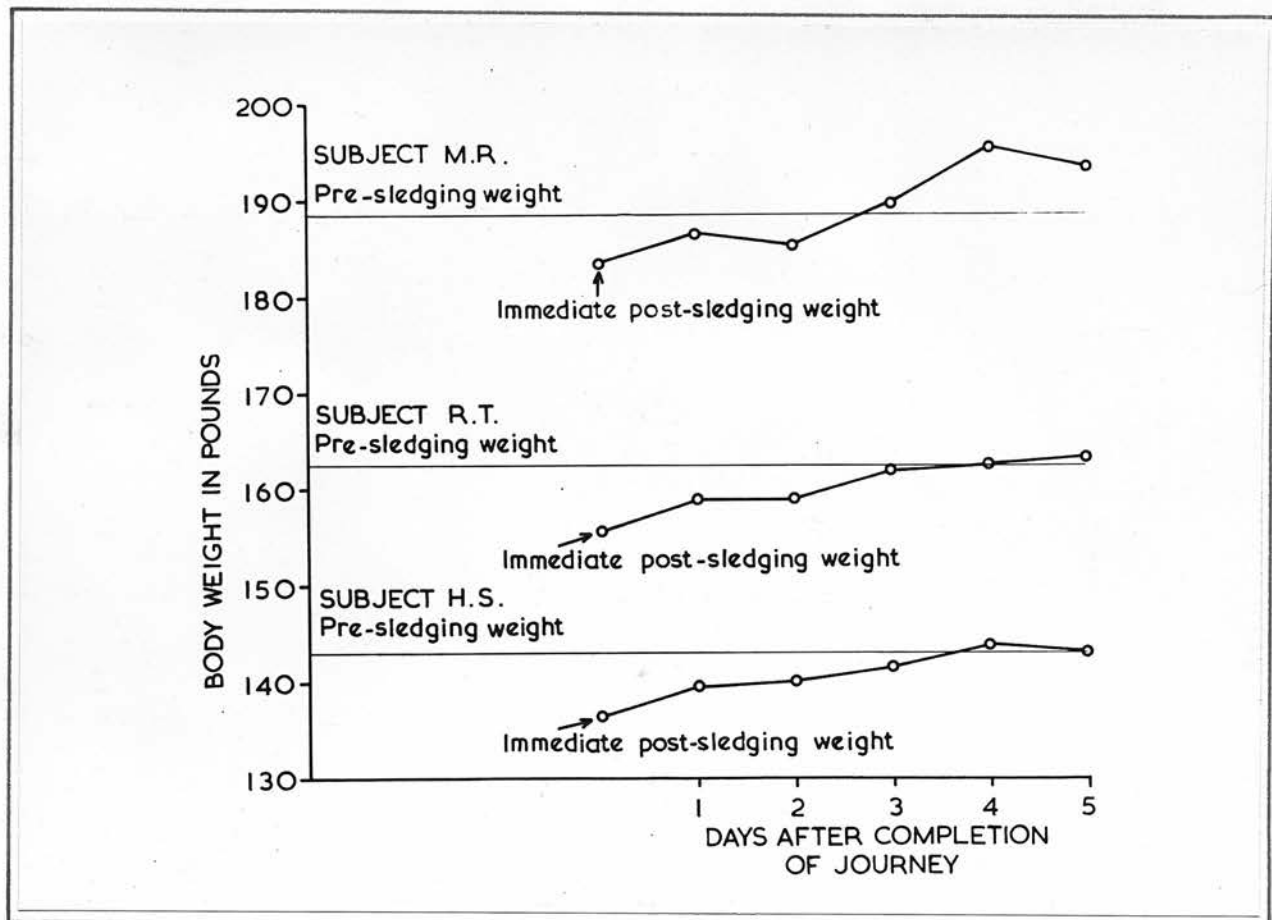
*Rifts are crevasses in the permanent sea-ice caused by currents, tides and glacier movement.

As a rough guide to the work output on this journey body weights were taken before and after. The diet contained 4200 calories/day. The weight deficit immediately on returning was -5 lbs; $-6\frac{3}{4}$ lbs., and $-6\frac{1}{2}$ lbs. After 24 hrs. this deficit had fallen to $-\frac{3}{4}$ lb., $-3\frac{1}{2}$ lbs., and $-3\frac{1}{4}$ lbs. If this initial one day weight gain can be attributed to water loss then the arrival dehydration was 2000 c.c.; 1500 c.c.; and 1500c.c. This is of interest since no water was drunk during the day's sledging and shows the importance of the normal routine of carrying thermoses of coffee. 17 miles had been sledged in order to incur this dehydration and these figures might be taken as a rough standard of any one-day's manhauling.

The actual body substance loss of $-\frac{3}{4}$ lb., $-3\frac{1}{2}$ lbs., and $-3\frac{1}{4}$ lbs. is small; it is concluded therefore that the work output on the journey was probably slightly over 4200 calories/day.

The eosinophil counts for the third man on this manhauling journey have been omitted at this stage, since they were very high. These counts may be found under the heading "An Illness encountered in Antarctica with Eosinophilia", (Section 6).

RESULTS FROM THE FOURTH JOURNEY 1957



"Sledging" indicates the manhauling journey when 3 men pulled a 300 - 400 lb. sledge 237 miles in 18 days mainly over sea-ice. 4,200 Cal/diet/man/day. Most of the weight gain in the first 24 hrs. is thought to be water.

3(d) Readings made on the same day as sledging.

It was difficult from the practical point of view to obtain eosinophil readings the same day as dog sledging. If one was on the journey the physical fatigue would have made the actual counting an extreme effort. There were however four occasions when sledging parties arrived on the base when I was already there. On these occasions the results were as follows:-

Subject	Base Hut Normal for Same Time of Day	No. of Readings	Immed. Post Sledging Level
K.B.	144/cu.mm. S.D. = $\bar{+}60$	14	21/cu.mm. = 13% -87%
L.R.	127/cu.mm. S.D. = $\bar{+}28$	8	49/cu.mm. = 38% -62%
			43/cu.mm. = 34% -66%
R.W.	65/cu.mm. S.D. = $\bar{+}14$	5	25/cu.mm. = 38% -62%
			Mean = 31% -69%

Since all the post sledging readings exceed 2 standard deviations they are considered as significant. Since all men are dehydrated on arrival these counts would err on the high side. These journeys were all a one day standard 17 mile run back from the satellite hut to the main hut and were unusually easy.

EOSINOPHIL COUNT TECHNIQUE AT A HIGH ALTITUDE.



Photograph taken at 16,500 (5,000 m.) looking through the sleeve entrance of a mountain tent. A venipuncture is being carried out on subject (left). The far door of the tent is open to let in more light. Good weather prevailing. Note tourniquet.

4. CHRONIC HYPOXIA.

Whilst returning to the United Kingdom from the Antarctic I spent several months in Peru with the Edinburgh Andean Expedition. The expedition spent its first month studying the effect of high altitudes on the eosinophil count.

In Peru it is easy to move to altitudes of up to 15,000' by car and since it is near the equator the temperature at this altitude is equable. A reasonably normal life can be led at the high altitudes and it is thought that this is a more physiological way of studying the effect of high altitude effects than use of a decompression chamber.

To make a satisfactory experiment one week was spent at each height and each eosinophil count was made at the same time each and every day. At the end of the week counts were made in the morning and in the afternoon we changed altitude by car. The final camp was attained with the help of porters on foot. In retrospect a further sea level base line was obtained in the liner on the way home. Throughout the experiments the daily activities would be social at sea level and moderate exercise, such as canoeing or riding, at high altitudes.

The blood haemoglobin level was measured in parallel with the eosinophil counts as an indication of the affect of

the hypoxia. The estimation was made with a Sahli haemoglobinometer bought in South America. Since the sea-level readings with this instrument were high it has been recalibrated against a blood sample whose haemoglobin content was known (Medical Research Council Scheme by Courtesy of Dr I. D. P. Wootton).

Results:-

	WEEK 1	WEEK 2	WEEK 3	WEEK 4	
Subject	Sea Level on Land (In Lima)	Altitude 12,750'	Altitude 15,000'	Altitude 16,500'	Liner at Sea (Retrospective)
W.W., MALE, aet. 26 y.	Mean	Mean	Mean	Mean	Mean
Eosinophils	222/cu.mm. ±SD of 36	247/cu.mm. ±SD of 60	187/cu.mm. ±SD of 41	160/cu.mm. ±SD of 33	236/cu.mm. ±SD of 46
Haemoglobin	100% (15.2g.)	103% (15.7g.)	140% (21.3g.)	143% (21.8g.)	131% (20.0g.)
No. of Readings	8	7	7	7	7
M.E., FEMALE, aet. 27 y.	Mean	Mean	Mean	Mean	Mean
Eosinophils	34/cu.mm. ±SD of 13	86/cu.mm. ±SD of 48	28/cu.mm. ±SD of 10	33/cu.mm. ±SD of 12	34/cu.mm. ±SD of 13
Haemoglobin	100% (14.7g.)	97% (14.3g.)	138% (20.4g.)	137% (20.2g.)	105% (15.5g.)
No. of Readings	7	7	7	7	6

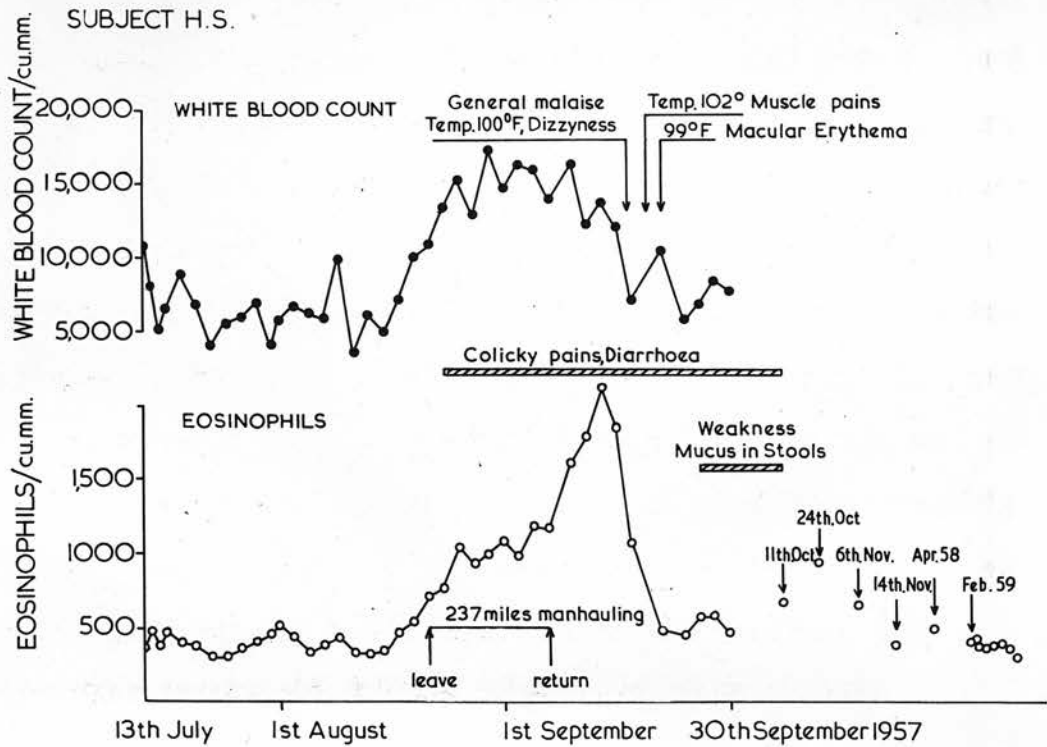
Consideration of the results for W.W. shows that at the two highest altitudes tested the mean eosinophil count is lower than those for sea level. These results are significant. (15,000' $P \leq .05$; 16,500' $P = .1$). The atmospheric pressure at 16,500' is nearly half that at sea level. In the first week at high altitudes there was no appreciable change in the haemoglobin but in the second and third weeks it had increased by over 30%. It is interesting that the haemoglobin was still increased in the liner at the end of the experiment; this was six weeks after being at a high altitude and it contrasts M.E's reading of 106%.

Consideration of the results for M.E. shows that there is remarkable similarity of the eosinophil count means at the two highest altitudes and the two sea level weeks; it would therefore appear that in her case the hypoxia did not activate the adrenal. This is of interest since the male subject tested had a greater physical reserve. In his case the question of mental stress in connection with the leadership of the mountaineering programme cannot be ruled out.

M.E. had a remarkable and significant rise of the count the week after moving from sea level to 12,750'. W.W. also recorded his highest mean during this week though the count is not significantly higher. The high standard deviations recorded for this height indicate the instability of the count. At this first sudden change of altitude there was a

noticeable systemic upset with anorexia, diarrhoea, breathlessness and insomnia. There was also a change of habit since at sea level activities were largely social and at 12,750' largely physical. Another factor is that M.E. had a one day illness with vomiting and diarrhoea immediately before we rose to this altitude and the question of disease cannot be ruled out.

AN ILLNESS ENCOUNTERED IN ANTARCTICA WITH
EOSINOPHILIA.



5. AN ILLNESS ENCOUNTERED IN ANTARCTICA WITH EOSINOPHILIA.

My own eosinophil levels during the manhauling journey have been omitted since it was soon evident that one was not recording a physiological level.

Reference to the white blood count and eosinophil levels (determined separately) shows that there was a slow rise of both over a period of 25 days to 17,000 per cu.mm. and 2200/cu.mm. It will be noticed that the eosinophilia continued to increase in spite of the 237 mile manhauling journey (which produced chronic eosinopenias in the other two subjects of 59% and 52% respectively). However, some retardation of the climbing eosinophilia is evident for the "stress" period. At the end of the manhauling there is a synchronised increase of eosinophils. The cell counts had begun to increase before the sledging started.

The interesting features of this illness appear to be:-

1. The 25 days with only a mild diarrhoea and colicky pain and no other symptoms. Meanwhile there was a steady increase in the cell leucocyte and eosinophil count of the systemic blood.

2. The white blood count leucocytosis leads the eosinophil leucocytosis reaching a zenith 15 days before the eosinophil zenith.

3. The sudden onset of pyrexia, dizziness and muscle

pains and the appearance of a generalised macular erythema preceded by subsidence of blood signs.

The aetiology of this disease has not yet been elucidated but the question arose as to whether it was a true Antarctic disease or whether it had been merely an intestinal infection transferred from one of the other eleven men. One of the men had a normal eosinophil count which sometimes exceeded 500/cu.mm. and he had seen R.A.F. service in the Middle East; it seemed possible that he might be a carrier for an intestinal infection either bacterial or parasitic. Any illness was, however, completely latent. No other man on the base had similar signs or symptoms though it was possible that one of the Argentines, with whom we had occasional meals, was a carrier.

There was also the possibility that the disease was associated with the Antarctic fauna. The meat of the following animals was eaten from time to time and was generally preferred to tinned meat:-

Birds:

Skua
Penguin
Sea-gull
Shag

Animals:

Weddel seal
Crabeater seal
Leopard seal
Elephant seal

Trichinosis is a common disease in Greenland and occurs principally in the muscle of the phoca barbata (the bearded seal) and also in foxes. The possibility of its occurrence

in the southern seal seemed worthy of more investigation. Samples of the muscle (including intercostal and diaphragm) of all the above except shag and elephant seal were brought home but the results (admittedly for only a few samples of each animal) were negative for trichineallae*. The trichinosis antigen skin test was negative. Salmonellae occur in the blood of penguins.

Another possibility was that the disease was spread by the huskies. It was inevitable that the faeces and urine of the dogs would find its way into the drinking water in small quantities. Blocks of snow are brought in daily to replenish the water supply and it is almost impossible to exclude the possibility that a block contains contaminant. It is the standing rule that all drinking water be boiled but this was occasionally forgotten. Post mortems are carried out on all dogs dying at the base and at one of these during the year the peritoneal surface of the gut and liver was found to be covered with tiny pin head concretions a few millimetres across.

* Specimens examined by Professor Kershaw of Dept. of Parasitology, Liverpool University.

POST MORTEM SPECIMENS OF THE HUSKY CHOPPER

D/3011/52.



Top left: Liver. Top right: Large bowel and mesentery. Centre: Healed rib. Note multiple small tiny concretions on liver and gut which are as yet unexplained (See text).

Relevant Extracts from the Medical Veterinary Report.

Chopper D/3011/52 became suddenly drowsy on 13/12/56 at View Point and died a few hours later. Poor sea-ice prevented the corpse being brought immediately to D for a p.m. but he was buried in a snow drift and sent around on the ship on 24/1/57.

He was a well built adult dog with long fur; the latter was easily pulled out. No external injuries but dried blood around the mouth and trickling from the left nostril. Paws in good condition. Normal tarry stool at anus. Left eye socket somewhat blood stained compared with the right. Teeth good except for a broken left lower canine.

An incision was made from mentis to pubis. There was very little subcutaneous fat except in the neck. Veins were congested. There was a small left haemothorax. The pleura was smooth. There was an old healed fracture of the sixth left rib. The two lungs appeared very congested as in the red hepatisation state of a pneumonia. No definite lobe was involved, but the left lung was more involved than the right and was almost solid with blood. There was no pus. The trachea contained many blood clots which were continuous with those in the mouth and those in bronchi. Cutting through the lung tissue showed it to be densely congested with blood.

On the other hand there was no blood in the oesophagus

or stomach, both of which were empty. Heart and pericardium n.a.d. The gut was empty except for a little fluid; no parasites were found. Many small white specks were seen on the mesentery - faintly gritty to the knife. They were about .25 - .5 mm. across. These were also present on the peritoneal surface of the liver. The latter was too autolysed for an opinion about the parenchyma.

The spleen was markedly congested. Kidney surface was smooth and interior appeared normal. Brain and leptomeninges n.a.d.

Specimens of the liver, gut with "tubercles", fractured rib and muscle (intercostal diaphragm etc.) have been preserved.

In conclusion the most striking feature was the congestion in all of the organs. It seems possible that there was a pneumonic process in the lungs as well. The concretions on the peritoneal surface of the gut suggest tubercles or the encysted phase of some parasite. The tentative diagnosis, therefore, is a bronchopneumonia following trichinosis infection, and consequent general respiratory weakness. Should the muscle specimens taken contain no parasites, alternative possibilities might be suggested by someone with more veterinary experience (e.g. fungus).

HUGH SIMPSON,
Hope Bay.
December, 1957.

Specimens of the husky intestine, liver, muscle etc. were given to Professor W.E. Kershaw, W.R.D., M.D., D.Sc., of the Liverpool School of Tropical Medicine. The relevant extract of his letter to me is enclosed:-

We discussed the clinical manifestations of the death of the dogs with the veterinary clinicians and compared it with the histology of the liver lesions, and this suggested that the illness might have been either a virus hepatitis or due to an infection of ictero-haemorrhagiae group of spirochaetes. Accordingly I sent tissue to Dr. Broom of the Wellcome Institute, who found no spirochaetes after silver impregnation, and he assured me that in all certainty a spirochaetal infection could be eliminated.

Professor Kershaw also examined the muscle.

In conclusion, neither disease has been elucidated but it is felt that some contribution to possible Antarctic diseases has been made.

6. EXPERIMENTS IN THE UNITED KINGDOM.6(a) Comparison of United Kingdom and Antarctic levels
in one man (same time of day).Subject: H.S.

ANTARCTICA	402/cu.mm. (mean of 16 readings)	$\bar{\pm}$ S.D. 73/cu.mm.
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UNITED KINGDOM	370/cu.mm. (mean of 6 readings)	$\bar{\pm}$ S.D. 36/cu.mm.
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(Readings on days with no
apparent stress)

Consideration of these two means shows clearly that they
do not differ from one another significantly.

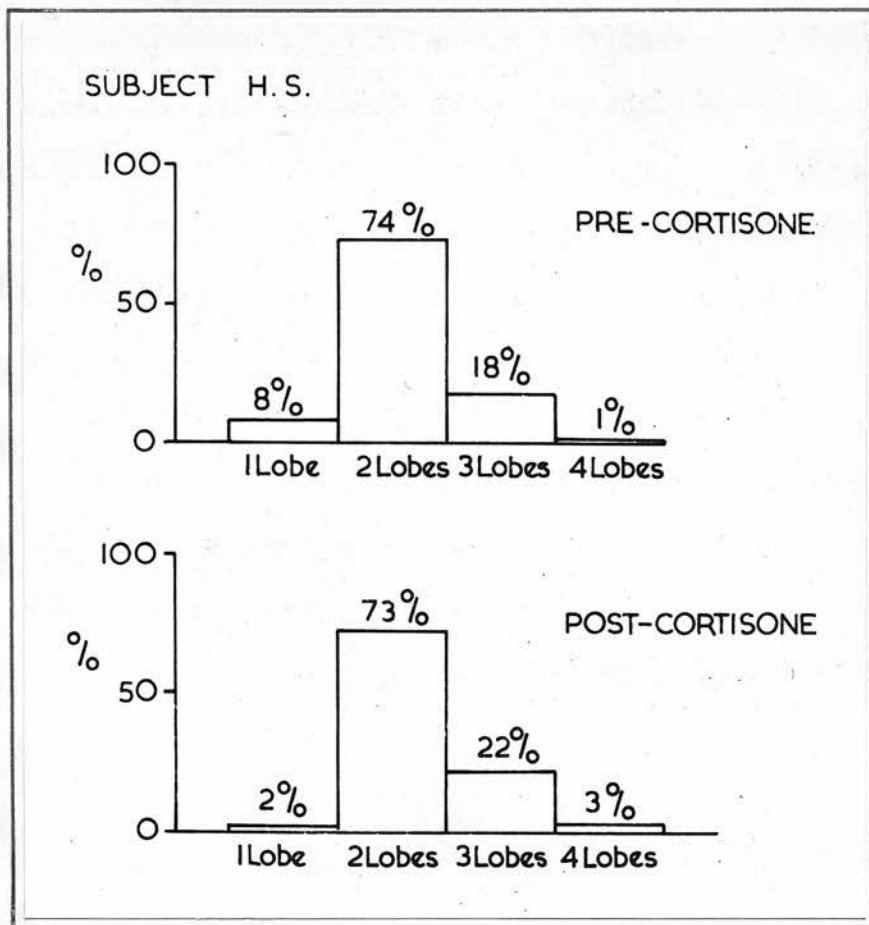
6(b) A study of the effect of oral cortisone (and a comparison with Antarctic stresses) on the count.

A study of the effect of 50 mg. of oral cortisone was undertaken in one subject to find the magnitude of the eosinopenia and to compare this eosinopenia with Antarctic stress eosinopenias.

		11.30 a.m.	3 p.m.	Percent of Initial Value
CONTROL	2.2.59	405	415	102%
NON STRESS DAYS	3.2.59	375	316	84%
50 mgm. of oral cortisone at 11.40 a.m.	12.2.59	360	190	53%
	3.3.59	305	165	54%

The post cortisone values of 160/cu.mm. and 195/cu.mm. were substantially lower than any eosinophil level ever recorded in Antarctica for this subject. The lowest Antarctic level (256/cu.mm.) was recorded during a period of apprehension before a dangerous sledge journey. Moreover, 120 Antarctic different day counts are available for this individual and it is therefore unlikely that any big eosinopenias were missed. The observation is, however, limited by the fact that no same day as sledging counts exist for this man. (Counts made on the same day as sledging meant 31% of normal in other men).

THE EFFECT OF CORTISONE ON THE
EOSINOPHIL ARNETH COUNT.



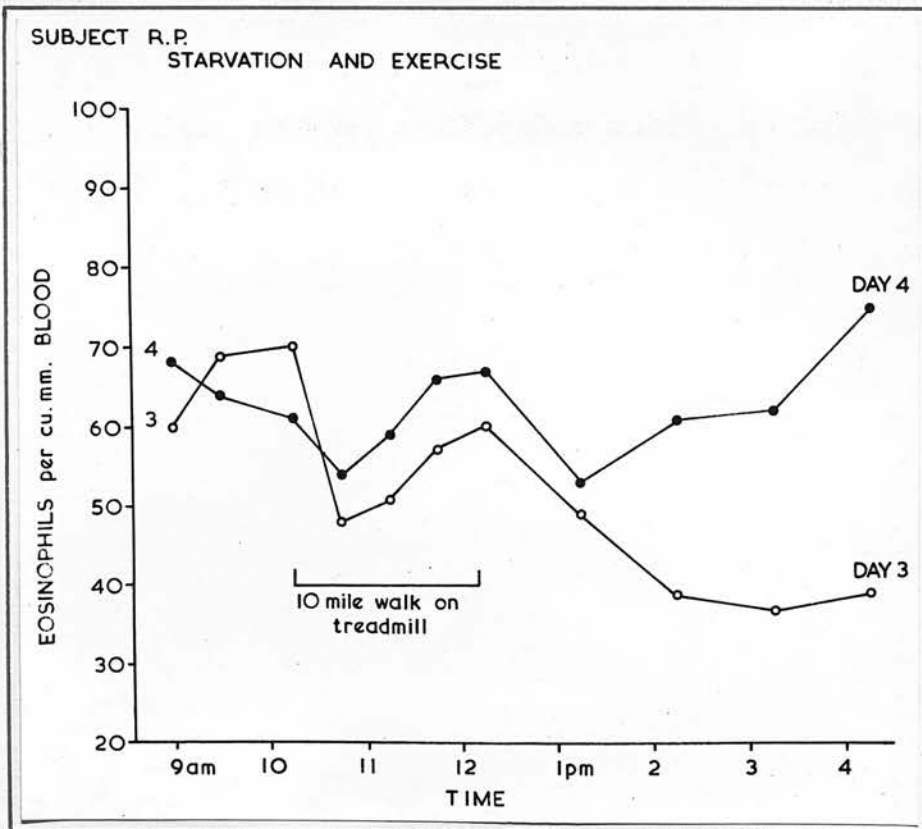
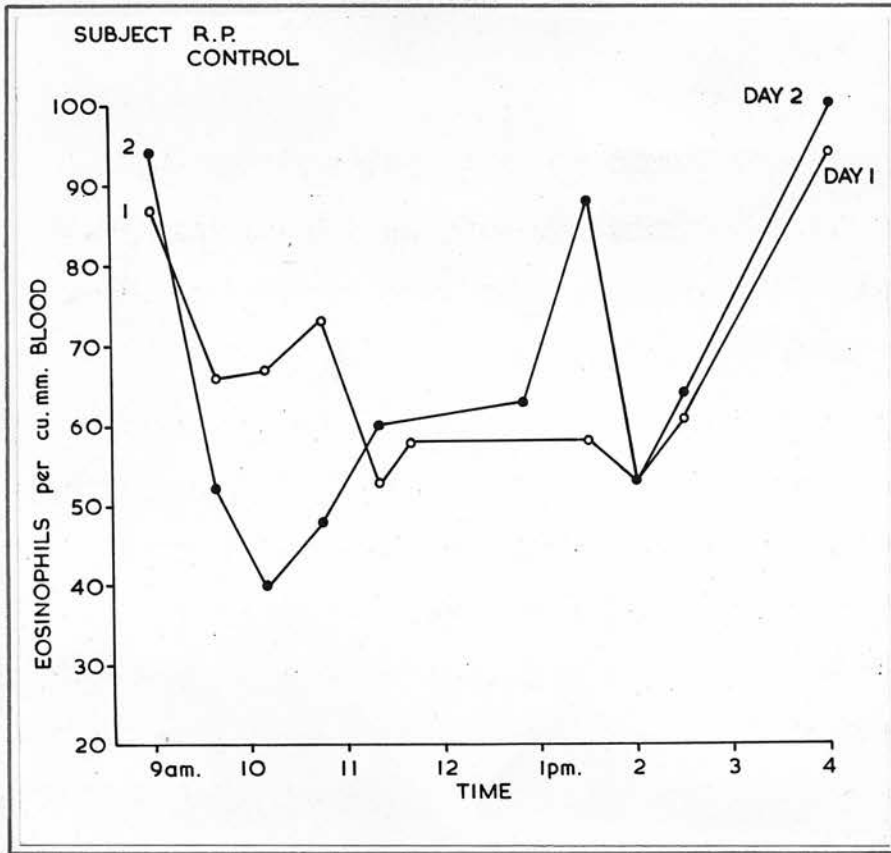
The post cortisone Arneth count was made when the absolute eosinophil count had been reduced to 53% by 50 mg. oral cortisone $3\frac{1}{2}$ hrs. previously.

6(c) The effect of cortisone on the eosinophil
Arneth count.

During the previous experiments in which cortisone eosinopenia was studied it was thought of interest to see if the ratios of 1-2-3-4 lobed eosinophils altered significantly when an individual's eosinophil count was nearly halved with cortisone by mouth. Blood films were stained May Grunwald-Giemsa and the eosinophil lobes were counted under oil immersion. 500 eosinophil of normal blood and 500 eosinophils of the individual's blood after oral cortisone were counted.

Consideration of the results shows that either cortisone induced no change in the ratios or that the post cortisone population is of older cells.

THE EFFECT OF STARVATION AND EXERCISE
ON THE EOSINOPHIL COUNT.



6(d) The effect of Starvation and Exercise on the Eosinophil count.

In order to study the effect of pure physical stress on the eosinophil count an investigation was carried out with R.P. on the Edinburgh treadmill. This investigation consisted of two control days and two exercise days in which there was also starvation. On all 4 days 10 - 11 eosinophil counts were made between 9 a.m. and 4 p.m. at intervals of $\frac{1}{2}$ - 1 hr. On the exercise days 10 miles were walked by the subject (R.P.) between 9.45 and 12.15. At other times he lay on a couch or was seated.

Consideration of the control days on the graph shows a U shaped diurnal variation with a morning fall and a rise in the afternoon. On the starvation and exercise days it is seen that the initial level is lower which is possibly the effect of the starvation. For the duration of the exercise there is little difference between the days, but after its completion it appears that the afternoon rise seen on the control days has been restrained.

DISCUSSION

If the mixing of blood and diluting fluid is adequate then the resulting dispersion of cells in a haemocytometer chamber is according to a Poisson distribution, i.e.

Number of cells counted = $\sqrt{\text{the standard deviation}}$
(Berkson et al 1940). This is the ultimate limitation of the haemocytometer method. It is seen that the precision of the result is dependent on the number of cells counted. In the present work, where low concentration of cells were anticipated, 6 chambers were filled and counted in order to obtain a satisfactory accuracy; it would perhaps have been better, and less tedious, to double or treble the concentration of blood relative to stain and to count a smaller number of chambers. It has been shown that mixtures of blood and stain do mix adequately in a small, shaken, tube since a Poisson distribution was obtained in the haemocytometer chamber. Using a standard haemocytometer pipette for dilution I have had difficulty in obtaining an accurate end point and overfilling necessarily leads to rejection of the sample. On the other hand, with the tube-mixing method, which was adopted, this difficulty was overcome and accurate pipetting made possible. Ordinary pipettes are, moreover, cheaper and easier to clean.

It may be thought remarkable that in some cases slightly more favourable distribution than Poisson was obtained (i.e. the average value of Standard deviation - mean = 0.971 over 30 chambers. However Berkson et al 1940, obtained a value of 0.92 for red cells which they attributed to over-crowding. Since the concept of the distribution is based on physical contacts between particles it seems probable that with biological material an exact replica will not be obtained.

The results of this work and other work makes a basis for the understanding of the normal eosinophil count. Each individual appears to possess a fundamental eosinophil level. This is so characteristic that in the present series it would normally have been possible to say which of the subjects had donated some unknown blood merely by counting the eosinophil content. The extremes of the normal eosinophil counts found in the present series were 34/cu.mm and 427/cu.mm. which is a remarkable span of magnitude. There are few other biological indeces which vary as much as this in physiological life. The mechanism of this "setting" of the eosinophil level is not known; there is general agreement that those liable to allergies may have high counts e.g. ashtamatics but this did not appear to be the factor in the present fit sample of "explorers". Individuals with hypoadrenal symptoms do not have significantly higher

eosinophil counts than controls (Recant et al 1950) and thus the level is unlikely to be under adrenal control. Age and sex do not appear to influence the level (Rud 1947). From all such evidence, and from the consideration of the condition "familial eosinophilia" (Stewart 1933) it would seem that the fundamental level is set genetically. It is worth noting that the subject with the lowest mean count (34/cu.mm.) is a radiographer.

The resting level is fluctuating about a mean in standard conditions owing to a diurnal variation. Although the daily variation of corticoid levels and eosinophil levels in the blood run parallel, there is also a great increase of the circulating adrenaline during the day and this also may be a factor in the control of the resting levels.

Even if diurnal variations are controlled by determining the eosinophil level at the same time of day over several days in standard conditions, there is still a residuum of variation which in most cases cannot be explained by experimental error. This may be seen in the analysis of variance and in the graph of the first sledge journey of subject L.R. and R.W. The former is a stable count and the latter unstable. Both of these subjects have a similar order of count and any difference in stability is likely to be biological. Any correlation between this instability and the mental

characteristics of the individuals concerned is a problem on which impartial judgement cannot be given in this thesis. The following table of diurnal variation of the eosinophil cells contains the pooled results of 14 authors using a total of 81 subjects.

9 am	12 noon	3 pm	6 pm	9 pm
100%	108%	112%	131%	141%

There is general agreement that in apparently non-stressed conditions the 24 hr. nadir occurs at 9-11 a.m. and thereafter there is a rise of the count towards midnight.

When 203 counts taken from six individuals living in the Antarctic hut with no other apparent stress were analysed for diurnal variation the following table is obtained:-

7-9 a.m.	9-11 a.m.	11-1 p.m.	2-3 p.m.	4-6 p.m.	8-10 p.m.
---	100%	92%	97%	92%	110%

By comparing these two tables it can be seen that the slow rise during the waking hours of the resting rhythm has

been replaced by a moderate fall during the working period of the day. It is suggested that this is reasonably explained by the large amount of work which is inseparable from base life. Not only must each man do the job that he was specifically employed for but also the general domestic work around the hut e.g. bringing in snow blocks for the water supply and feeding the 50 dogs. Agreement with this suggestion comes from the work of Wake, Graham and McGrath 1953 who found that on an 'exercise' day of badminton, basketball etc. the eosinophil trend was downwards until mid afternoon contrasting the upward trend of the same 21 subjects on a rest day. In the present work it is shown that a 10 mile treadmill walk also flattens the daily rise from 9 a.m. onwards.

In stress associated with Antarctic field work there was evidence to suggest that the individual's eosinophil level reacted in a manner characteristic of the individual. For example subject L.R. had a significant eosinopenia whilst merely living in a tent and this was not true of other individuals. Subject R.W. during two journeys over the same ground, had eosinopenia covering a difficult initial stretch and eosinophilia when the going was easy. Subject H.S. had eosinophilias on all 3 occasions when returning from a journey, the counts rising to a peak on the third post sledging day. This was not shown by any other

individual. One would expect these results if the eosinophil level was an expression of the impact of any stress on any individual if his attitude towards it had not changed after the exposure. While this is thought to be the correct interpretation there is also the possibility, in some cases, that the eosinophil reaction is modified by the magnitude of the initial count; Esselier et al (1954) showed that the higher the basal eosinophil count the longer it took to induce an absolute eosinopenia whatever the dose of cortisone.

Selye has introduced the word "adaptation" and "failure of adaptation" as descriptions of the response of the animal to a prevailing stress. Rats, for example, which are exposed to cold are more resistant to lethal cold than normal rats and are therefore said to be "adapted". If this interpretation is allowed then clearly subject L.R. is an example of "failure of adaptation" to living in the field in Antarctica. As already stated merely living in a tent caused statistically significant eosinopenia and the longer the period spent in the field the more pronounced the eosinopenia. As base leader subject L.R. was on the 1st, 3rd and 6th journeys 1957 and spent more than 200 days of the year in the field. If his unstressed level is taken to be that found in Summer whilst at the base hut it is probable that, from the comprehensive results of the

first journey and the isolated results of subsequent journeys, he had a morning eosinopenia for 200 days of 1957. Morning eosinopenia is emphasised since it appears that the low level has persisted through the night. That these low eosinophil counts were indicators of internal stress in this subject was strongly supported by clinical impressions. The responsibility of leadership on this base is any case a great burden to any man: L.R. resigned his base leadership and preferred, for the second half of his Antarctic stay, to occupy the position of surveyor only. On the other hand subject R.T. appeared to be an example of a subject who "adapted" to sledging and this was also in accordance with clinical impression (compare his results for the first and second half of the first sledge journey 1957, his first experience of sledging). At the end of his Antarctic duty his application to stay on for a third consecutive winter was accepted. This privilege is only granted on recommendation from the medical officer and the base leader. It might be argued that R.T. did not adapt since on the subsequent fourth sledge journey (manhauling) he had a significant eosinopenia. It is thought that the physical stress on this journey was so great that complete adaptation was unlikely; unlike dog sledging one cannot keep up large daily mileages indefinitely.

The finding that a radio signal with exciting news

causes a stress eosinopenia is an expected one (see results Section 2(H)). The psychological effect on a base of any signal is much exaggerated because of the small contact with the outside and the absence of distractions. The individual in which this effect was demonstrated was not particularly given to exaggerated emotional reactions. Similarly the eosinopenic effect of cooking is not unexpected for cooking has to be done as well as the individual's main job. On such weeks, one is generally exhausted by the end of the day. Sledgers have to make up their cooking weeks when they return from the field, since sledging was considered a privilege.

A disease in which there was a marked eosinophilia has been described. Had no blood research been carried out, this illness would have been dismissed as an unremarkable intestinal infection. This disease may have occurred in other bases in Antarctica and passed unnoticed. Any intestinal illness occurring again on the bases should be investigated by blood films. It is only in this way that any potential Antarctic hazard (e.g. the meat of the carnivorous leopard seal infected by trichinellae) will be discovered.

The object of studying the effect of altitude was to observe the eosinophil level in an easily measurable physical stress i.e. the hypoxia associated with low

atmospheric pressure. It seemed of interest that at half atmospheric pressure there was only a moderate, but significant, fall in the one fit man studied and no fall in the one woman studied. The low oxygen tension of the blood and the alkalaemia consequent on the hyperventilation might contribute to the stress. It is difficult to know whether the small effect observed is due to adequate adaptation or whether it represents a depressive hypoxia of the adrenal gland. Burril and Ivy (1949) found a decrease of ketosteroid excretion when exposing humans intermittently to a simulated 18,000 ft., but this might have been due to depression of testicular steroid. Burkhardt, Criscuolo, Coulson, Hale, Flickinger and Adler (1951) found with exposing dogs daily to 20,000' that the eosinophils after a fall, rose to normal on the 30th day. Cukier (1952) found an average of 56% eosinopenia when he took 21 subjects up to 14,750' in cars but this was an acute experiment. In view of the fact that the subjects in the present experiment subsequently climbed strongly to 22,200' it is felt that the minimal eosinopenia is attributable to adaptation other than failure to supply adrenal hormone.

The finding that there was no difference in the home and Antarctic eosinophil levels for one man is an expected one. If, in fact, men were in a state of stress merely with the Antarctic environment then it would seem unlikely

that the normal tour of duty would be as long as 2½ years. However it would be of interest to do more of these duplicate tests.

Uters, Hofschlaeger, Anton and Zimmerman 1951 made a brief study of meteorological conditions and the output of 17-ketosteroids. They concluded that a meteorological warm or cold front can be associated with a rise in 17-ketosteroids. The present study could find no correlation between various weather indices and the eosinophil level and it would be hard to find a region where the weather fluctuations were more violent than Antarctica. Similarly there was no adequate evidence of chronic eosinopenia in the Winter.

When eosinophil levels were determined the evening of an easy 17 mile sledge journey, lower levels were recorded than during any other stress during the year (i.e. a mean eosinopenia of 31% compared with control levels). Since this was not a severe journey one would anticipate lower levels for sledging across unknown country or greater distances. In the present work it was impracticable to obtain results at the end of such strenuous days and in this case one had to be content with observations on the subsequent day. The easy run mentioned above was between the satellite hut to the main hut. It should be pointed out that it is unusual to finish a day's sledging in a

completely exhausted state since this would detract from the next days progress. This should be borne in mind when comparing the results with competitive athletics. In a Harvard boat race the mean crew eosinopenia was 26% of control (Reynold et al 1951). Other stress eosinopenias which it is interesting to compare with these results are channel swimmers, mean 2% (Pace et al 1956); drivers in the Indianapolis speedway, mean 22% (Frost and Dryer 1951); candidates in the final dental exam, mean 10% (Kerr 1956); a day of resort skiing, mean about 36% (Pugh 1959).

Since no serious difficulty was experienced in obtaining blood and making blood counts whilst in the field for a period of 2 months it is suggested that more physiological work is practicable under these conditions than is generally realised. Techniques take longer in the field but the life is simple and one has more time and less distraction than while on the base or in a laboratory.

From the result of this thesis, and from the work of others, it is possible to build up a provisional outline of the eosinophil count changes occurring in the men of an Antarctic base.

The well described daily rise of the count from the late morning to the time of retiring is flattened. This is probably due to the stress of the work in the general

running of the base. This effect does not seem to accumulate since there was no tendency for counts to fall from day to day except during duty cook weeks. Exciting radio signals or the anticipation of sledging were examples of stress that caused a lowering of this daily level.

In the field one man had a continuous stress eosinopenia; the others had continuous eosinopenias while dog sledging but only when the going was hazardous. In contrast, manhauling was associated with continuous eosinopenia. The lowest levels of all during the year were recorded immediately after sledging.

After returning to base there was evidence of a variable rebound eosinophilia.

S U M M A R Y.

The level of eosinophil polymorphonuclear leucocytes circulating in the antecubital venous blood was used as a measure of the impact of diverse Antarctic and other stresses on 12 individuals.

A fall in the eosinophil level in stress is thought to be due to a stimulation of adrenal cortical and medullary tissue; in general it is thought that acute stress is predominately associated with adrenaline eosinopenia and chronic stress with corticoid eosinopenia.

In all, 715 eosinophil counts were made, and the stresses studied included sledging, hypoxia, bad weather, treadmill walking, and activities in the base hut. A study was also made of normal levels and diurnal variations. The effect of oral cortisone on the eosinophil lobe ratios was also studied. A disease, with intestinal symptoms, and associated with marked eosinophilia, has been described.

A technique was evolved for estimating the venous eosinophil level which proved satisfactory even when on sledge journeys of over 2 months duration.

Various Antarctic activities were found to be associated with an eosinopenia. Eosinophil blood levels estimated immediately after sledging 17 miles averaged 31% of control counts. An interesting finding was evidence that one man

had a continuous eosinopenia for 200 days of field work in 1957. These continuous low readings have been attributed to mental stress. More generally, dog sledging only appeared associated with eosinopenia when the journey was hazardous. A manhauling sledge journey of 18 days was associated with a continuous eosinopenia. Daily base activities and treadmill walking appeared to flatten the diurnal rise of the count. Cooking duty and radio signals about the arrival of relief ships appeared to induce an eosinopenia. In contrast, the physical stresses of the cold and winds produced no detectable change. At a high altitude, with the atmospheric pressure about half that at sea level, only one of two subjects had a significant eosinopenia although the haemoglobin was increased to over 21 g. per cent.

C O N C L U S I O N .

The men on an Antarctic base are being subjected to continual stresses which are sufficient to cause diminution of the circulating eosinophil cells.

The daily routine causes a depression of the count which rises to normal after a night's sleep. Exciting radio signals or rescue operations cause significant depression below this daily level. Sledging is the biggest stress in Antarctic life and a chronic morning eosinopenia may appear during a whole journey. The level of this eosinopenia depends on the mental and physical strain of the journey and its mental and physical impact on the individuals.

A C K N O W L E D G E M E N T S.

I am indebted to the men of the Falkland Islands Dependencies' Survey and to the members of the Edinburgh Andean Expedition for their unfailing cooperation as subjects in these experiments. Some of these men submitted to more than 100 venipunctures in difficult circumstances.

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APPENDIX (see p. 55).ANALYSIS of VARIANCE(a) Between Chambers (Experimental error) (σ_B^2)(b) Between Days (Biological variation) (σ_A^2)Subject: M.R.

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chamber	44	54	25	30	47	25	38	29	38	34	49	25	34	42	33	41
Chamber	37	32	30	42	32	24	38	22	45	44	45	24	38	37	28	38
Chamber	44	68	27	30	34	29	43	27	46	37	35	32	38	53	32	40
Chamber	54	49	41	28	34	33	39	24	54	38	37	27	43	31	40	24

Sum of Squares of individuals = 91943 1

Column totals, squared, Squares summed and divided
by number of individuals in each column
= $358811 \div 4$ = 89702.8 2

Sum of all individuals, squared and divided by
total number of individuals
= $\frac{2355^2}{64}$ = 86656.6 3

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	Components of Variance
Between Days	2-3 = 3046.2	15	203.1	$n\sigma_A^2 + \sigma_B^2$
Between Chambers	1-1 = 2240.2	48	46.7	σ_B^2
Total	1-3 = 5286.4	63		

If σ_A^2 exists significantly then the between days mean square must be significantly greater than the between chamber mean square. The ratio of 203.1 to 46.7 is 4.4 to 1. There are 15 degrees of freedom for the larger variance and 48 for the smaller variance. From the table of the variance ratio (page 183 of "Industrial Experimentation") it can be seen that the Between Days mean square is significantly greater $P = .001$

We have then:

$$n \sigma_A^2 + \sigma_B^2 = 203.1$$

$$\text{and } n = 4 \text{ and } \sigma_B^2 = 46.7$$

Therefore:

$$\sigma_A^2 = \frac{203.1 - 46.7}{4} = 39.1$$

$$\sigma_A^2 = 39.1$$

$$\sigma_B^2 = 46.7$$