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PRELIMINARY INVESTIGATIONS INTO THE
REPRODUCTIVE WATER DRIVE OF BUFO CARENS SMITH
AND THE
HUMIDITY REACTION OF RANA NATALENSIS (SMITH)

by

J. C. Poynton

Department of Zoology, University of Natal,
Pietermaritzburg.

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Pietermaritzburg.



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RESUME

1. Preliminary investigations into the reproductive water drive of Bufo carens Smith and the humidity reaction of Rana (Pyxicephalus) natalensis (Smith) have been undertaken.
2. The literature is briefly reviewed to show that the sex hormones play an important rôle in behaviour associated with sex, and from the information presented it is concluded that the possibility of an effect of hormones on the reproductive water drive of Bufo deserves investigation.
3. The apparatus and methods used in measuring and recording the water drive in Bufo are described.
4. An increase in the water drive following injections of pituitrin, testosterone, chorionic gonadotropin and estrogen was not observed, but it is concluded that more extensive and better controlled experiments may yet show that injections of the sex hormones have an effect on the water drive of Bufo carens.
Suggestions for the best procedure that should be adopted in further work are given.
5. Humidity chambers designed for studying the humidity reactions of Rana natalensis are described. The method of removing the microclimate around these

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animals by introducing moving air into the chambers is given.

6. A method for measuring and recording the behaviour of Rana is described.
7. It was found that moving air affects the behaviour of Rana both quantitatively and qualitatively.
8. A humidity reaction was found to exist in these animals, in that their activity increased with lowered humidity.
9. The behaviour of these animals in the humidity chambers is described, and it is shown that most of the activity consisted in attempting to get out of the chambers.
10. An approach to further studies on the humidity reaction of Rana, taking into account the complications arising from the microclimate of these animals and the effect of moving air on their behaviour, is given.

GENERAL INTRODUCTION

The humidity reactions of various invertebrates have received a considerable amount of attention in recent years. From this work has come the knowledge that species may evolve simple innate behaviour patterns which, in addition to morphological adaptations, assist in the avoidance of an excessive loss of water. Among the land vertebrates, only scant attention has been paid to this problem. The Amphibia suggest themselves as being a starting-point for humidity reaction studies in this phylum, for, owing to an integument which is freely permeable to water (Gray, 1928), they will be subjected to a strong selection pressure favouring behaviour mechanisms which help to prevent the body water level from becoming dangerously low. Consequently any such humidity reactions that may exist should be particularly suitable for experimental investigation in these forms. In this connection, work has been done by Shelford (1913) on frogs and salamanders, where it was apparent that these animals possessed a well-marked humidity reaction, but no attempt has yet been made to make a detailed study of the behaviour mechanisms involved in the reaction. It was therefore considered desirable that work should be undertaken on the humidity reactions of a local amphibian, in which the same techniques that have been developed in work on the humidity reactions

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of invertebrates would be employed, in order to analyse the reactions more closely, and to obtain some indication as to how far studies of this nature could be carried in this group.

The peculiar water relationships of the Amphibia suggest that this group might also provide material for work on water drives, or "hydrotropisms" (Savory, 1930), as distinct from the humidity reactions, or "hygrotropisms" (Savory). It appears that toads in particular possess a form of water drive, in that they are attracted to water mainly during the breeding season (Blair, 1946; Noble, 1931). In order to investigate this phenomenon, it is necessary to look to the seasonal changes occurring both in the external and internal environment of these animals, since it is apparent from the literature that the release of most of the behaviour patterns associated with breeding is normally governed by the condition of the internal state of the animal, as well as by the nature of the external stimuli (See Part I, Introduction). Each of these factors can be studied separately, if the other is properly controlled. As regards those changes in the internal environment which might influence the water drive, it is known that the alternation in the level of the sex hormones, whether experimentally induced or occurring in the normal seasonal cycle, produces a marked effect on the release of

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behaviour patterns associated with breeding (See Part I, Introduction). The existence of some hormonal influence on the water drive would be of fundamental importance in the understanding of the drive, so it therefore seemed desirable that preliminary work on the water drive should be directed towards finding out if changes in the hormonal level in toads is one of the factors determining the appearance of this drive. In addition to this importance, the information gained from such work would add to the existing knowledge of the effects of hormones on the behaviour of the Anura - a field of behaviour studies that has so far received comparatively little attention.

The work on local Anura recorded in the following pages was thus directed towards determining the nature of the behaviour mechanisms involved in the humidity reaction, and towards discovering if any hormonal factors are involved in the seasonal water drive.

PART I. THE WATER DRIVE

INTRODUCTION

G.K. Noble has suggested that the spring migration of amphibians to water prior to breeding "may be considered a secondary sexual character found in both sexes" (Noble, 1930, p.402), and in addition he points out that the sex hormones, which determine the appearance of the anatomical secondary sexual characters, may also influence the appearance of the seasonal movement to water. Blair (1946) also considers that what he terms the "mating reaction" of Bufo fowleri, which consists of the spring migration to water and the subsequent breeding activities, are under hormonal control. He was, however, unable to elicit any of these reactions by hormonal treatment. Other workers have recorded that the clasp reflex can be induced in Bufo by pituitary implants (e.g. Houssay, Guisti & Lascano-Gongales, 1929), but there appears to be no report in the literature of the induction of the complete sexual behaviour in Bufo by the injection of the sex hormones. It seems that in this respect Bufo is very peculiar, since a review of the literature shows that it is characteristic of animals studied from all the vertebrate classes that sexual behaviour

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is as much a part of the reproductive equipment of the animal as are the organs of reproduction, and, like these organs, it falls under a marked and specific influence of the sex hormones. Blair and Noble believe that there are also environmental factors which influence the seasonal water drive and sexual behaviour, in addition to the hormonal factors, and certainly while it is clear from the literature that the environment, acting on the central nervous system, is of critical importance in the appearance of sexual behaviour, the level of the sex hormones must also be a critical factor in its appearance. The literature indeed shows that sensory stimulation and the sex hormone level (among other internal factors) appear to be complementary elements in the evocation of sexual behaviour, and it is therefore reasonable to look for the effects of the sex hormones on the breeding behaviour of Bufo, in spite of the largely negative results that have so far been obtained. It is, moreover, evident from the literature that the development of the cerebral cortex in the vertebrate series seems to be inversely related to the indispensability of the sex hormones in the appearance of sexual behaviour. Because of the relatively poor development of the anuran brain, it is further to be expected that Bufo should show any responses to sex hormone treatment that might exist in

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a particularly well marked manner. A review of the literature does, in fact, show that there is ample justification for further investigations into the effects of the sex hormones on the general breeding behaviour of toads, and the following review will be concerned with the two main considerations given above, namely that i) sensory stimulation and the sex hormone level appear to exert a dual rôle in the evocation of sexual behaviour, and that ii) the influence of the sex hormones on behaviour seems to be inversely related to the development of the cerebral cortex.

The evidence for the dual rôle of the effect of hormones and nervous excitation on sexual behaviour comes mainly from the rat. The importance of the effect of hormones themselves is shown by Stone. He found (Stone, 1927) that castrated adult male rats ceased copulation after the operation, some within a week, others within periods of up to nine months, after castration. If, however, the hormone secreted by the testes is replaced by injections, sexual behaviour can be restored to normal levels. Stone (1939) showed that this behaviour can be quickly restored in castrated adult male rats by injections of testosterone propionate. In the more active animals, copulatory and ejaculatory frequency was restored to the pre-castrate level. When injections were discontinued, ejaculations

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immediately disappeared, and copulatory activity gradually waned. Control injections of oil failed to reactivate the castrates, but renewal of the testosterone injections speedily restored the sexual behaviour. Additional obstruction tests showed the presence of a sex drive, which was also controlled by the androgen level. Other investigators have consistently confirmed Stone's results, as may be seen in the extensive review of the literature on the effects of hormones on behaviour by Beach (1948).

A similar effect of hormones on mating behaviour has been shown in the female rat. In the spayed female, sexual behaviour is practically absent (Nissen, 1929). This behaviour returns after the injection of ovarian extracts and synthetic estrogenic compounds (Hemmingsen and Krarup, 1937). The response to estrogen is, however, slight when compared with the response to an injection of progesterone following a priming of estrogen. Using spayed virgin females, Beach (1942a) found that 14% of these animals showed lordosis after estrogen injections, compared with the 74% which showed lordosis after injections of estrogen followed by progesterone. The induced receptivity was closely comparable to the receptivity shown by normal females. The correlation between the behaviour and the hormone level is so close in the estrous cycle that Young,

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Boling and Blandau (1941) have concluded that the "identification of such endpoints as the beginning and end of heat, the beginning of preovulatory swelling and the time of ovulation is generally more accurate when reference is made to the behavioural responses associated with estrus rather than to the vaginal condition."

Nervous impulses from the accessory sexual organs are not involved in the hormonal induction of sexual behaviour, since removal of the seminal vesicles, prostate, and the associated ducts in the male rat brings about no loss of sexual aggressiveness (Shapiro, 1937), and Ball (1934) reported normal sexual behaviour in females with the prepuberal removal of the uterus and vagina.

Gonadectomy in the rat does not, however, completely eliminate sexual behaviour in either the male (Beach, 1942b and 1944a) or the female (Ball, 1939). The behaviour exists to a limited extent in a proportion of the gonadectomised animals, and the injection of the sex hormones appears to activate a pattern already present, and not specifically to organise new patterns. Thus the onset of adult mating behaviour is manifestly not due to the formation of new behaviour patterns at this time, since the production of a normal adult level of the sex hormones in immature and senile animals by injections of these hormones brings on a

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normal, although untimely, occurrence of sexual behaviour. For example, Stone (1940) found that precocious copulatory activity could be induced in immature male rats by injections of testosterone, while Minnick, Warden and Arieti (1946) have recorded that injection of testosterone into senile male rats significantly increases their sexual activity. Similarly, Wilson and Young (1941) found that precocious sexual receptivity can be induced in immature female rats by injections of estrogen, while Slonaker (1927) induced mating behaviour in old female rats by the injection of estrogen, even though the animals were no longer fertile. This effect of the hormones of activating a pattern already present is also shown in the influence of the sex hormones on the occurrence of inverted sexual behaviour, where an animal of one sex displays the sexual behaviour characteristic of the other sex (Morris, 1952). Normal males are occasionally found to show the exact reaction of a female in heat, when mounted by another male (Beach, 1938; Stone, 1924), and Beach (1942c) has reported that a normal multiparous female rat showed the complete copulatory pattern, and that seven sexually inexperienced females mounted and palpated males when extremely excited (Beach, 1938). Injections of sex hormones bring about a marked increase in the occurrence of inverted sexual

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behaviour. Ball (1940) has reported that masculine sexual behaviour was considerably increased in young adult female rats injected with testosterone, and that after three weeks of injections, the complete masculine copulatory pattern began to appear. This complete pattern was not found in untreated controls. Masculine behaviour fell off rapidly in the experimental animals after the injections were discontinued, the copulatory pattern disappearing within nine days. Kun (1934) found that castrated male rats exhibited perfect female copulatory behaviour when injected with estrogen. These experiments therefore suggest that the sex hormones serve to activate patterns that are already present. Heterologous sex hormones do not simply induce inverted sexual behaviour, however, for each sex hormone is capable of increasing the frequency of the occurrence of both male and female mating patterns in either sex. The occurrence of female mating behaviour in the male rat can readily be brought on by large doses of testosterone (Beach, 1941; Engel, 1942), as well as by large doses of estrogen (Beach, 1942b; Ball, 1939). Ball's testosterone-injected females (Ball, 1940) continued to accept aggressive males, and she also found (Ball, 1937) that if injections of estrogen were given to castrated male rats before the drive disappeared, the sexual activity of these animals rose, but the activity was typically masculine. Beach

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(1942a) found that the injection of testosterone into prepuberally spayed female rats raised the level of masculine mating activity from a weak, incomplete degree to a much higher degree, as shown by the greater proportion of complete copulatory responses. But the femal receptive behaviour, which was absent before the injections of testosterone commenced, also appeared after the injections. Similarly, Ball (1939) implanted estrogen pellets into prepuberally castrated male rats, and these animals showed a limited amount of male sexual activity, as well as female mating responses when mounted. Much more estrogen was required, however, to bring out the female pattern in the males than in spayed females. It can, then, be concluded from the above investigations on the effects of sex hormones on the sexual behaviour of immature and senile animals, and on the occurrence of inverted sexual behaviour and the effects of heterologous sex hormones, that the injection of the sex hormones in some way raises the excitability or reactivity of already-existing behaviour patterns.

Having to some extent clarified the general effect of hormones on behaviour, the problem must now be investigated more closely in order to see how the effect of hormones and nervous excitation play a dual rôle in the evocation of sexual behaviour. It therefore becomes necessary to form

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some picture as to how the qualitatively different actions of external stimuli and hormones can affect the motor organisations in the same quantitative way, so as to give the complementary effect on behaviour that was noted at the beginning of this Section. The first step towards forming this picture must consist in trying to discover if the target organs of the sex hormones in these cases are nervous or non-nervous organisations.

K.S. Lashley, in a much-quoted paper (Lashley, 1938), reviewed the then existing literature to find if the effect of hormones on behaviour lay in 1) an ability to stimulate growth or formation of new nervous connections; 2) an ability to increase the general level of excitability in the organism; 3) an ability to induce specific changes in various non-nervous organs associated with sex; or 4) an ability to act upon the central nervous system to increase the excitability of the sensori-motor mechanisms specifically involved in the instinctive activity. The first possibility was rejected because of the immediate response to the injection and withdrawal of sex hormones at successive periods of treatment (e.g. Stone, 1939). As has been suggested above, there is every indication that the "neural mechanism is already laid down before the action of the hormone, and the latter is only an activator, increasing the

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excitability of a mechanism already present" (Lashley, 1938, p. 460). The second possibility was also rejected on the grounds of the specificity of the action of the hormones on behaviour patterns. The removal of the genital organs, referred to above, excludes the third possibility, although it must be recognised that the genital sensations contribute an important source of sexual stimulation (Beach, 1947), and that the external parts are essential for the possibility of the complete behaviour pattern. (Beach and Holz, 1946). Lashley therefore accepted the last possibility, and his conclusion is still generally accepted at the present time (e.g. Tinbergen, 1951, p.74).

Having determined the target organ of the sex hormones in the induction of sexual behaviour, the question of the interaction between the hormone level and sensory stimulation in this target organ, the central nervous system, must now be considered.

When comparing the effects of a high hormone level and a strong sensory stimulation on sexual behaviour, it appears from the literature that the characteristic effects of sex hormone injections can be produced by an increase in excitation through purely physical stimulation, leading to a rise in action-specific energy. Farris and Yeakel (1943) have reported that stimulation of male and female rats by

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sound from an air blast resulted in a significant increase in pregnancies. Stone (1924) reports that the rare occasions when adult male rats mount the normally inadequate stimulus object of another male rat, occur most frequently when strange males are introduced into a home cage of a group of males, or when several males are brought together again in the home cage following periods of prolonged copulation. On these occasions the males show a high degree of excitement. Two unusually excited males showed the normal feminine receptive behaviour when mounted by aggressive males. These two males showing the receptive behaviour normally showed unusual masculine sexual vigour and aggressiveness. Inverted sexual behaviour in male rats under conditions of great excitement has also been reported by Beach (e.g. Beach, 1944b). Beach (1938) has also reported masculine mating patterns in extremely excited virgin female rats. These effects, which are manifestly due to high levels of action specific energy due to external stimulation, are identical to the effects of high levels of the sex hormones upon behaviour. If the androgen level in the male is raised above the normal level, the sexual excitability is markedly increased, and the male reacts to normally inadequate stimuli (Beach, 1942e), to the extent of attempting to mate with male rats and even small female

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guinea pigs (Beach, 1942f). This suggests that the hormone has the same effect on the motor centres concerned as has intense physical stimulation, and that these qualitatively different sources of excitation act in the same quantitative manner on these centres. In this manner, sensory stimulation and the hormonal level appear to be complementary in the evocation of sexual behaviour.

A picture of how the action of external stimuli and hormones jointly affect the motor organisation in the same quantitative way has been developed by Beach along Sherringtonian lines. He postulates (Beach 1942e) the existence of a Central Excitatory Mechanism (CEM), which responds maximally to excitation arising from the particular pattern of multisensory peripheral stimuli which constitutes the mate, and which is also activated by the presence of the sex hormones in the blood stream. The CEM is thought to lie in the forebrain, and is connected efferently with the motor centres organising the mating patterns, as well as afferently with the peripheral receptors. The tonus of the CEM, which is proportional to the level of the sex hormones, is augmented by sensory impulses from several receptor systems, and exerts a facilitative effect on the executive circuits, which lowers the threshold of these circuits to the more direct stimuli from peripheral

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receptors. In this model, mating behaviour may be seen to occur in the absence of sex hormones when intense stimulation raises the excitatory level of the CEM, and such stimulation may also act directly on the executive motor circuits. On the other hand, normal amounts of androgen in the male maintain the excitatory level so that the animal tends to react to a normal receptive female. If the hormone level is raised, however, the sexual excitability is markedly increased, and the male reacts to normally inadequate stimuli. This same reaction to normally inadequate stimuli may also take place when the hormone level is normal, if sensory stimulation is intense enough to raise the excitatory level of the CEM above normal. Beach has produced much experimental evidence, of an outstandingly high quality, to support his postulate.

The removal of areas of the neopallium in rats lowers the occurrence of copulatory behaviour, and furthermore, the post-operative reduction in the proportion of the males which still copulate is proportional to the amount of cortex removed (Beach, 1940). What is more, Beach (1940) records the significant observation that those males that did copulate did so in a perfectly normal manner. This demonstrates that the organisation of the motor pattern itself was not affected by the injury, but that the peripheral sensory stimuli no longer had the excitatory

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effect, for Beach (1944a) has shown that these lesions did not seriously interfere with the reception of stimuli. Therefore, since the cortical lesions neither seriously affected the reception of the stimuli nor the ability to execute the motor acts of sexual behaviour, strong evidence for the existence of a cortical mechanism acting on lower centres by "distant facilitation, stimulating and increasing the activity in the executive centres" (Beach, 1944a) is available. Now this loss of sexual responsiveness due to the cortical lesions is entirely similar to the loss of responsiveness shown by castrates. In some elegant experiments Beach (1944a) did in fact inject his partially decerebrate animals with testosterone, and the injections brought about a return of responsiveness to a normal level. Moreover, the amount of testosterone required to bring back responsiveness varied proportionately with the extent of the brain injury. The effects of castration and cortical injury were additive and, unless the brain injury was very extensive, replacement injections of testosterone could still bring about a return of responsiveness. Here, then, is experimental evidence that testosterone acts synergically with this cortical mechanism. Beach (1942e) suggests that testosterone lowers the thresholds of the motor centres to peripheral stimulation in addition to increasing the

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excitability of the CEM.

Beach uses his model of the CEM in describing how one sex shows the mating patterns typical of the other sex (Beach, 1942e and 1948). This account is important for the present purposes, since it is the best illustration of how behaviour can be a function of both the internal and the external environment, in addition to bringing into order the complicated information about inverted sexual behaviour and the effect of heterologous sex hormones on behaviour. He proposes that each sex possesses the motor centres for both the male and the female pattern. In both sexes androgen and estrogen raise the level of excitation in the CEM. In addition, androgen lowers the threshold of the masculine motor centre to external stimulation, and estrogen lowers the threshold of the feminine motor centre. The thresholds to the motor centres are not the same, the threshold to the male motor centre being lower in the male, while the threshold to the female motor centre is lower in the female. Thus in a normal male, the CEM will activate the masculine motor centre when the stimulus of a receptive female raises the level of excitation above the threshold to that centre. Estrogen can also activate the masculine motor centre, only the peripheral excitation will have to be greater, because, since estrogen is held not to lower the threshold to the masculine motor centre, the threshold will

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be relatively higher. However, high doses of estrogen can lower the threshold to the female motor centre in the male to such an extent that the excitation discharges through this centre and produces typical female behaviour. The female mating pattern can also be released in a normal male if the stimulus situation does not provide for the release of a high level of excitement in the CEM through the execution of the normal masculine pattern. If stimuli normally eliciting the feminine pattern are brought to bear on such a highly excited male, such as mounting, then the excitation will be released through the execution of the feminine pattern (e.g. Beach, 1944b). The high level of excitement in the CEM, whether it is due to high hormone levels or to the character of the external stimuli, or both, will thus "spark over" to the female mating pattern if the entrance to the masculine motor centre is blocked by the stimulus situation. Beach (1938) emphasises that the mating behaviour displayed by rats of either sex is very greatly influenced, if not entirely determined, by the behaviour of the partner. He suggests (Beach 1942e) that the stimulus situation might account for the retention of sexual behaviour in male rats for some time after castration (e.g. Stone, 1927), in that the males were subjected to conditioning or learning during the sex tests occurring

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before and immediately after castration.

The organisation in the female is thought to be similar, although he considers (Beach, 1947) that in view of the fact that external stimuli tend to influence the female's responsiveness to sexual arousal to a much smaller extent than in the male, the nervous element in sexual arousal is less important in the female than in the male. Beach's model admirably serves its purpose of bringing order into the very complex information about the arousal and maintenance of sexual behaviour, both normal and inverted, and it shows many close parallels with the models which have been developed, apparently independently, in the Lorenz-Tinbergen school.

Beach is associated with the American school of Comparative Psychology, which works to a very great extent with the rat as the sole experimental animal, and information is drawn mostly from specific laboratory experiments. The Lorenz-Tinbergen school, on the other hand, is characterised by the method of obtaining information by observations on widely differing vertebrates ~~in the field~~, and most experiments carried out have direct relevance to phenomena observed in the field. In fact most of their experimental work is carried out in natural surroundings. The model put forward recently by Tinbergen (1951) is characteristic of the

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thinking of this school, and in this work he indicates the similarities with Beach's concepts. Tinbergen differentiates the causal factors controlling innate behaviour into internal and external kinds. The internal factors, which include hormonal effects, internal stimuli, intrinsic factors and many external stimuli, do not evoke the overt response, but determine the threshold of the response to the remaining external stimuli. The internal factors are therefore qualitatively different, but they act quantitatively with the external factors on the motor centres. Beach's CEM, in so far as it goes, essentially corresponds to this collecting pool. Tinbergen's model is considerably more elaborate and complex, but both authors recognise that the qualitatively different effects of hormones and external stimuli act in the same quantitative manner on the responsiveness of the executive motor centres responsible for the overt behaviour.

Returning now to the beginning of this Section, it was suggested there that it was reasonable to look for effects of the sex hormones upon the breeding behaviour of Bufo in spite of the evidence in the existing literature on this animal, since external sensory stimulation is not necessarily the sole controlling factor in the appearance of breeding behaviour, but rather external stimulation and

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the internal state of the animal, and in particular the sex hormone level, appear to be complementary factors in its appearance. The above summary of work done on the rat in this connection is given to support and illustrate this idea, and to give some indication as to how the internal and external environment may interact to produce such behaviour, since practically all of the critical work has been done on the rat. But, since this work is primarily concerned with the Anura, it is still necessary to give a comparative account of the effects of hormones on behaviour, in order to see if this situation is generally found in the vertebrates, or whether it is peculiar to the rat. A comparative account of the effects of hormones on behaviour is also necessary to support the other statement made at the beginning of this Section, namely that the development of the cortex in the vertebrate series seems to be inversely related to the indispensibility of the sex hormones in the appearance of sexual behaviour. If this is the case, then a strong reaction to hormonal treatment may be expected in Bufo. These two questions may be dealt with together, and only a brief comparative survey of the literature on the effects of hormones on vertebrate behaviour follows, since Beach (1948) has recently published an extensive review of the literature concerned with the problem.

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Turning from the rat to the primates, where the neopallium is enormously enlarged, it is evident that the nervous element in the control of sexual behaviour has also increased enormously in this group, to the extent that "some evolutionary shift in the physiological control of such behaviour is clearly indicated" (Beach, 1947, p.303). In the case of man, there are great differences of opinion as to the extent of the control that the hormones exert over behaviour (e.g. Beach, 1948), which indicates that the effect is hardly recognisable. Howard and Vest (1939) have recorded a case in the male where libido and potentia were increased with equal effectiveness either by injections of testosterone or by injections of oil coupled with the appropriate suggestion. In the female, interesting work has been done by Benedek and Rubenstein (1939), where psychoanalytic records showed that during the menstrual cycle the presence of estrogen corresponds to the presence of active heterosexual libido, and that the presence of progesterone corresponds to a passive, receptive tendency. A selective advantage may be seen in this phenomenon, in that before the time of ovulation, when the estrogen level is rising, the female becomes sexually aggressive; and when ovulation has occurred and the progesterone level rises, she becomes predominantly receptive. Nevertheless, the

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effects of hormonal injection on the sexual behaviour of women are as unpredictable as they are in men, and it is evident that in Homo the size of the cortex produces a very different state of balance between hormones and the nervous element compared with the rat.

In birds, the opposite situation is shown, for in this group the effects of hormones on mating appear to be more striking and complete than in mammals. This is in keeping with their more stereotyped behaviour responses, a characteristic, attributable to the small size of the neopallium, that has made birds the main field of research in the Lorenz-Tinbergen school. The effect of hormones on the behaviour of birds has been reviewed by Beach (1948) and Bullough (1945), but there are some important points that are relevant to this work, which have not been fully discussed in these reviews.

Noble and Wurm (1940) have found that both sexes of the Black-crowned Night Heron display the same courtship behaviour during mating, with the exception that the male has two additional ceremonies. The injection of testosterone into immature males induces the complete pattern, including the two typically masculine ceremonies. The injection of testosterone into immature females has precisely the same effect. In one-month-old chicks injected with testosterone, the voice became more guttural, as in mating adults, and

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territorial defence, nest-building, all the male ceremonies, copulation and brooding, were induced. The effect of the hormone was remarkable for its strength and completeness. Estrogen failed to stimulate breeding behaviour in either sex, or to affect the secondary sexual markings. The authors presume that both sexes react to low levels of the male sex hormone at the beginning of the breeding season, when both sexes behave in the same way, and that only later do increasing amounts of androgen in the male shift the male's behaviour pattern in such a way that pairs can form and breeding follow. This report is particularly interesting in that it gives an example of an animal in which the breeding behaviour of both sexes is potentially the same, and in which the actual difference in behaviour between the two sexes is due only to a different quantity of a single hormone. The quantity of this hormone determines the completeness of the appearance of the full behaviour pattern, but this is a characteristic effect of hormones on behaviour (see for example Beach and Holz, 1946; Berg, 1944). The same situation also seems to be true of the Penguin, since Roberts (1940) reports that at the beginning of the breeding season, the behaviour of both sexes approximates that of the male alone later on in the season, and that copulation depends on what Roberts calls the "relative masculinity"

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in pairs, and not on male and female behaviour per se. According to Roberts, this method of pairing can lead to the formation of homosexual pairs.

At first sight this type of breeding behaviour, and its hormonal control, might seem totally different to that typically found in the rat and other mammals. A consideration of the reports of inverted sexual behaviour, and the effects of heterologous sex hormones, however, suggests that the behaviour of these birds is only the specialisation of one particular response shown in the rat. The report of Ball (1940) described earlier gives an account of this very phenomenon in female rats, where continued injections of testosterone brings on the complete masculine behaviour. Specialisation of this response in the female, so as to exclude the other forms of response, would produce the condition described by Noble and Wurm, and Roberts.

The absence of any effect of estrogen does not appear to be typical of all birds, however, although this effect has been studied in very few birds. In the domestic fowl, estrogen induces squatting in the female (Davis and Domm, 1941). Estrogen also induced squatting, together with masculine behaviour, in capons. Injections of androgen also induced masculine mating behaviour, and the reaction was more complete than in estrogen-induced masculine

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behaviour. In these respects the hormonal induction of mating behaviour in the fowl closely resembles the induction of this behaviour in the rat, although in the fowl this induction is much more strongly shown. Estrogen also tends to lower aggressiveness, and consequently the social status, of treated female birds in a flock (Allee and Collias, 1940). The injection of testosterone leads to an increase in aggressiveness, and consequently a rise in social status in hens (Allee, Collias and Lutherman, 1939), and it also induces masculine behaviour (Domm, Davis and Blivaiss, 1942). Further, estrogen induces squatting in immature female chicks (Noble and Zitrin, 1942), and the complete adult masculine behaviour was induced in male chicks at their tenth day of life (Hamilton, 1938). As in rats, therefore, the sex hormones can bring about the release of adult sexual behaviour before the behaviour is appropriate in terms of the animal's morphological development. This is particularly impressively shown by the fact that Hamilton's chicks began striking at objects in the same manner as an adult strikes at objects with its spurs, although at this early stage of development the spurs had not yet grown. This behaviour furnishes a good illustration of Lorenz's doctrine that "behaviour patterns are not something which animals may do or not do, or do in different ways, according to the requirements of the occasion, but something which

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animals of a given species 'have got', exactly in the same manner as they 'have got' claws or teeth of a definite morphological structure" (Lorenz, 1950, p.238). The phenomenon is all the more remarkable because the organ and the behaviour pattern employing it are under the control of the same hormone.

Work done on prolactin in birds gives an example of behaviour which is under fairly direct genetic control through the genetic control of hormone production, and so shows how the effect of hormones on behaviour may become a factor in microevolutionary changes which result in nice adjustments of a population towards its greatest state of biological efficiency - an aspect of the internal control of behaviour that is normally overlooked in the literature. Broodiness appears to be controlled both by sex-linked and autosomal genes, and the intensity of broodiness is proportional to the number of genes present (Roberts and Card, 1933). The fewer genes for broodiness that are present, the greater is the quantity of prolactin required to induce the brooding behaviour (Nalbandov and Card, 1945), and these authors presume that these genes augment the production of prolactin. If this is the case, then the intensity of brooding behaviour is shown to be under fairly direct genetic control, and consequently the intensity of

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the brooding behaviour will be readily open to changes on a microevolutionary scale.

The effect of prolactin on the behaviour of male cocks is so remarkable that it warrants further attention. It illustrates the phenomenon of a complete reversal in behaviour due to the alteration in the level of a single hormone, given the appropriate external conditions. It should first of all be noted how important the external conditions are in the evocation of the brooding behaviour. Nalbandov (1945) has concluded, from experiments on cocks, that prolactin does not bring about brooding behaviour by a direct action on the central nervous system, but that it affects behaviour through its anti-gonad action, an action of prolactin that has also been found by Riddle, Hollander, Lahr, Smith and Marvin (1941-1942) to be present in rats. According to Nalbandov (1945), broodiness in cocks results from an interaction of i) the absence of androgen in the internal environment, and ii) the presence of a certain group of external stimuli, namely a warm, dark nest. This conclusion is borne out by Goodale's observation that capons show the brooding behaviour in warm, dark, closed places (Goodale, 1916). Here there appears to be a reversal of the complementary effect of hormones and nervous stimulation on behaviour, as it is found in the rat; the hormone level

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and the sensory stimuli appear to be antagonistic in the induction of brooding behaviour. Returning to the reversal in behaviour, this is best illustrated by the work of Nalbandov (1945) on cocks. He measured the broody response of cocks to chicks by giving each animal five chicks each day in warm, dark nests, and he recorded how many of these chicks were either killed or adopted. He found that with one injection of prolactin per day per animal, the general reaction of his cocks was as follows:-

	No. of chicks killed	No. of chicks adopted
Day of 1st injection	5	0
Day of 2nd injection	5	0
Day of 3rd injection	3	2
Day of 4th injection	1	4
Day of 5th injection	0	5

These results demonstrate a complete reversal of the behaviour of the cocks towards the chicks given to them upon the injection of prolactin. A more precise interpretation of these results would be that differences in the level of androgen brought about a complete reversal of the behaviour of the cock towards the same external object, namely the chick.

Reversals in the behaviour towards the same object ~~also appear~~ in the females of many mammalian species

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(Hartman, 1945) along with changes in the hormone levels during the estrous cycle. Such females are indifferent or even antagonistic towards males, and may even attack and kill them, at times other than when the estrogen level is high (Hartman, 1945). During the period when the estrogen level is high, the female's social behaviour completely reverses, and she becomes co-operative and receptive when in the presence of males. Koster (1943) points out this change in the rat, and remarks that when the estrogen level is raised, "a different set of stimulus-response mechanisms is now functioning in the female". It appears that the reversal of behaviour towards the same object in this case is due to a change in the estrogen level.

This phenomenon of the reversal in an animal's behaviour towards the same object, when brought about by different levels in a single hormone, is important, because it suggests that not all the effects of the sex hormones on the nervous system can be accounted for simply in terms of a raising or lowering of excitation in a single CEM, such as appears to be the case in the changes in sexual responsiveness as described by Beach. For, as Koster points out, the effect of hormones in reversing behaviour is due to different stimulus-response mechanisms being brought into play, or, in other words, a different set of internal releasing mechanisms starts reacting to the same stimulus animal, and

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consequently a different behaviour pattern, excited by different CEM's, is being released. It does not seem possible to regard the complete reversal in behaviour, as described above, as representing the minimum and maximum end-points of a quantitative range of action-specific energy from one CEM; the behaviour is qualitatively different. In Beach's model, no modification in the number of CEM's is necessary to accommodate the phenomenon of inverted sexual behaviour, since this behaviour occurs in conditions of great sexual excitement, and appears to be an instance of a true displacement activity (c.f. Morris, 1952), in which the same CEM is involved. A quotation from Beach (1942e, p.193), makes this apparent. "If . . . the maximally aroused male is mounted and palpated by a vigorous copulator of his own sex he may exhibit the feminine mating response. In the absence of some other animal to be used as a female, the first male cannot employ the masculine pattern. At the same time there is a strong tendency for the CEM to discharge into some efferent channel. If discharge is to occur it must pass into other available neural circuits, - those in which the feminine motor pattern is organised. Therefore when excitability is high and the appropriate stimuli are applied, such a discharge takes place, and in many cases is strong enough to overcome the

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high threshold in the neuromotor mechanisms mediating hopping, crouching, lordosis and ear vibration." Such "spark-over" behaviour is termed a "reversal" in sexual behaviour by the American workers (e.g. Beach, 1938), but it is an entirely different phenomenon to the reversal in behaviour shown by prolactin-treated cocks and the estrous and anestrus female mammal, as described above. For in these cases there is no indication of there being any displacement activities involved in the reversal. This distinction appears to be important when the effects of hormones on behaviour are being considered, since it points to at least two different ways in which the sex hormones can affect behaviour. For it appears that the effects of hormones on behaviour are not due solely to their seeming ability to alter levels of excitation; they also appear to be able to alter the reactivity of the sets of internal releasing mechanisms which control the flow of impulses from different excitatory mechanisms.

In leaving this section on the effects of hormones on bird behaviour, it may be concluded that such effects in those birds that have been studied show no important difference from these effects in mammals. In general, however, birds show a clearer and more complete response to hormonal treatment than do mammals.

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Work on the remaining vertebrate classes on the effects of hormones on behaviour is sufficient to show that essentially the same mechanisms are at work in the lower vertebrates as in the higher vertebrates. The literature connected with this field has recently been reviewed by Beach (1948). Much more work, particularly systematic work, is badly needed in the lower vertebrates on the effects which hormones have on behaviour in the normal life of these animals in the field. In the reptiles, valuable work has been done by Noble and Greenberg (e.g. Greenberg and Noble, 1944) and Evans (e.g. Evans, 1936) on Anolis, where the behaviour associated with territory and mating has been studied. Noble and Greenberg (1941) report that the implantation of pellets of testosterone propionate into immature males of this species induces both male and female sexual behaviour, a situation found in both mammals and birds. Regarding fish, Noble and Kumpf (1936-1937) have shown that spayed females of Betta splendens and Hemichromis bimaculatis show no sexual behaviour, but that injection of ovarian extracts brings about a return of this behaviour. Castrated males of this species did not, however, show a reduction in mating behaviour. The nuptial coloration and the genital tube appeared at each spawning in these castrates, and it is not impossible that the adrenals were replacing the testis hormone. Suggestions that the

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adrenals play a part in the appearance of sexual behaviour are not uncommon in the literature (e.g. Ring, 1945), but the rôle of the adrenal hormones in the endocrinology of behaviour is, as it is in general endocrinological phenomena, largely undetermined. Injections of carp pituitary extracts induce spawning in goldfish outside the breeding season (Hasler and Meyer, 1942).

In the amphibia, Noble and Aronson (1942) have found that injections of anterior pituitary extracts into Rana pipiens induced the normal sexual behaviour, except calling, at any time of the year. Calling could only be induced in the breeding season. Mating behaviour was induced in male and female Xenopus by injections of testosterone and progesterone (Shapiro and Zwarenstein, 1937). Of particular interest in connection with this present work is Chadwick's investigations into the water drive of Triturus. The life cycle of this animal is composed of three stages, namely i) an aquatic larval stage, lasting about 3 months; ii) an immature land stage, lasting for two and a half to four years; iii) an aquatic adult stage (Reinke and Chadwick, 1939). Chadwick (1940) showed that if the immature land animals were injected with prolactin, they sought and entered water 5 to 10 days after the injection. Controls of the same age would leave water if put in it. The drive could be induced in gonadectomised animals. Blair (1946)

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injected prolactin in Bufo, but the only effects of this hormone which he reported were an increased amount of throat skin, associated with the vocal sac, and an increase in the number of melanophores (which remained highly contracted) in both sexes. In Triturus, Chadwick (1940) has reported profound changes in the external morphology and colour resulting from the injection of prolactin, in addition to its effect on the water drive, so it would appear from Blair's work that its effect in Bufo is very limited on the morphological level, and absent as far as the water drive is concerned. Unfortunately, it is not stated in Blair's paper how the water drive was measured. It should be noted, however, that the water drive as described by Chadwick for Triturus is not the same phenomenon as the water drive connected with the spring migration of Bufo. The appearance of the water drive in Triturus marks a definite ontogenetic stage in the life history of this species, in that the land-living stage of the immature animal gives way to the aquatic adult stage. On the other hand, the reproductive water drive of Bufo is a transitory seasonal phenomenon of the breeding adult. Thus the apparent absence of an effect of prolactin on the water drive in Bufo, as reported by Blair, does not contradict Chadwick's work on Triturus. It is interesting to note

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that the implantation of Bufo anterior pituitaries into the land stage of Triturus induced the water drive and the assumption of the adult features in these animals (Chadwick, 1941). Bufo evidently secretes prolactin, but this hormone seems to be quite without the function that it has in Triturus. The response of the efts of Triturus to prolactin is remarkable for its completeness.

Even though the small amount of information available on the effects of hormones on the behaviour of Bufo is largely negative, the above review of the effects of hormones on behaviour in other vertebrate animals strongly indicates that it is worth while conducting further investigations into the effects of hormones on the behaviour of Bufo. It is evident that the response of breeding behaviour to the sex hormones is characteristic of the vertebrates in general, and that such effects should be discoverable in Bufo, however important the effects of the external environment may be.

With these considerations in mind, the following investigation into the effects of hormones on the reproductive water drive of Bufo were carried out.

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MATERIAL

Bufo carens Smith was the species chosen for the investigations into the water drive. Members of this species are to be found in considerable numbers at almost any distance from water in certain localities in Natal, during nights when the temperature is above about 18°C., regardless of the relative humidity of the air, and the presence or absence of surface water from rains. These animals are just as common on a warm night before the spring rains have started to fall as at any other time. The majority of animals collected away from streams and ponds are females, while the majority of males are to be found calling in patches of water. This unequal distribution of the two sexes appears to be a characteristic condition both in B. carens and B. regularis. During the daytime, these animals usually conceal themselves under stones and banks, although breeding activity does take place in water at this time. B. carens was chosen for the study of the water drive because the recourse of this species to water apparently takes place primarily for the purposes of breeding, since, judging by their distribution, the toads seem able to survive on surface water provided by rains and

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dew alone. Therefore, if the water drive that appears to be connected with breeding is under some hormonal control, the effect of the hormones would be well marked in this genus.

All the animals were kept for at least a week before experiments on them were commenced, in the constant temperature laboratory in which the experiments were conducted. This was thought to be necessary because the behaviour of frogs and toads undergoes conspicuous changes when these animals are brought into the laboratory, in that sexual behaviour is practically eliminated. Noble and Aronson (1942) have found that injections of anterior pituitary extracts bring this behaviour back to normal intensities in laboratory stocks of Rana pipiens. It is clear that a change in some sensory and/or physiological factors controlling behaviour takes place under laboratory conditions, and consequently a week was allowed for acclimatisation. The temperature of the laboratory was set at 24°C. This temperature was chosen because toads emerge in greatest numbers in the field when night temperatures reach about this level, and so it was presumed to be the best temperature for measuring these animals' activity. The laboratory was illuminated by a fluorescent tube, which was left on permanently. The tops of the vivaria were made of glass, so that the animals were constantly lighted.

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Each individual was given a piece of meat of a size that it could just swallow once a week, and the swarming forms of termites were used to supplement the diet whenever possible. The animals seemed to be in perfectly good condition during their period of captivity, and no deaths occurred in the stock.

APPARATUS AND METHODS

The water drive of an animal can be expressed quantitatively as the time spent in water during a unit period. In this present investigation, a distinction has to be made between the time spent by a toad in water in order to meet the purely physiological need for water, and the time spent in water, over and above the time required to meet the physiological need, during the breeding season. The water drive manifested to meet the physiological need will here be termed the "normal water drive", and the additional drive associated with breeding will be termed the "reproductive water drive". The term "drive" is admittedly unsatisfactory, but it has a general usage in the literature.

The normal water drive of Bufo was measured by the proportion of a fixed time that was spent by control animals in water, and the reproductive water drive would therefore be shown as an increase in the time spent in water above the control time. The fixed time was one or two weeks. The apparatus used to measure the time spent in water consisted essentially of a dish of water, set up so that an electric circuit including a kymograph signal-marker was closed when a toad occupied this dish, and broken when the

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toad was not in the dish. The resulting kymograph trace would thus show the time spent in water.

The dish was a 2 lb. jam tin, whose sides were cut off about 5 cms. from the base. The interior of the dish was painted with duco in order to prevent any reaction between the metal and the water from taking place. This dish was placed on one end of the beam of a balance (Fig.1; see also Fig. 3) which was built out of Meccano parts. The beam (B), 32.5 cms. long, was pivoted on a rod (P). The other end of the beam was counter-balanced by a tension spring (S), which was attached to the base of the whole structure. When an empty dish was placed in position, the spring was fully contracted, and the beam lay about 9° off the horizontal. At the dish-bearing end of the beam, a vertical rod (R) was fixed so that when the beam was horizontal it touched a metal plate (I), which was secured to the base of the balance, but insulated from it. Wires were fitted to the rod and to the insulated plate, and connected through a kymograph signal-marker to a two-volt accumulator. When the dish was weighed down so as to bring the beam into a horizontal position, the circuit through the rod and the insulated plate was closed, and this caused a change in the position of the signal-marker. This change was recorded on a kymograph drum. The dish was inserted through a hole in the floor of a cage, whose area was about 26 cms. x 20 cms.,

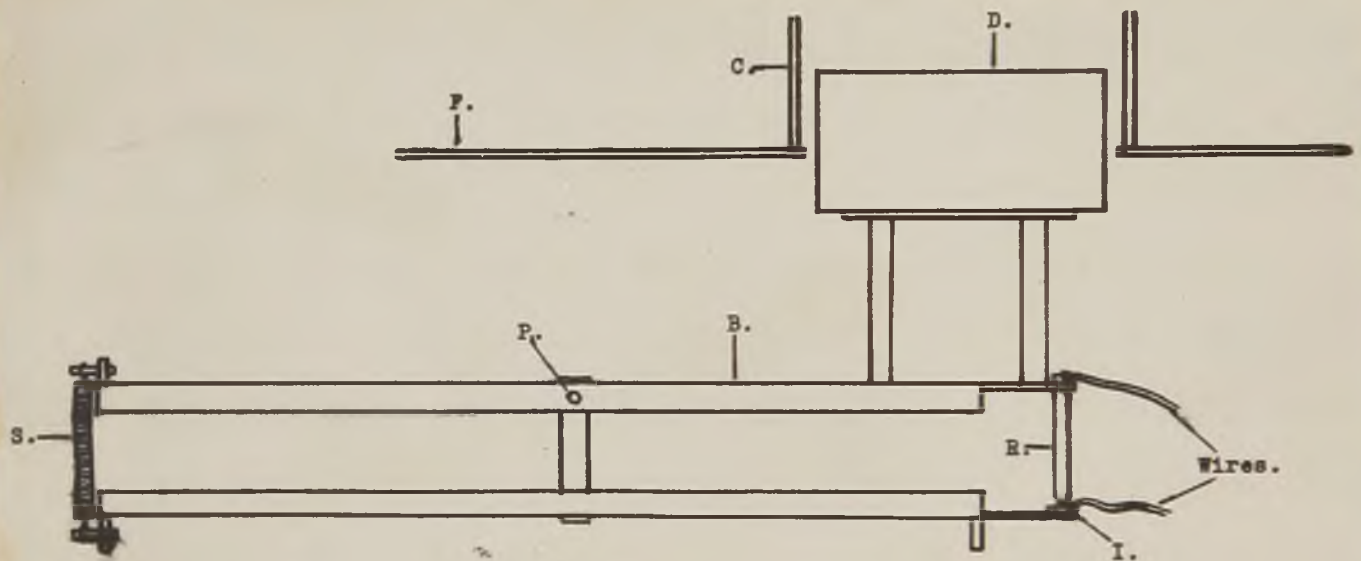


Fig. 1. Balance for measuring the water drive of Bufo oarens.

- | | |
|-----------------------|--------------------|
| B. Beam | I. Insulated Plate |
| C. Protecting Barrier | P. Pivoting Rod |
| D. Dish | R. Contact Rod |
| F. Floor of cage | S. Tension Spring. |

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with a height of about 19 cms. The top of the dish projected about 2.5 cms. above the level of the floor. A space of about 0.5 cm. lay between the dish and the encircling cut edge of the floor, in order to allow the dish to move freely up and down through the floor. Only one toad occupied the cage during experiments. When the apparatus was set up for an experiment, a weight approximating the toad's weight minus 10 grams was placed on the dish end of the beam, and the dish was put on the balance in a position so that it moved freely through the hole in floor of the cage. Water was then poured into the dish until contact was made by the two terminals. The ~~10 g.~~ weight was then removed and this parted the terminals again. If the toad now entered the water, its weight, being 10 g. greater than the weight required to bring the terminals together, would close the circuit, and the extra 10 g. would constitute a factor of safety against the toad splashing water out, and against any loss of water due to evaporation.

It was found necessary to place a protecting barrier around the dish, since the toad, while moving about the cage, often knocked the dish and this movement sometimes resulted in a mark on the kymograph trace, although while making the mark the toad had not entered the water at all. A ring of perforated zinc sheeting, 5 cms. high, was

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therefore attached to the floor of the cage above the edge of the hole in the floor (Fig.1 (C)). Wood could not be used as the flooring of the cage, since water splashing out of the dish made it warp. The floor was therefore also made of perforated zinc, and the protecting ring was soldered on to it. With this arrangement the toad was able to get in and out of the dish without any difficulty, but the dish was protected against the general activity of the toad. The sides of the cage were made of wood, and the top was covered by a plate of glass. Four such cages and balances were used in obtaining information about the water drive.

A time-marker recorded 12-hour periods on every kymograph trace. An alarm clock was used as the time keeper, and the alarm mechanism was altered so that instead of the alarm hammer being set off at the required time, a circuit leading through a signal marker was closed for about half an hour, so that a mark was made on the trace. The marks were made at 6 a.m. and 6 p.m. The four signal-markers and the time-marker recorded simultaneously on the same trace, and the stylet points were placed as nearly as possible in a vertical straight line. An excerpt from a kymograph trace is shown in Fig. 2. Frontal writing levers were used to draw the traces, as a precaution against minor displacements and accidents, which might occur during prolonged experiments. A photograph of the complete

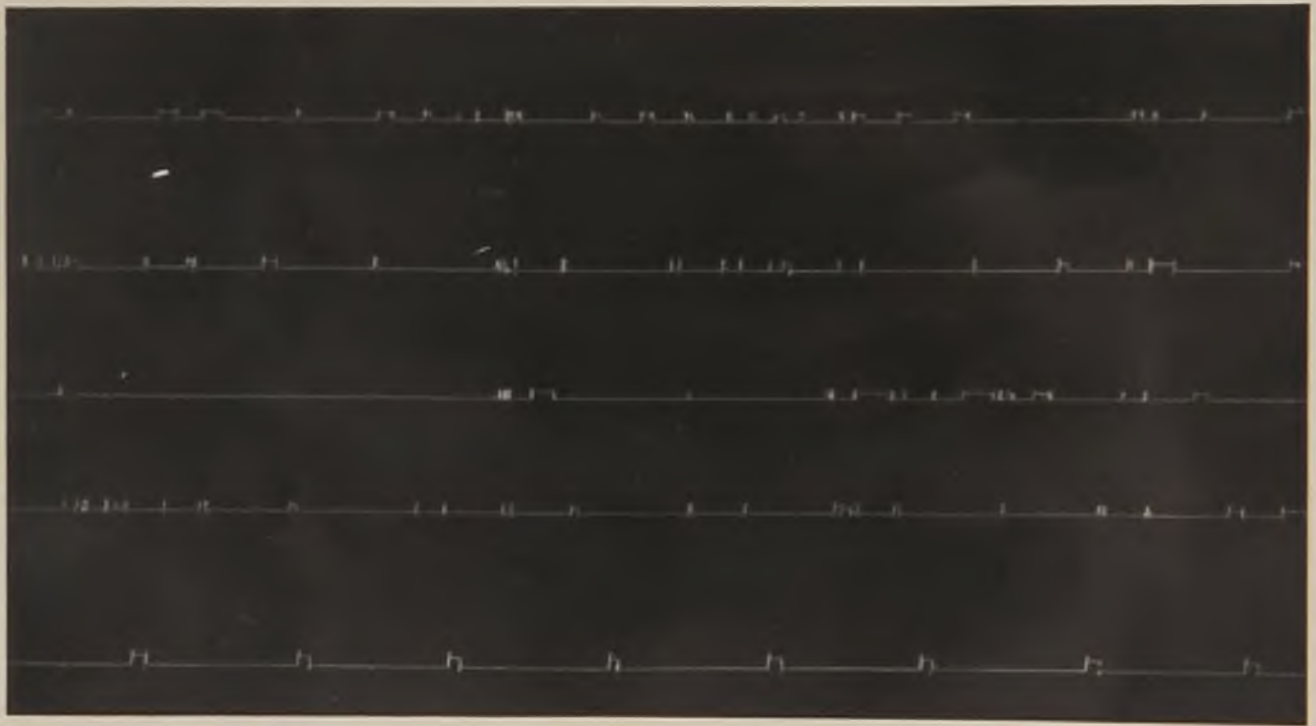


Fig. 2. Excerpt from a kymograph trace of control Bufo carens.
The lowermost line is marked at 12-hour intervals.

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apparatus is shown in Fig. 3, although here only two balances and cages are shown.

Water was changed every 24 hours during the course of an experiment, in order to prevent the water from becoming fouled. Each dish was refilled so that, as described above, with the load of the weight of the toad minus 10 g., the contacts of the balance just met.

A record of the relative humidity (R.H.) of the laboratory was made by hanging an Edney hygrometer next to the cages. There was no means of controlling the R.H. of the laboratory, and consequently the rate of evaporation from the toads was not controlled.

The time spent in water by the toads was calculated in the following manner. In Fig. 2 it may be seen that the final traces consisted of vertical deflections of each pointer for variable lengths along the line for each toad. All deflections along the line of each individual toad for a length greater than 1 mm. were measured and summed. Each individual total was then expressed in units of time (hours). An average was then taken of the time spent in water by all the toads of one series of treatment, and the standard errors calculated. The result of each series was therefore expressed as the mean time spent in water by all the animals of that series. The total number of deflections of less

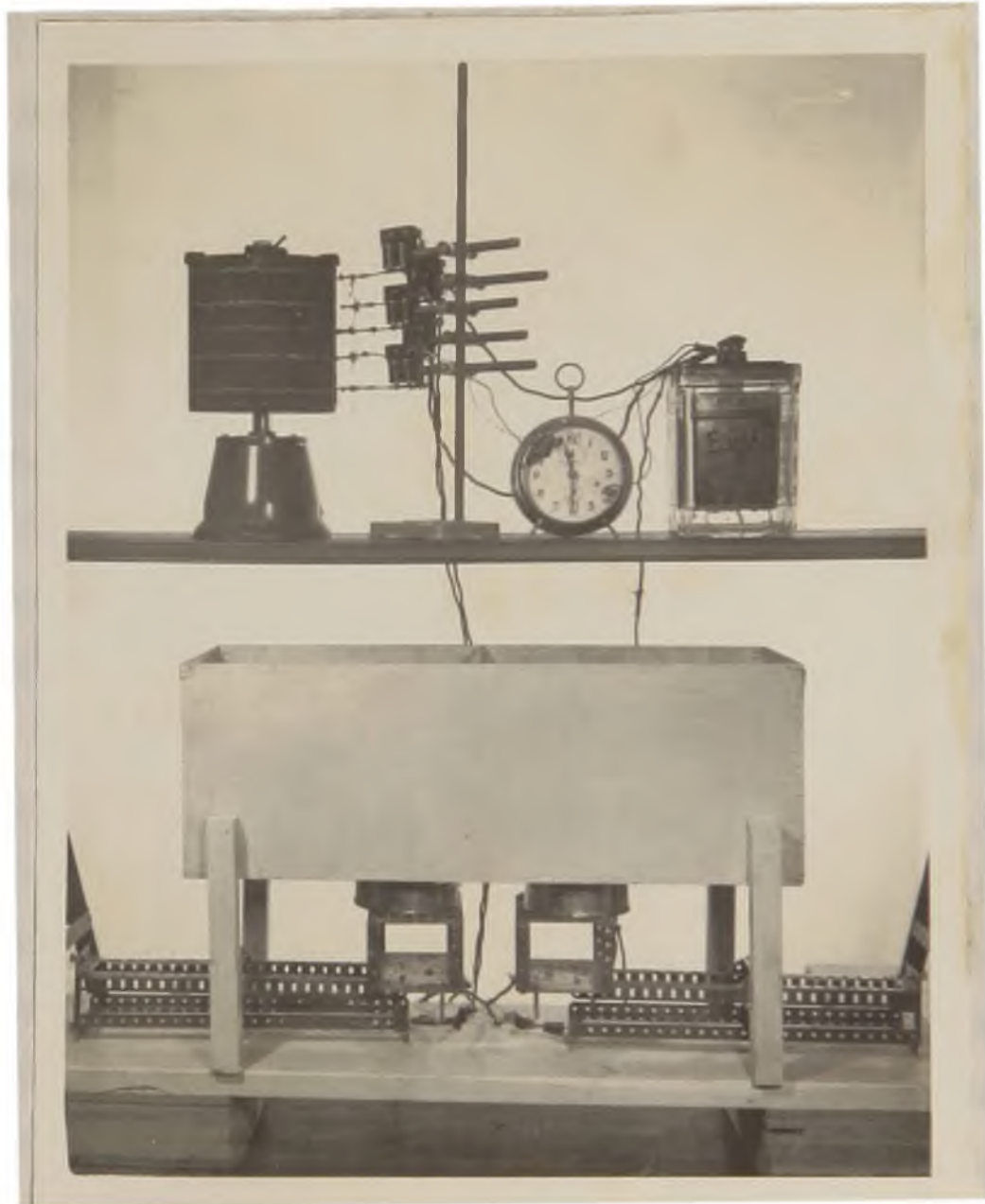


Fig. 3. Apparatus used in measuring the water drive of Bufo carens. (For explanation, see text.)

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than 1 mm. for each toad proved to be so enormously variable that these totals were not included in expressing the water drive.

As to the experimental procedure, control records of the water drive were first of all taken. Two controls were used; one series in which the animals were not handled at all, and another series in which the animals were given daily injections of mammalian Ringer. Each of the two control series consisted of two week-long records, and a different set of four toads was used for each week's record. The volume of Ringer given was equal to the volume of pituitrin that was to be injected in the first series of experiments, namely 1.5 ccs./100g. body weight/day.

The effects of the posterior pituitary hormones on the water drive were then investigated, before work was commenced on the hormones associated with sex, since it has been shown by Ewer (1952) that B. carens shows an increased water uptake, together with an anti-diuretic response, upon the injection of pitressin. It was therefore of interest to see what effect the posterior pituitary hormones had upon the time spent in water. Two week-long records were made, with each toad receiving 15 i.u./100g. body weight/day of Pituitrin (Parke Davis). This is a heavy dosage (c.f. Ewer, 1949).

The water drive of four male toads, injected with testosterone propionate (Oreton, Scherag), was then studied

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for two successive weeks. The dosage was 0.2ccs./100g. body weight, injected every three days. Blair (1946) showed that this dosage was sufficient to bring on the precocious development of various morphological secondary sexual characters in juvenile male and female B. fowleri, and so it was considered that this dosage would be sufficient to bring out any changes in the toads' water drive. A control series of four female toads were given the same dosage during a week's recording. Following this, four females were injected with chorionic gonadotropin (Antuitrin, Parke Davis) for a week. The dosage was 218 i.u./100g. body weight, injected once every three days. Blair (1946) showed that this dosage also brought on the precocious development of various morphological secondary sexual characters in juvenile male B. fowleri.

All the work done up to this stage had been carried out over the winter with animals collected in the autumn of 1953. At this stage, which was in the spring of 1953, fresh stocks were brought in from the field. Since these animals were collected in the spring, they would be expected to have a different hormone balance from the animals collected in the autumn, and so it was considered necessary to use some of these new animals as controls, and to compare the results of these controls with those of earlier controls.

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Consequently the gonadotropin experiments were continued by a record, lasting for two weeks, of two injected animals and two non-injected animals taken simultaneously. Each group consisted of a male and a female.

A two-week record was finally taken of the effect of estrogen upon four spring females. The water drive of these animals was recorded for a week before injections commenced as an additional control, and then each was injected with 1,900 i.u./100g. body weight (= 380 r.u./100g.) every three days. Blair (1946) injected juvenile B.fowleri with about twice this dosage, but as all his injected animals died within a month, it was considered that his dosage was not physiological.

A check was made of the normal level of the water drive in the animals injected with testosterone, and those females used in the first gonadotropin series, by leaving them untreated on their first day in the cages, and recording the time spent in water.

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RESULTS

The most striking feature of the water drive records is the great toad-to-toad variation. In view of the very small sampling taken in this work, these results can therefore only be regarded as a general indication of the effects that the hormones had on the water drive. The results are set out in Table 1. The number (N) of animals used for each treatment is given, with the mean time spent in water by the toads of each recorded period, and the standard error (S.E.) of the mean. The length of each record was a week in each case, but some treatments lasted two weeks.

Table 1. The effect of various hormones on the water drive of B. carens. (Mean number of hours spent in water)

<u>Treatment</u>	<u>N</u>	<u>Mean No. of hours</u>	<u>S.E.</u>	<u>Length of Record</u>
1. Unhandled (Autumn group)	8	21.35	2.71	1 week
2. Injections of Ringer	8	21.46	5.02	"
3. " " Pituitrin	8	19.67	4.31	"
4. " " Testosterone (Males)	4	18.95	5.50	"
" " " (2nd week)	4	12.6	1.95	"
5. " " " (Females)	4	27.03	5.30	"
6. " " Gonadotropin (Females)	4	40.37	17.30	"
7. " " " (Male & female)	2	12.47		"
" " " (2nd week)	2	21.70		"
8. " " Estrogen (Females)	4	29.39	9.18	"
" " " (2nd week)	4	20.99	5.14	"

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The standard errors of the second gonadotropin treatment, and the later control groups are not given in this Table, but will be dealt with separately.

The figures in the Mean No. of Hours column in Table 1 show considerable variations, but the standard errors are very large. The most conspicuous inconformity in the means is that of the females treated with gonadotropin (Treatment 6). The pre-injection mean, however, shows that these animals possessed an abnormally high water drive (the pre-injection day mean was 9.03 hours compared with the daily mean during treatment of 5.77 hours), and consequently the high mean in the table does not necessarily indicate an effect of gonadotropin on the water drive.

With regard to the testosterone results with males (Table 1, Treatment 4), a difference is shown between the first and second weeks of treatment. But if the difference of these means is tested for significance with Fisher's t test, P comes to about 0.6; and so the difference is not significant. If the score for the second week is tested for significance with the unhandled controls, using Fisher's t test, P comes to about 0.2, which again is not a significant difference. The increase in the mean of the females treated with testosterone (Table 1, Treatment 5) over the control mean cannot be considered to be due to the effects of the hormonal treatment, since the mean of these

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females on the pre-injection day was abnormally high, and did not differ from the daily mean during injections.

Only two animals were injected in the second gonadotropin series (Table 1, Treatment 7), and so the standard error was not taken. The results are shown individually in Table 2, and they are here compared with the results of the two control animals (see Methods).

Table 2. A comparison on the water drive of toads injected with gonadotropin with that of controls.

<u>Toad No.</u>	<u>Sex</u>	<u>Mean time spent in water (hours)</u>	
		<u>First week</u>	<u>Second week</u>
C38	Male } Control	22.16	34.99
C39	Female }	7.20	7.55
C40	Male } Experimental	16.62	24.00
C41	Female }	8.31	19.39

The mean for the first and second week scores respectively were:

	<u>First week</u>	<u>Second week</u>	
Controls - - -	14.68	21.27	hours
Experimental -	12.47	21.70	"

Since the controls spent more time in water during the second week, as well as the injected animals, the gonadotropic hormone could not be said to be responsible for the increase in the time spent in water during the second week of

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injections. Changes in the R.H. of the laboratory were not noted during these recordings, and the increase in the water drive seems to be inexplicable. The difference between the male and the female means may be noted. This difference will be discussed later.

As was stated in the Methods, the water drive of the estrogen-treated animals was measured for a week before the injections were commenced, in order to obtain a control record for the spring level of the drive. The mean time spent in water for this period was 34.71 hours, with a standard error of 1.93. If the difference of this mean is tested for significance with the mean of the original unhandled controls (Table 1), using Fisher's t test, P comes to about 0.4, and so the water drive of these two controls are not significantly different. Therefore there appears to be no significant difference between the water drive of toads collected in autumn and spring. This conclusion is supported by an absence of a conspicuous difference between the mean of the toads collected in spring, which were used in the gonadotropin experiments (Table 2), and the mean of the toads collected in autumn for the original control records. The water drive of the original unhandled controls was measured during August, when the R.H. recorded in the laboratory was $37\% \pm 10\%$ R.H., and the majority of the unhandled controls from the spring collection were recorded

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during January, when the R.H. of the laboratory was 60% \pm 9% R.H. Since the means of the two control groups did not differ significantly, the seasonal changes in the R.H. of the laboratory may be said to be excluded as an important source of error. But this matter is more complicated, and it will be dealt with in the following discussion. It can be seen from Table 1 that when the spring group of four controls were injected with estrogen (Treatment 8), the mean time that they spent in water became increasingly closer to the autumn control mean.

The difference in the means shown in Table 1 cannot, then, be said to demonstrate that any of the hormones used had a significant effect on the water drive of Bufo carens. The differences are merely the reflection of the fact that a very small number of animals were used to measure a very variable character. These results will be considered further in the next Section.

It was intended to keep the two sexes separate in the first two control series, in order to see if there were any differences in the water drive of the two sexes. It was thought that the sexes could be distinguished by the colour of the throat, as they can be distinguished in B. regularis. The animals were therefore segregated according to this character for the control and pituitrin records. The animals receiving testosterone were autopsied after the

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experiments in order to see what the effect of the hormone had been on the gross anatomy, and it was found that the coloration of the throat is completely unreliable as a sex-distinguishing character in B. carens. Consequently the first two control series and the pituitrin series are not sexed. The sexes were subsequently distinguished, with a high degree of success, by the girth and general appearance, and it was possible to distinguish the spring animals immediately at sight (see Discussion).

Unfortunately, prolactin was not obtainable during the course of the present work, and so it was not possible to reinvestigate Blair's work with this hormone (see Introduction).

The following effects of the hormonal treatment on the gross anatomy were noted. The males injected with testosterone were found to have very warty, reduced testes. This effect is in agreement with Blair's results (Blair, 1946), and it shows that this hormone was injected in physiologically effective doses. These males were also found to have a greenish tinge to their skins, a character which is found in the male during the breeding season (see Discussion). Most of the females injected with testosterone appeared to be normal, but two animals showed slight haemorrhage in the limb muscles, and their livers were slightly granular. Haemorrhage following this treatment

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was reported by Blair. The gross anatomy of all the animals receiving chorionic gonadotropin injections appeared normal. The females injected with estrogen showed varying degrees of haemorrhage in the limb muscles, and two animals had many small cysts in their livers. These effects of the injection of estrogen were not as serious as the effects that Blair obtained with twice the dosage given (see Methods), but they are sufficient to show that the hormone was given in physiologically effective doses.

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DISCUSSION

The problem of interpreting the results obtained in the water drive experiments is not made any the easier by a lack of information on the actual nature of the spring migration to the water, which Blair (1946) considers to be a constituent of the general breeding behaviour. The only information about anuran water drives collected during the present work relates to observations carried out on Rana natalensis stocks in vivaria. Twenty-three frogs were collected on the night of January the 30th, 1953. These animals were left in an unlighted laboratory, near a large window. They were heard calling at a normal intensity during the night of their capture, and indeed males were calling while being transported to the laboratory in a box. Mating pairs did not break up in transit, and on the following morning many eggs, which proved to be fertile, were found in the water of the vivarium. But virtually no calling was heard on the following nights, and sexual behaviour was discontinued. It was also noticed that while the males came out of their burrows at night, only very rarely did one enter the water. On the night three weeks after their capture, all the males sitting on the soil surface (7) were coaxed into the water. Half an hour later, only one male ~~was still in~~ the water, and all had returned to the land

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after a further quarter of an hour. The vivarium was then moved to an exposed situation on the roof of the laboratory building, which received sunlight until about 11.00 hrs. every morning. At 18.00 hrs. on the day after the tank was put on the roof, two males were found in the water, and at 21.15 hrs., five males and one female were in the water. No males of a stock of 24 frogs, which had been caught on the previous night, and which were taken to the constant temperature laboratory, were in the water at this time, although the laboratory light was turned off every night. At 23.15 hrs. of the same night, nine males were ^{water in} in the exposed tank and four were in the water in the laboratory tank. On the following day at 20.45 hrs., eight were in the water of the exposed tank. Calling was first heard in the exposed tank 10 days after it had been moved. By this time it was clear that the frogs kept in the laboratory spent their time on the soil surface, or buried in the wet soil, while the animals in the exposed tank showed a definite reproductive water drive.

These observations therefore first of all give a clear example of a reproductive water drive. The two groups of frogs were kept in the same type of vivarium, and since the frogs in the laboratory vivarium apparently did not need to enter the water provided to maintain a correct water level,

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because they could take up water from the wet soil, it is reasonable to suppose that the frogs in the exposed tank did not enter the water simply to keep their water level in order, but that they entered it for some other reason, presumably for breeding. According to the definitions used, this behaviour constitutes a reproductive water drive. Secondly, these observations show that the reproductive water drive is apparently strongly affected by external stimuli, as is the general level of sexual behaviour in these animals. It is shown in this case, and it has been found repeatedly on later occasions, that the environment of the laboratory puts an almost complete stop to calling, mating and the reproductive water drive. Experiments undertaken to discover the factors involved in this inhibition of breeding behaviour have so far proved unsuccessful because of the great difficulty encountered in obtaining normal behaviour in the control animals as a preliminary to experimentation. Even in this work, however, the hormonal factors involved in the evocation of sexual behaviour should be kept in mind, since, as has been mentioned before, Noble and Aronson (1942) have induced sexual behaviour in laboratory stocks of Rana pipiens following anterior pituitary injections. This matter will be considered further in the following Section.

Turning to the hormonal experiments, it was stated

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in the Results that the water drive of the unhandled controls recorded from autumn and spring stocks were not significantly different. It was concluded from this that the difference in the R.H. of the laboratory was not an important source of error in the water drive records. Another interpretation may, however, be given to these results. If the R.H. has an effect on the time spent in water, then the time spent in water by the spring stocks would be less than the time spent in water by the autumn stocks, because the higher R.H. of the laboratory during the summer would permit the animal to stay out of water without drying so rapidly. This would result in an apparent lowering of the water drive. Now if there was in fact an increased water drive in the spring animals, this drive would appear to be lowered by the high R.H., and so the effect would be masked. Because neither the effect of different R.H.'s on the water drive, nor the effect of spring and autumn levels of hormones on the drive is known, it is therefore not possible to say that the results obtained with spring and autumn animals are comparable until controlled experiments have been conducted. The considerably greater amount of time spent by the control spring stocks in water compared with the time spent in water by the control autumn stocks might be taken to

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indicate that, given an adequate control of R.H. conditions, a greater number of readings would stabilise this difference and make it significant.

On the other hand, results of this nature might appear unlikely, because any such hormonal differences would be unlikely to survive the acclimatisation to the laboratory. There is, however, evidence that some seasonal changes which have a hormonal basis are not affected by acclimatisation to the laboratory. As was noted in the Results, it was found that it was not possible to sex the stock of toads collected during the autumn with certainty by using external features alone, but that animals collected during the following spring and summer could be immediately sexed at sight. This summer character was that the dorsal surface of the males was grey-green in colour, while that of the females was brick-red. It was noticed that the males of the autumn stock which had been injected with testosterone had a greenish tinge to them when they were autopsied two weeks after the injections were discontinued. (see Results). These observations indicate that the colour of the male is controlled by the level of testosterone.¹ If this is the

¹ Greenberg (1942) has found that this effect is present in the American Cricket Frog, Acris gryllus. It is difficult to see what advantage this difference in colour between the male and the female has in B. carens, since field

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case, then the male becomes indistinguishable from the female in autumn because of the fall in the level of testosterone at this time. If acclimatisation to the laboratory involves a fall in the sex hormone level, as is suggested by the fall in sexual behaviour, then it would be expected that the acclimatised animals would take on the autumnal colouring, as well as show the autumnal level of sexual behaviour. But males of the stock collected in spring have been kept in the laboratory for three months, and at the end of this period were still indistinguishable from males found in the field at this time (January). Therefore this seasonal difference, which appears to have a hormonal basis, survives the acclimatisation of the laboratory. A similar survival seems to be shown by the persistence of the seasonal cycle in Rana natalensis stocks, which were kept in the constant temperature laboratories for a year (See Part II, Material). Similarly, Noble and Aronson (1942) were only able to induce calling by the

observations carried out on this animal in the course of this work show that pairing normally takes place at night, and, as with most anurans, pairing appears to take place on the basis of the passiveness and girth of the female, compared with the reactivity and slimness of the male and the spent female. Sex recognition therefore appears to occur after clasping, when the mounting male cannot see the toad beneath it.

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injection of anterior pituitary extracts in laboratory stocks of Rana pipiens during the breeding season, which also suggests the presence of a hormonally controlled seasonal difference which survives acclimatisation to the laboratory. Therefore the seasonal difference in the water drive, if it existed, might well survive acclimatisation to the laboratory and be successfully recorded, provided the R.H. were properly controlled. It is therefore certainly worth while repeating the recordings of controls taken from the field during autumn and spring, and seeing if more replications will bring out a significant difference between the two groups when the R.H. conditions are controlled. The discovery of such an effect would be a starting point for a properly orientated investigation into the effects of hormones on the water drive of Bufo.

The difference between the male and the female water drive in the second gonadotropin series (see Table 2) was noted in the previous Section. These animals belonged to the spring stock, and it would be interesting to compare a large number of males and females during the breeding season to see if there is a significant difference between the male and the female water drive. Work on this problem might go far towards arriving at an understanding of the unequal distribution of the males and females with respect to water, described in the section on Materials. The

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fact that the two males showed the stronger water drive in the above experiment is a hopeful sign that a definite effect might be found, which would have a strong bearing on the observed distribution in the field. This work might also provide valuable clues for the investigation of a spring-autumn difference.

All that can be said of the work so far done on the reproductive water drive of Bufo carens, however, is that there are at present no good grounds for considering that a difference does exist between the spring and autumn drive, or that any of the hormones tested affect the level of the drive shown by controls. But on the other hand, the possibility of some effect of the seasonal fluctuation in the level of these hormones on the drive is by no means excluded, and greater numbers of animals under better controlled conditions may show that there is in fact a significant difference between the water drive of toads collected in spring and autumn, and that there is a sex difference in the spring drive. The number of experiments is enough, however, to show that an effect such as the trebling of the intensity of the water drive, which was the one originally looked for in this preliminary survey of the effects of the available hormones on the water drive, probably does not exist.

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CONCLUSIONS

At the end of this present work on the reproductive water drive of Bufo carens, it is evident that the rôle that hormones play in the induction of this drive is still far from being clear, although it appears that the hormones studied do not evoke a drive as intense as, for example, the drive induced by prolactin in Triturus (Chadwick, 1940). Methods for measuring the reproductive water drive have been developed, however, and the best procedure that should be adopted in more extensive and controlled investigations has become clear. The importance of the environmental factors in the induction of the reproductive water drive has been clearly shown in Rana natalensis, and it is entirely probable that the environment is of as great importance in Bufo. The experimental approach to this whole problem has therefore been clarified, and orientation given to its investigation. It is evident that this work completes a definite stage in the investigation of the reproductive water drive.

It can be concluded that the next step in the investigation of this drive must be to compare the drive of normal males and females collected during the breeding

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season, and to determine whether the drive at this time of the year differs, in any or both of the sexes, from the drive of animals collected in autumn and winter. The R.H. in these comparisons must be carefully controlled. If positive results are obtained in this work, then work may be resumed on hormonal treatments, using what clues that may be picked up in the comparisons as a new guide. It is also necessary to investigate the effects of changes in the external environment on the intensity of the drive. The method used up to now of counting the number of animals in water as a measure of the intensity of the drive is useful in giving first approximations. However, the method of measuring the intensity of the drive by measuring the time spent in water by isolated animals, as it has been used in work on the effects of the internal environment on the drive, must be used for critical investigations into the effect of the external environment on the water drive.

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PART II. THE HUMIDITY REACTIONMATERIAL

It was suggested in the General Introduction that in starting work on the humidity reactions of the Anura, it is desirable that the same techniques that have been developed in work on the humidity reactions of invertebrates should be employed, since it is from work on the invertebrates that practically all our knowledge of humidity reactions and their measurement has been gained. The animal selected for this present work was Rana (Pyxicephalus) natalensis (Smith), since the members of this species could easily be fitted into the standard size apparatus used in determining the humidity reactions of invertebrates.

This species is a very common frog over most of Natal, characteristically occurring in and around ditches which contain water from about the end of August to April. The length of a typical male is about 3 cms., with a weight of about 2.5 g., while the female is larger. Only males were used in the experiments, because the females were outside the suitable size range. Observations conducted on specimens in vivaria left in the open, and on this species in the field, show that during the day they bury

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themselves in the soil within a few feet of their pond or stream, (except in wet weather, when they may be found well away from established bodies of water), and that they come out into the open at night. During the night the males (~~usually~~) sit at the water's edge, or more usually in shallow water, and emit a highly-pitched call.

The experimental animals were kept in the same constant temperature laboratory that was used for the water drive work. They were housed in glass tanks, whose floors were covered in soil. The soil surface was sloped from one side of the tank to the opposite side, and water was added so that a pool was always available where the soil was shallowest. Tenebrio larvae and the swarming forms of termites were provided so that each animal could feed at least once a week. All the frogs, apart from eight used in final experiments, were collected from January to March, 1953. The majority of these animals buried themselves and went into hibernation from April to October, so they did not eat during this time. This hibernation period lagged about one month behind the hibernation period in the field, although calling in those frogs of the laboratory stock which did not hibernate commenced a fortnight before calling was heard in the field, which was on the night following the first heavy rain of the season (August the 26th). While calling in the laboratory was very much more

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infrequent and softer than was the calling of a group of a similar number of animals which was kept under observation in the field, it is evident from the hibernation period and the onset of calling that the seasonal cycle was still operating to a certain extent in the stock kept under constant conditions.

The presence of this cycle did not complicate the present investigation, however, since technical difficulties did not permit work on the animals during the winter months.

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APPARATUS AND METHODS

It was originally intended to study the humidity reactions of Rana by the use of the alternative humidity chamber, introduced by Gunn and Kennedy (1936), and the uniform humidity chamber. The alternative humidity chamber consists of a sealed dish containing a perforated false floor placed above a set of petri dishes in each half of the chamber, each set containing a solution which will subtend a desired humidity. Solutions of different subtending values, when placed in respective halves of the chamber, will thus cause a gradient to be developed between the two halves of the chamber, and will present the animal with a choice between the two humidities at each end of the gradient. The humidity reaction of an animal placed in this chamber is measured by the relative times spent in each half of the chamber, or by the relative frequency with which the animal is found in each half of the chamber. The uniform humidity chamber differs from the alternative humidity chamber only in that the subtending solutions are of the same values in both halves of the chamber.

While this type of chamber is satisfactory for animals such as small arthropods, which do not give off a large amount of water vapour from their bodies, it was thought

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advisable that the extent of the microclimate of Rana in these chambers should be determined before work on their reaction was commenced, since the skin of these animals, being moist, would be expected to influence the R.H. of the surrounding air to a greater extent than would the integument of arthropods. A frog was therefore put in a uniform humidity chamber with an R.H. of 5%, and it was found that an Edney hygrometer which had been lying next to the animal for 28 minutes recorded an R.H. of 49.5%. A hygrometer lying in the opposite half of the chamber recorded an R.H. of 12% at this time. Further investigations showed that the influence of the frog's microclimate on the R.H. of the chamber was complicated by the fact that the effect varied with the different original R.H. values established in the chamber. This effect is shown in Table 3.

Table 3. The effect of different R.H.'s on microclimate of Rana natalensis.

Original R.H.	Maximum R.H. increase	Average R.H. increase	No. Readings	Length of Experiment
5%	44.5%	25.8%	16	60 min.
35%	15.5%	10.3%	86	90 "
65%	19.5%	6.2%	42	90 "
95%	1.5%	1.3%	16	60 "

The table shows that the influence of the frog on the

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R.H. of the chamber is greatest at the lowest humidity and least at the highest humidity. A further complication was found, in that the effect of the microclimate varied with the activity of the animal. When the animal was still, it was surrounded by a localised microclimate of a high R.H., but when the animal was continually moving, the R.H. of the chamber as a whole was raised to a lesser extent.

The results showed that the subtending solutions of the chamber could not eliminate the frog's microclimate effectively, thereby introducing a major source of error into the apparatus. Consequently some other means of establishing stable R.H. conditions had to be sought, which would be able to reduce the error due to the microclimate to an inconsiderable amount. Now the extent of the effect of the microclimate on the humidity of the chamber is the distance between the surface of water vapour which is of the required concentration, and the surface of the frog. This being the case, the desired reduction in the effect of the microclimate on the humidity of the chamber may be achieved by introducing a flow of air, whose humidity could be controlled, into the chamber. The effect of this would be to maintain a surface of water vapour of the required concentration at a controllable distance from the frog (Ramsay, 1935). The greater the velocity of the air

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passing the frog, the shorter will be the distance (Ramsay, 1935), and consequently the smaller will be the effect of the microclimate on the chamber. Therefore by using moving air, it was thought that the effect of the microclimate would be brought under control, and so it was decided to attempt to construct a humidity chamber which could have air of controllable humidities passed through it at controllable rates. In view of the enormous microclimate of these frogs, it seemed that in no other way could the humidity reaction of these animals be studied, if the humidity chamber method was to be used.

In setting about the construction of a piece of apparatus to meet these requirements, it was first of all necessary to decide upon some method for conditioning the air blown into the chamber ^{to} ~~of~~ any desired R.H. There are two possible means of doing this. Either air can be bubbled through subtending solutions which give a known R.H., and changes of the air can be effected by changing the densities of these solutions, or else the stream of air can be divided before entering the chamber so that a part of it passes through a drying unit, while the remainder passes through water. Using this latter method, changes in the R.H. of the air entering the chamber can be altered by varying the the relative amounts of moist and dry air entering it. The latter method was adopted, firstly because it was foreseen

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that, at least in the preliminary work, accidents such as spillings, and the solution being sprayed out of leaks, would not involve such a waste of material and time due to the loss of carefully prepared solutions, and to putting right the mess and damage that such solutions would cause; and secondly, because it would have been necessary, with the former method, to dismantle the apparatus at intervals to renew the solutions, whose composition would be rapidly altered by the passing air, or to replace them with other solutions. As work progressed, the superiority of the method that was employed over its alternative became increasingly apparent.

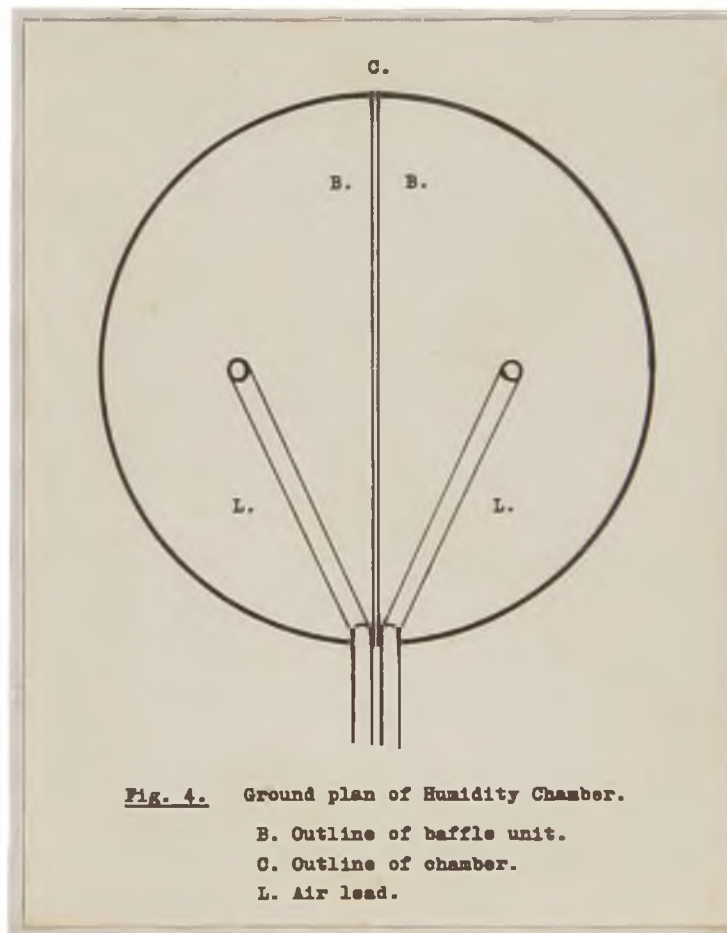
It was then necessary to adapt the pre-existing type of humidity chamber to meet the new requirements. In order that the direction of air flow should interfere as little as possible with the behaviour in relation to R.H. gradients set across the chamber, it was decided to have the flow passing at right angles, in the vertical plane, to the gradient. This was achieved by passing the air up through a perforated false floor bearing the experimental animal. The principal difficulty that presented itself was that of how to diffuse the air stream, which had to be introduced into the chamber by fairly thin leads, so that there would be an even air flow across the whole area of the chamber at the level of the false floor. This was

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overcome by having the openings of the air leads into the chamber situated at the bottom of the chamber, and inserting a number of baffle plates between these openings and the false floor.

A detailed description of this modified type of humidity chamber now follows.

Air was introduced into the chamber by two leads, which ran vertically together down the inner surface of the side of a circular glass pneumatic trough, 25.8 cms. in diameter and 13 cms. high. Each lead could be supplied with air of any R.H. The method of obtaining the different R.H.'s will be described later. Upon reaching the bottom of the dish, the leads diverged across the floor (Fig. 4). The upward-pointing openings lay on one diameter of the dish, each opening being in the centre of each half of the dish. Two semicircular baffle units, whose combined area equalled the area of the dish, were placed above the outlets, so that the apposed vertical surfaces of a pair of units lay on the diameter of the dish at right angles to the diameter upon which the two lead openings formed two points (Fig. 4). Each baffle unit was made separately from perforated zinc sheeting with a mesh gauge of 28, with 1.5 mm. holes. A strip of sheeting, 6.5 cms. wide, was cut and then bent along its width so as to form the outline of the semicircle. Three semicircular plates of perforated



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zinc were then soldered into position inside this vertical support, lying parallel to each other and to the bottom of the dish, and each placed about 1 cm. from each other. The lowermost plate lay about 2 cms. above the bottom of the dish, and about 1 cm. above the opening of the inlet pipe. Before insertion, the lowermost plate of each unit had three bands of bitumen painted on it, each being about 5 cms. wide. The bands were equally spaced, and ran from the base of the semicircle, and at right angles to it, to the rounded edge. The bitumen blocked all the holes in these areas. The inlet pipe opened onto the middle band. The middle plate had four strips of bitumen painted on it, each being about 1.25 cms. wide and equally spaced, and running parallel to the base of the semicircle. The uppermost plate was not painted. All the flat side of each baffle unit, and the curved side below the insertion of the lowermost plate, were painted with bitumen, and when the unit was inserted in the dish, a large amount of paraffin jelly was smeared on the bottom edges. With these precautions, one unit did not allow exchange of air with the other unit of the same dish, so that with this arrangement the dishes could be used as alternative humidity chambers.

The baffle units appeared to be effective in completely diffusing the air flow, since no eddying or unequal flow

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could be detected either when smoke was added to the air stream entering the chamber, or when smoke was blown into the chamber from above. A perforated protecting lid was placed on top of the dish in order to prevent the humidity conditions from becoming disturbed by currents in the air surrounding the dish. The lid was made of thick glass, with 19 holes, 0.6 cm. in diameter, drilled along four equally-spaced diameters, so that each hole was isometrically placed in relation to adjacent holes. There was a space of 5.5 cms. between the perforated floor and the lid. A small nick had to be cut in the lid and the perforated floor to accommodate the inlet leads, and corresponding nicks were cut in the sides of the baffle units. In order to prevent a disproportionate amount of air from travelling up these nicks, they were plugged with cotton wool.

Having built the chamber and decided upon the method to be used for conditioning the air, the next step was to determine the rate of the flow of air needed to reduce the effect of the microclimate to a satisfactory degree. Gunn and Kennedy (1936) have noted that at one set R.H., the readings of Edney hygrometers vary irregularly by about $\pm 3/4$ R.H., and a similar degree of error has been observed in the present work, so it was considered that if the increase in the original R.H. due to the frog's microclimate could be brought to within about 3% R.H. in a moving air

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chamber, i.e. to an extent roughly within the limits of reliability of the recording apparatus, the error due to the microclimate would not be particularly serious. The method employed to determine the rate of flow necessary to reduce the microclimate to this extent was to place hygrometers close to a frog, and to increase the flow of air entering the chamber until the hygrometers showed a deflection of less than about 3% R.H. above the original R.H.

Since the effect of the microclimate is greatest at low humidities (Table 3), it was decided to determine the rate of air flow necessary to reduce the microclimate to the desired amount at the lowest humidity which was intended to be used in experiments using moving-air chambers, and then to use the same air flow for higher humidities, so as to provide an internal control for the effect of air flow on the animals when activity in different humidities was to be compared. Projected experiments were not intended to be made at an R.H. of less than 20%, and so it was decided to attempt to reduce the frog's microclimate to not more than 23% R.H. at this R.H.

One chamber was used in the following attempt to reduce the microclimate to the desired amount. The humidity conditioning apparatus consisted of one series of bottles

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containing KOH pellets, and a parallel series of distilled water bottles, which supplied each lead to each half of the chamber. The air was provided by an air compressing unit which was installed outside the constant temperature laboratory. A coil of 5 m. of copper tubing was inserted into the lead from the pump to the laboratory as it entered the laboratory, in order to bring the air to the right temperature. The air entering the chamber was of the same average temperature as that of the room ($23.9^{\circ}\text{C}.$). After passing through the copper tubing, the air entered a pint milk bottle containing a wad of cotton wool, in order to remove oil droplets (Fig. 5, O), and then the stream divided to supply each half of the chamber. The system is shown in Fig. 5. Each supply line (S) branched into a pair of conditioning lines (C). In one of these lines, three pint milk bottles were inserted, the entrance lead being taken to the bottom of the bottle, and the exit lead commencing just below the rubber bung which held the leads in position. Two of these bottles (K) were half-filled with KOH pellets, and the third (Kt), the one furthest from the air compressor, contained a wad of cotton wool to trap particles of KOH. The other conditioning line had four pint milk bottles inserted into it, with the "in" and "out" leads arranged as in the other line. The three bottles nearest the air compressor were filled to about $\frac{3}{4}$ of their

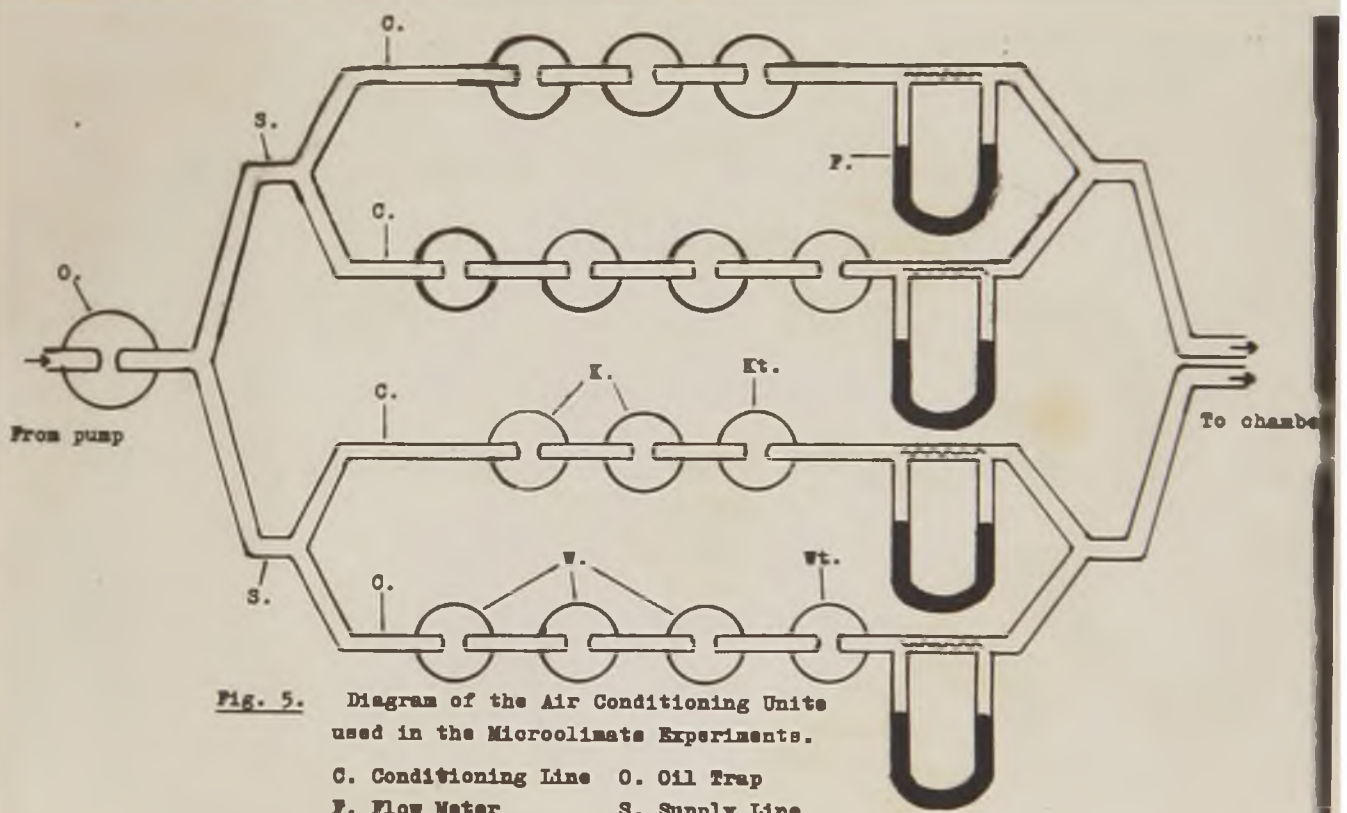


Fig. 5. Diagram of the Air Conditioning Units used in the Microclimate Experiments.

- | | |
|----------------------|------------------|
| C. Conditioning Line | O. Oil Trap |
| F. Flow Meter | S. Supply Line |
| K. KOH Bottles | V. Water Bottles |
| Kt. KOH Trap | Wt. Water Trap |

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volume with water (W), and the last bottle (Wt) contained a wad of cotton wool to trap water spray. Before the conditioning lines met again before entering one half of the chamber, a flow meter (F) was inserted into each line. Each flow meter consisted of a mercury manometer, with both ends set in the line, and a capillary tube of a known length and internal diameter inserted between the two manometer ends. The pressure built up by the air passing through the capillary tube was recorded by the manometer, and by using Poiseuille's formula, the rate of air flow in each line could be calculated by measuring the displacement of the mercury. The formula used was

$$\text{Rate of flow} = \frac{\pi a^4 P}{8 \eta l}$$

The rate is given in ccs./sec. when a = the radius of the resistance in cms., P = the pressure across the resistance measured in dynes/ccs., η = the viscosity of air and l = the length of the resistance in cms. The conditioning lines then joined, and the supply lines entered the chamber as described above. The leads and the manometers were made of glass tubing, 0.7 cm. in diameter, held together by rubber sleeving. The air flow was controlled by screw clips on pieces of rubber tubing, which were fitted into the circuit after the initial branching into the supply

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lines, and again before each flow meter in the conditioning lines.

Since the humidity passing through each half of the chamber had to be the same for the microclimate experiments, it was not necessary for each half to have a separate conditioning unit. A double set of units is only necessary if the chamber is to be used as an alternative humidity chamber. Separate units were installed for each half, however, in order to see if the conditioning units of this type did work in practice, and to practise operating this type of conditioner.

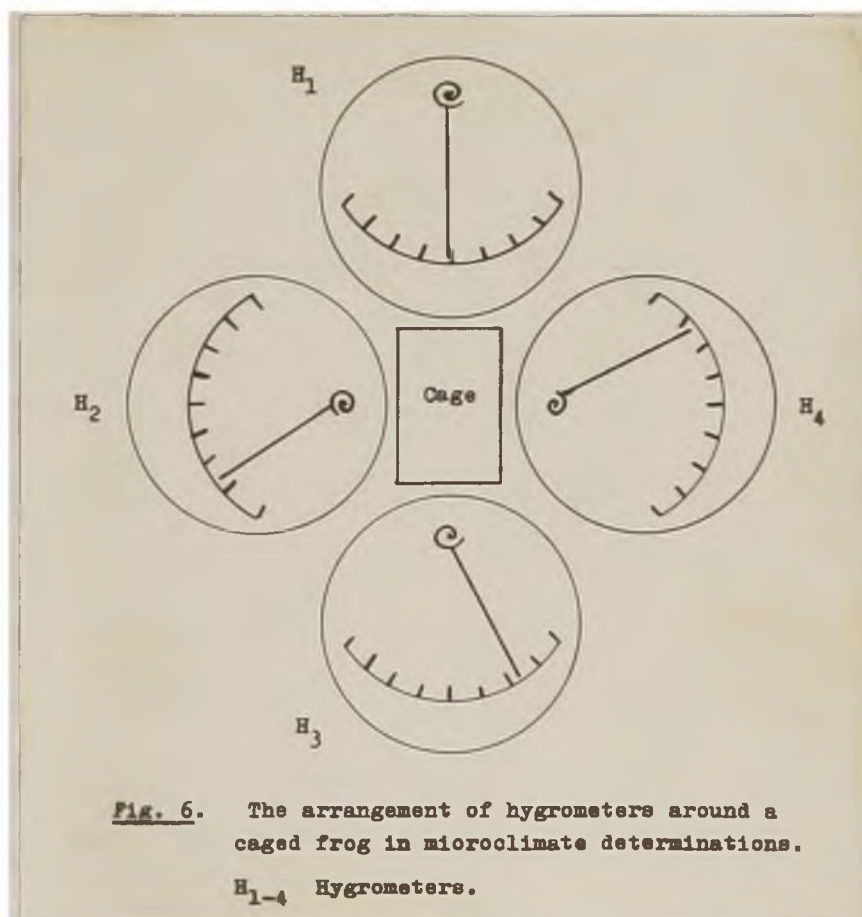
The R.H. of the chamber was measured by placing 7 to 10 Edney hygrometers, which had been calibrated over sulphuric acid solutions of known densities, in the chamber, and allowing them to equilibrate. Any necessary adjustments to the R.H. were then made by altering the proportions of wet and dry air entering each half of the chamber by suitable adjustments to the screw taps.

With a flow of 52.9 cc's/sec. entering the chamber, a frog sitting about 0.5 cms. from ~~(the)~~ hygrometers deflected them by up to 27% R.H., and a deflection of 16% R.H. was shown with a flow of 182.2 cc's/sec. entering the chamber. With a flow of 274 cc's/sec. entering the chamber, the deflection shown was 13% R.H. After these results had

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been obtained, the determinations were more properly conducted by putting the frog into a wire mesh cage, just big enough to contain it, and then placing four hygrometers around the cage (Fig. 6). Each one lay about 0.25 cms. from one side of the cage, three of them (Fig 6, H₂ to H₄) orientated so that their coils were as close as possible to the cage, while the fourth had its coil away from the cage. Six other hygrometers were placed in the chamber at different distances from the cage. An average was taken of the readings of the three hygrometers with their coils close to the cage, when they had equilibrated. These hygrometers recorded the maximum influence of the micro-climate on the original R.H. of the chamber, and it was aimed at reducing the deflection of these hygrometers to less than 3% R.H. A check was kept on the general R.H. of the chamber with the other hygrometers. Using this method, the following determinations were made.

Air entering chamber (cc's/sec.)	Average increase of the 3 hygrometers nearest the frog. (% R.H.)
1,873	5.1
1,960	5.0
2,024	4.6
3,474	4.3
4,558	4.1
6,301	3.0
7,369	2.3
9,711	3.0



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This table shows that there was a lessening of the effect of the frog's microclimate on the original R.H. of the chamber with an increase in the amount of air entering it, and that the deflection of the hygrometers fell below 3% when the air flow rose to about 7,000 cc's/sec. Since there was an unaccountable variation shown by the hygrometers, the determination of the effect of the frog's microclimate was repeated twice with the rate of 7,369 cc's/sec., and as the error due to the microclimate fell within the desired limits at this rate, it was decided that this rate should be used for the projected behaviour experiments. The fact that at a considerably higher flow rate (9,711 cc's/sec.) the hygrometers gave an average reading of an increase of 3% R.H. indicates that with air entering the chamber at rates greater than about 7,000 cc's/sec., the increase in R.H. due to the frog's microclimate does in point of fact lie within the limits of reliability of these hygrometers. With air entering the chamber at the rate of 7,000 cc's/sec., the rate of air flow passing the frog was calculated to be about 0.4 miles per hour (16.55 cms./sec.)

It should be noted that the Edney hygrometer is not the most suitable instrument for measuring very localised R.H. differences, such as those which occurred around the experimental animal, since the holes allowing for the

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movement of water vapour into it are on the side of the instrument, and so lie parallel to the air flow.

Consequently there is a comparative stagnation of air in the hygrometer, and so the R.H. of the air around the coil is not necessarily the same as the R.H. of a point at the same distance from the frog which is fully exposed to the air flow. This defect is particularly noticeable when the readings given by two coils at equal distances from the frog are compared, but when the container of one is up against the side of the frog (Fig. 7, H_2), and the other container (H_1) is orientated so that it is as far from the frog as possible, the hygrometer with the container lying against the frog (H_2) will record a higher R.H. than the hygrometer lying away from the frog (H_1). An instrument with holes in the floor around the coil and without a glass top to it, would be more critical in these conditions. Thus the changes shown by the hygrometers when they were being affected by the microclimate must be considered to be only rough approximations to the actual changes in the R.H. taking place around the frog. In addition to this source of error, there is some hygrometer-to-hygrometer variation in recording a single change in R.H., which is probably not completely compensated for by taking an average of three hygrometers for one reading. The shortcomings

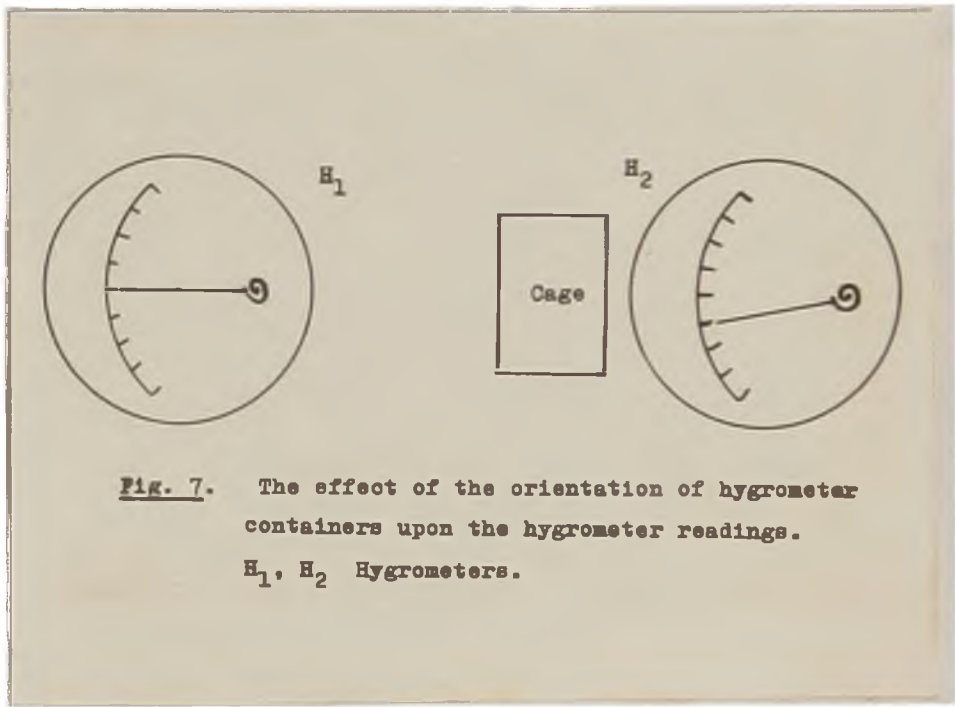


Fig. 7. The effect of the orientation of hygrometer containers upon the hygrometer readings.
 H_1 , H_2 Hygrometers.

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in the absolute reliability of the hygrometers do not, however, affect the main issue, namely that the increase in the R.H. of the chamber due to the frog's microclimate was reduced to within the range of error of the recording apparatus used. The 2.3% R.H. rise constitutes an error of 11.5% of the original R.H. of 20%.

At an R.H. of 60%, with air passing into the chamber at the rate of 7,567 cc's/sec., the average increase of the three hygrometers with their coils next to the cage was 1.1% R.H., that is, an error of 1.8% at 60% R.H.

Having determined the flow of air necessary to diminish the microclimate in order to conduct satisfactory experiments on the effect of humidity on the behaviour of R. natalensis, it was then necessary to discover if moving air itself had any effect on the behaviour of these animals. It was decided to determine if any such effect existed by observing an animal's behaviour at different R.H.'s in still and moving air, and by finding out if any preference towards one half of a chamber existed when air was blown into each half at different rates.

The conditioning units used in the microclimate experiments were unable to carry enough air to supply more than one chamber, and, since two chambers could be used simultaneously in most of the observations on behaviour, and since many chambers were required for work on preference

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for different air flow rates to get significant results in a reasonable time, a new unit had to be built to meet the need for an increased flow of air. This new unit included a modification of the pre-existing plan, in that only one series of drying and wetting bottles were able to serve the different requirements of each half of one or more alternative humidity chambers. This modification was necessary since, owing to the fact that seven and ten litre aspirators were to be used in the place of the pint milk bottles, the size of the conditioning unit was limited by the available space in the laboratory.

Air was supplied by the air compressor used in the microclimate experiments. The air entered the laboratory through rubber tubing with an internal diameter of about 1 cm. Oil droplets were removed by passing the air through the length of a sealed tin, 35 cms. long and 17 cms. wide, which at the outlet end had five discs of perforated zinc sheeting, whose diameter was equal to the internal diameter of the tin. The discs were separated by layers of cotton wool, each about 3 mm. thick. Cotton wool was packed between the edges of the discs and the inner surface of the tin, so that all the air passed through the filters. The space between the inlet pipe and the filter was filled with loosely-packed wood wool to break up the main stream of air entering the tin. After leaving the oil filter,

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the air stream divided to supply a single wetting and drying unit. The wetting unit consisted of a series of four aspirators, the three nearest to the oil filter being filled to about four-fifths of their volume with water, and the fourth acted as a water trap. Air entered each of the three water bottles through a length of copper tubing, which was inserted into the lower opening of the aspirator, so that it lay horizontally across the diameter of the aspirator. The furthest end was plugged with solder, and three rows of holes, each hole being about 1.5 mm. in diameter, and spaced at 7 mm. intervals, were drilled along the length of the part of the tubing in the aspirator. When the tube was in position, the central row of holes was on the crown of the tubing and the other two rows lay just above the mid-lateral line on both sides. The air was taken out by a tube opening just below a rubber bung in the top of the aspirator. It was found that this method was much more efficient than the method of passing air through a vertical tube to the bottom of the bottle, because the air came out as a stream of small, widely-dispersed bubbles, in contrast to the rush of air that was delivered up the outer side of the vertical inlet. Consequently the surface area of a unit volume of air was considerably greater when the perforated horizontal inlet was used, giving a greater efficiency, which reduced the number of bottles required.

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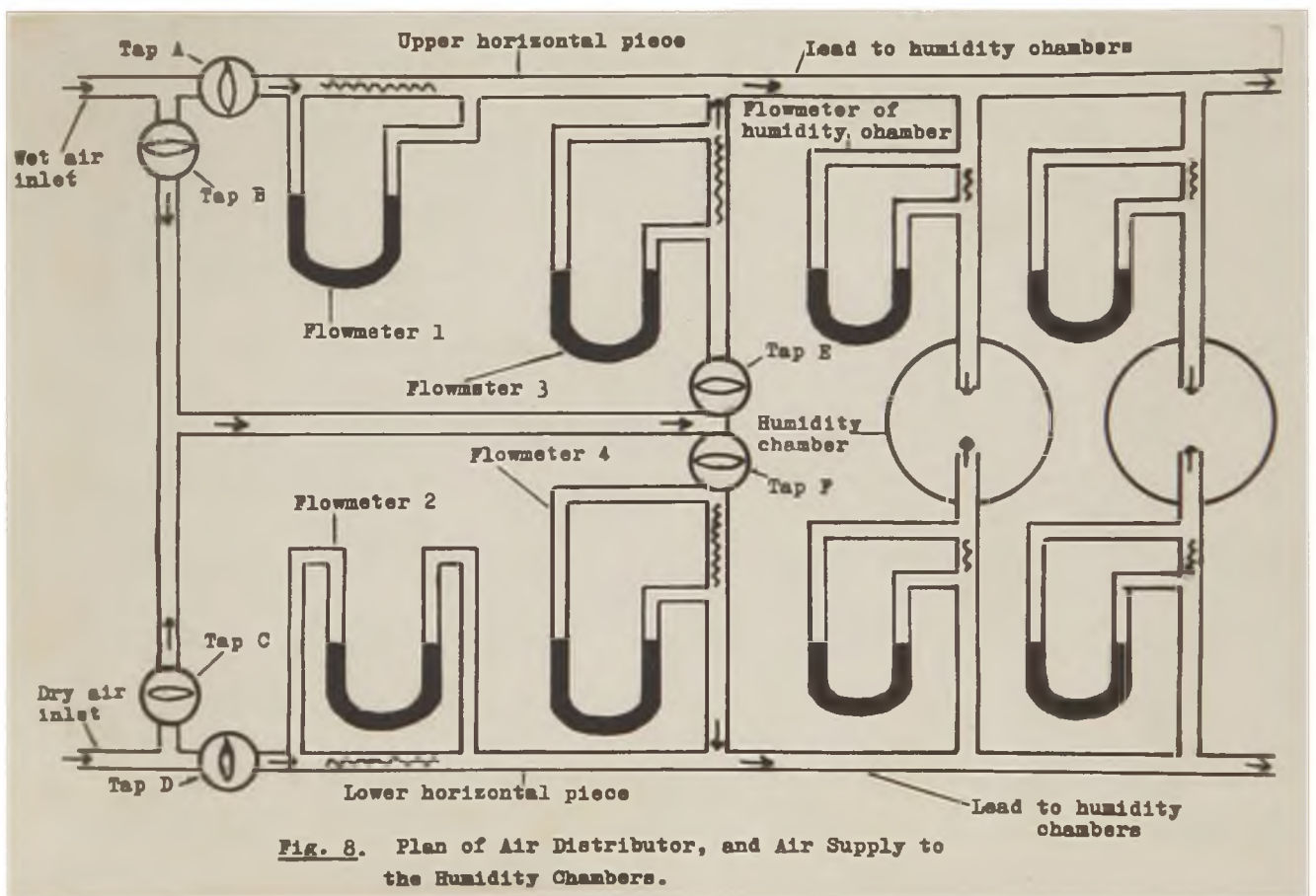
In addition, this method reduced the turbulence at the top surface of the water, so that the aspirators could be filled to a relatively higher level without so much splash being blown out of the ^{out}inlet pipe. This method of using horizontal perforated inlets was thought of by Mr. P. Metcalf, who also designed and constructed these inlets. The fourth aspirator had the inlet and outlet tubes secured in the top opening by a rubber bung, with their openings just below it, and it acted as a water trap. A sealed tin, 15 cms. long and 6.5 cms. wide, was fixed into the outlet tube so that the air passed through its length. It was loosely packed with cotton wool, in order to remove water droplets. Owing to the back pressure formed in the water aspirators by the heads of water produced when the unit was in use and the inlet pipes were cleared of water, water was forced back along the lead between the wetting unit and the oil filter after use. This water was collected in a pint milk bottle inserted in this part of lead, in which the outlet tube opened at the bottom of the bottle, so that the water collected was blown back into the first aspirator when the unit was re-used. The drying unit consisted of two aspirators containing fused CaCl_2 , and a pint milk bottle containing a wad of cotton wool, set up as for the drying units used in the microclimate experiments. All the glass tubing used had an internal diameter of about

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1.2 cms., and the connections were made fast with rubber sleeving.

The leads from the wetting and drying units were then connected to an air distributor, whose plan is given in Fig. 8. The function of this distributor was to deal with air from the wetting and drying units in such a way that only a single unit of each conditioner was required in catering for the different requirements of each half of one or more alternative humidity chambers (see above). The main part of the distributor was made of pieces of cast iron gas piping and glass tubing, whose internal diameter was about 0.8 cm., and gas taps, whose minimum internal diameter was about 0.4 cm. Four flow meters of the type used in the microclimate experiments were included, and they were made so that the resistances could be removed and replaced by others according to the rate of air flow to be used.

The distributor consisted essentially of an H-piece, standing vertically, with a height of about 75.5 cms. and a width of about 48.5 cms., with two horizontal pieces connected to the top and bottom ends of the H. Each horizontal piece had a flow meter inserted into it, and flow meters were also placed in the vertical piece nearest to the outlet. The taps are shown in the Figure. Wet air entered the distributor by the uppermost horizontal piece,



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and dry air by the lowermost horizontal piece. Air left the distributor by the continuation of these horizontal pieces. Each horizontal piece continued into a lead which gave off branches supplying the halves of a series of chambers. If it was desired to pass only air from the wetting unit into both halves of the chambers, taps A, B and F were opened and the rest closed. The rate of air flow could be regulated by the open taps, and this rate would be measured by flow meters 1 and 4. If only the driest air was required for both halves of the chambers, then taps C, D and E were opened, and the rest closed. The rate of flow would be measured by flow meters 2 and 3. If an intermediate R.H. of the same percentage in both halves of the chambers was required, then taps B, C, E and F were opened, and the rest closed. The rate of flow would be measured by flow meters 3 and 4. With this arrangement, the air from both conditioning units was mixed in the central horizontal piece. The humidity was then altered by altering the setting of taps B and C. In determining the suitable adjustments for obtaining the intermediate humidities, at least six Edney hygrometers were put in one chamber, and the taps were altered until, by trial and error adjustments, the hygrometers showed that the desired R.H. had been obtained. In order to have the halves of the chambers at different R.H.'s, a different set of tap

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adjustments had to be used. If completely wet and completely dry air was wanted, respectively, in the two halves of each chamber, then taps A and D were opened, and the rest closed. The rate of flow would then be measured on manometers 1 and 2. If on the other hand, a fairly high R.H. was wanted in the one half of the chambers and a fairly low R.H. was wanted in the other halves, say 60% and 20% R.H. respectively, then all the taps were opened to different degrees. Mixed air from the middle horizontal piece would be mixed with wet air from tap A, and, with correct tap adjustments, would leave the uppermost horizontal piece at 60% R.H., and the mixed air from the middle horizontal piece would also be mixed with dry air from tap D, and would leave the lowermost horizontal piece, given the suitable tap adjustments, at 20% R.H. Determination of the suitable tap adjustments would again be done empirically by observing hygrometers in one of the chambers.

A difficulty arose in obtaining air which could be shown by the hygrometers to have an R.H. of 100%. While air from the wetting unit did not have to be completely laden with water vapour when intermediate R.H.'s were required, additional apparatus had to be inserted into the circuit to saturate the air fully before experiments requiring 100% R.H. could be undertaken. By placing a hygrometer on top of the cotton wool in the tin that was

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inserted into the wet lead between the distributor and the water-trapping aspirator, it was found that the air leaving the wetting unit was at 100% R.H., and in fact water droplets sometimes collected on the inner or outer surface of the glass top of the hygrometer. But air entering a chamber after having passed through the distributor (which had taps C and D closed) and about 3.5 m. of glass tubing, showed an R.H. of about 87%. It was also found that this drop in R.H. was associated with a consistent drop of temperature of 0.5°C . A consistent drop of 0.2°C . was recorded across the distributor, and a hygrometer placed in the circuit immediately after the distributor showed that a drop of about 9% R.H. occurred when the air passed through the distributor. As there was a slight drop in the temperature of the air after it left the wetting units, it seemed unlikely that the temperature differences were the cause of the great drop in R.H., since it would be expected that a decrease in temperature would serve to raise the R.H. of the air after it had left the wetting units. Thus it was supposed that the cause of the drop in R.H. was the condensation of the water vapour onto the sides of the distributor, conducting leads and the baffle units. In order to overcome this difficulty, an aspirator filled with distilled water was inserted into each lead immediately before the side-branches left for the halves of the

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humidity chambers, and the baffle units in the chambers were sprinkled with water. To prevent the water from reacting with the zinc, the baffle units were covered with a layer of shellac. With this arrangement, hygrometers in the chambers recorded an R.H. of approximately 100%.

The halves of the humidity chambers were supplied with air by lengths of copper tubing, with an external diameter of 0.5 cms., which were connected to the two main leads from the distributor by glass T-pieces set into the leads. A flow meter was set into each copper lead, and the rate of the flow of the air entering the chambers was read from these manometers, and not from the distributor manometers, which were used only as guides for the total flow to all chambers.

With this apparatus, which was able to eliminate the microclimate of Rana as a source of considerable error in a number of humidity chambers, it was then necessary to determine the nature and extent of the effect of moving air on the frogs' behaviour, before it could be put into use for investigating the humidity reactions of these animals in a critical way. As has already been stated, it was decided to determine if any such effect existed by observing the behaviour of these animals at different R.H.'s in still and moving air, and to find out if any preference existed when air was passed into each half of the humidity chambers

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at different rates.

Observations on the behaviour in still and moving air were first of all undertaken. The R.H.'s of 100, 60 and 20% were chosen for this work. If air movement in itself had no effect on behaviour, then at 100% R.H. there should be no significant difference between the behaviour of frogs in still and moving air. At lower humidities, however, the microclimate would be virtually absent in moving air, and therefore, if a humidity reaction existed, it would be shown much more strongly in moving air because of the lower R.H. of the air around the animals - provided that the moving air in itself had no effect upon behaviour. Thus the critical comparisons of the level of activity in still and moving air would be at 100% R.H., and the results obtained at lower humidities would help to confirm the conclusions arrived at from the 100% R.H. observations.

To compare the activity in still and moving air, it was necessary to devise a quantitative method of measuring the activity of the animals in the chambers. Use was made of the fact that the overt movements of Rana are characterised by short bursts of activity, separated by pauses of an extremely variable duration. At first it was attempted to record the duration of each burst of activity, and to express the activity of an animal in terms of the summed time of activity over a unit period. But as the

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bursts of activity were so short, rarely lasting longer than a second, it was not possible to measure the activity really accurately by this method. Another method was therefore used, in which the number of bursts of activity during a given period of time was used to measure the activity of the animals. This method was particularly effective, since each burst of activity is almost invariably a single discharge of one of a limited number of behaviour patterns, or else a recognisable combination of these patterns. The behaviour could therefore be expressed both quantitatively and qualitatively - quantitatively by recording the frequency of the occurrence of the bursts of activity in a unit period of time, and qualitatively by recording the relative frequency of the occurrence of each pattern. The total behaviour of a frog is therefore expressed as the number of times each pattern occurred during a unit^{period} of time. Fourteen behaviour patterns were distinguished, three of these being combinations of other patterns. These were classified into six groups. This classification, and the definition of the patterns, is set out below.

GROUP I. TRANSLOCATORY PATTERNS. Movement from place to place across the floor of the chamber.

1) Hopping. Animal lifted completely off the ground and

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propelled by the simultaneous extension of both hind limbs.

- 2) Crawling. Animal not completely leaving ground, and all four limbs engaged in carrying the body, with the hind limbs not moving together.

GROUP II. ESCAPE PATTERNS. Scrambling against the sides of the chamber. "Trying to get out" behaviour.

- 3) Escape Hopping. Hopping against the sides of the chamber, the animal completely leaving the ground.
- 4) Escape Crawling. Crawling in a vertical or semi-vertical position, with at least one limb on the side of the chamber and at least one limb on the ground.
- 5) Nosing. All limbs on the ground, and the nose pushed against the side of the chamber, as though trying to push through it.

GROUP III. READJUSTING PATTERNS. Change in orientation or posture without a change in place.

- 6) Turning. A turn of the body to face a new direction.
- 7) Bowing. Anterior part of the body raised or lowered by stretching or bending the fore-limbs.
Hind limbs not moved.

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- 8) Crouching. Whole body lowered onto ground, and limbs drawn up to the body.

GROUP IV. LIMB MOVEMENTS. Patterns only affecting individual limbs.

- 9) Digging. Shovelling movement of the hind limbs, used to bury animals in soil.
- 10) Leg Movements. Movements of hind and fore limbs that do not appear to be associated with any other pattern.

GROUP V. COMBINED PATTERNS.

- 11) Bow-crawl. Bow accompanied by a short crawl.
- 12) Turn-crawl. Turn accompanied by a short crawl.
- 13) Bow-turn. Simultaneous bow and turn.

GROUP VI. MOULTING PATTERNS. Consist mainly of each limb wiping the body surface within its reach, and passing the shed skin to the mouth.

- 14) The typical moulting pattern; the hind limb of one side wipes its area of skin, then the fore limb of the same side wipes its area, after which both eyes sink into their sockets and the mouth opens. One such cycle last about 5 seconds.

The patterns were recorded in the succession in which they occurred, and grouped into the successive two-minute intervals. This method of recording behaviour permitted

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an analysis to find if there were any general patterns in the occurrence of the single patterns, and it also allowed histograms, recording the frequency of the occurrence of the patterns throughout the period of observation, to be made.

The patterns were worked out in 23 frogs for some months, and then the observations on the effect of still and moving air were carried out on a fresh group of eight frogs, which had been taken in from the field one week previously. Each frog was observed for a one-hour period in the still- and moving-air chambers, set at the three chosen humidities. The still-air chambers were moving-air chambers with the baffle plates and air leads removed, and with the perforated top replaced by a glass cover, which was sealed to the dish with paraffin jelly. This cover contained a small hole through which the frog was inserted into the chamber at the beginning of an observation period. At other times this hole was covered by a small glass plate, which was sealed to the cover with paraffin jelly. KOH was used as the subtending solution in the 60% and 20% R.H. still-air chambers. The solution was held in dishes which were supported by plasticine stands so that the height of the level of their tops was the same height as the baffle units. The surface of the KOH solutions was thus held as

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near the frog as was possible, in order to bring about the greatest reduction in the extent of the microclimate that was possible in a still-air chamber. The perforated floor was placed on top of the dishes, and so the distance between the floor and the lid was the same in both types of chamber. The KOH solutions were replaced by distilled water for the 100%^{R.H.} still-air observations. The still-air chambers were well ventilated between each observation period, and were allowed to equilibrate for at least 24 hours before being used.

Each frog was left in water for at least half an hour before being put into a humidity chamber, in order to control the water level of the experimental animals, but it was not possible to empty the bladder with a cannula due to the small size of the cloacal opening. Consequently the control over the initial amount of water in the animals was ~~due~~ by no means complete. The weight lost by the frogs during every observation period was recorded in order to give some indication of the amount of water lost at the different humidities (see Appendix II). The behaviour of each frog was recorded one minute after it had been placed in the chamber.

The results of these observations on the activity of Rana are given in the following Section.

RESULTS

It was noted in the preceding section that the effects of moving air on the behaviour of Rana had to be investigated before critical work on this animal's humidity reactions in moving-air chambers could be carried out. To determine these effects, the behaviour of frogs in still and moving air at different R.H.'s was studied, but time did not permit any investigations to discover if any preference existed when air was passed into each half of a humidity chamber at different rates. The comparison of behaviour in still and moving air was, however, sufficient to show that great complications existed.

Since the effects of still and moving air were studied at different humidities, the data collected gave some information about the humidity reactions under these conditions, in addition to information about the effects of moving air on behaviour. Consequently both of these problems may be considered in this Section. Furthermore, the method that was employed in recording the behaviour also makes possible an account of the general characteristics of the behaviour of frogs in the humidity chambers. This Section will therefore be divided into three parts; the first dealing with the effects of moving air on behaviour, the second with the humidity reaction, and the

third dealing with the behaviour in the chambers as a whole.

Part 1. The Effect of Moving Air on the Behaviour of Rana natalensis.

The behaviour of Rana is expressed as the frequency of the occurrence of the behaviour patterns set out in the previous Section. The moulting patterns are omitted in this Section, since preliminary observations showed that differences in humidity or air movement did not affect the frequency of the occurrence of these patterns, and moulting was not observed in the final group of eight frogs.

Table 4 shows the number of times that the behaviour patterns, grouped according to the classification in the previous Section, occurred in the one-hour observation periods, the total for all eight frogs being given. Figure 9 shows these data graphically.



Table 4. The Frequency of Occurrence of the Behaviour Patterns of Rana natalensis, grouped.

Condition		Trans-locatory Patterns	Escape Patterns	Read-justment Patterns	Limb Movements	Combined Patterns
Still air	100% R.H.	55	288	490	17	65
	60% "	235	836	750	56	161
	20% "	346	1692	939	28	209
Moving air	100% "	78	295	303	8	59
	60% "	75	304	264	25	59
	20% "	182	694	491	16	112

Figure 9. 1-5. Graphs of the frequency of occurrence of the behaviour patterns of Rana natalensis, grouped. The means of the totals of the eight frogs are plotted, and the length of each vertical line represents twice the standard error of that point.

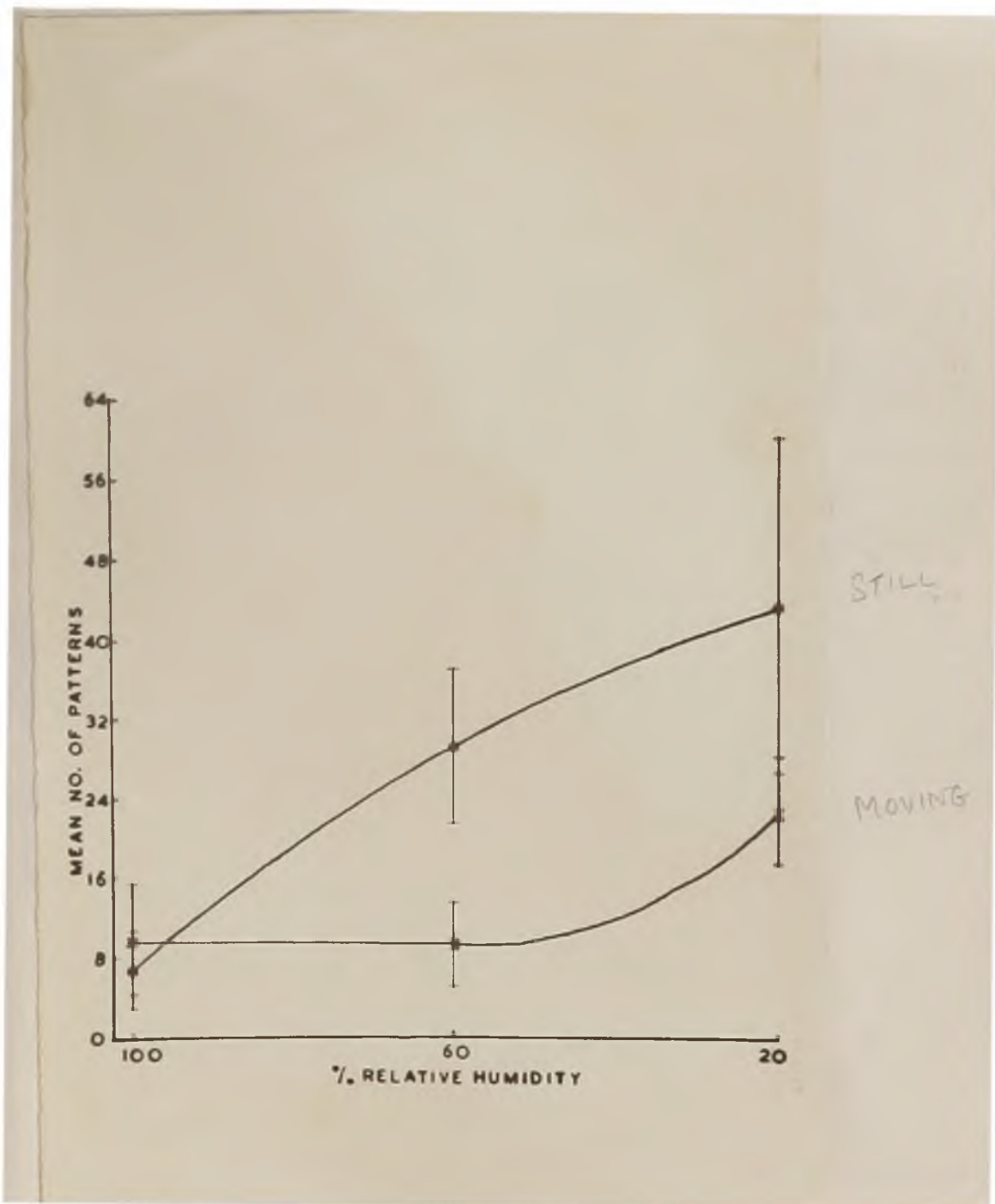


Fig. 9. 1. Translocatory patterns.

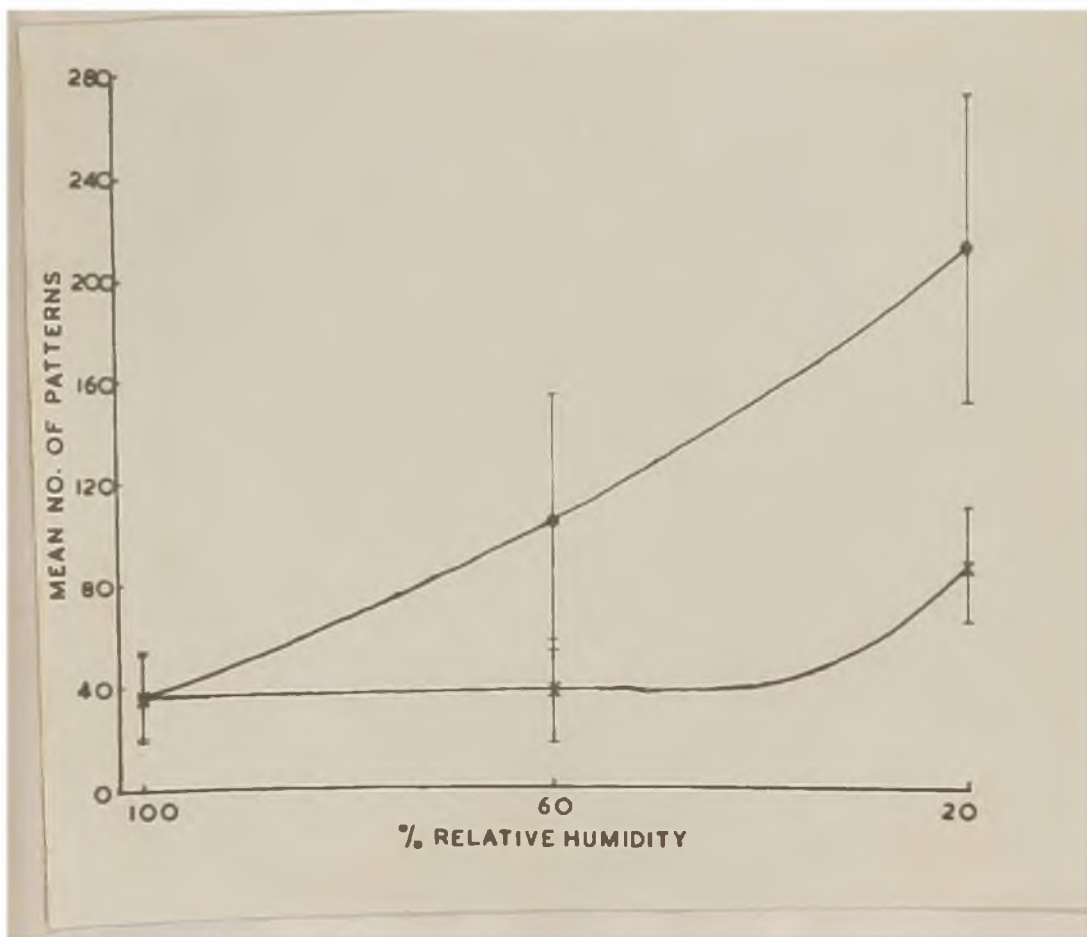


Fig. 9. 2. Escape patterns.

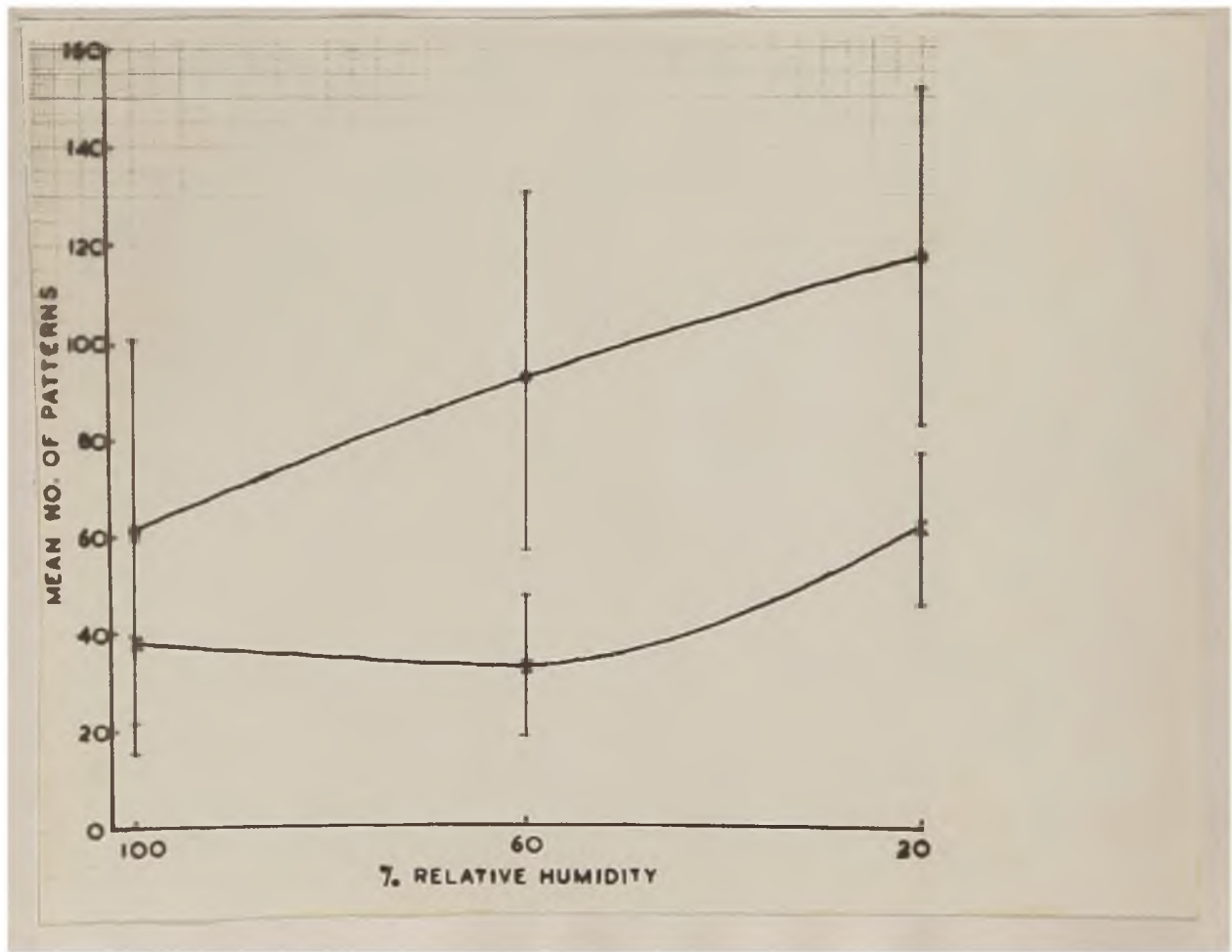


Fig. 9. 3. Readjusting patterns.

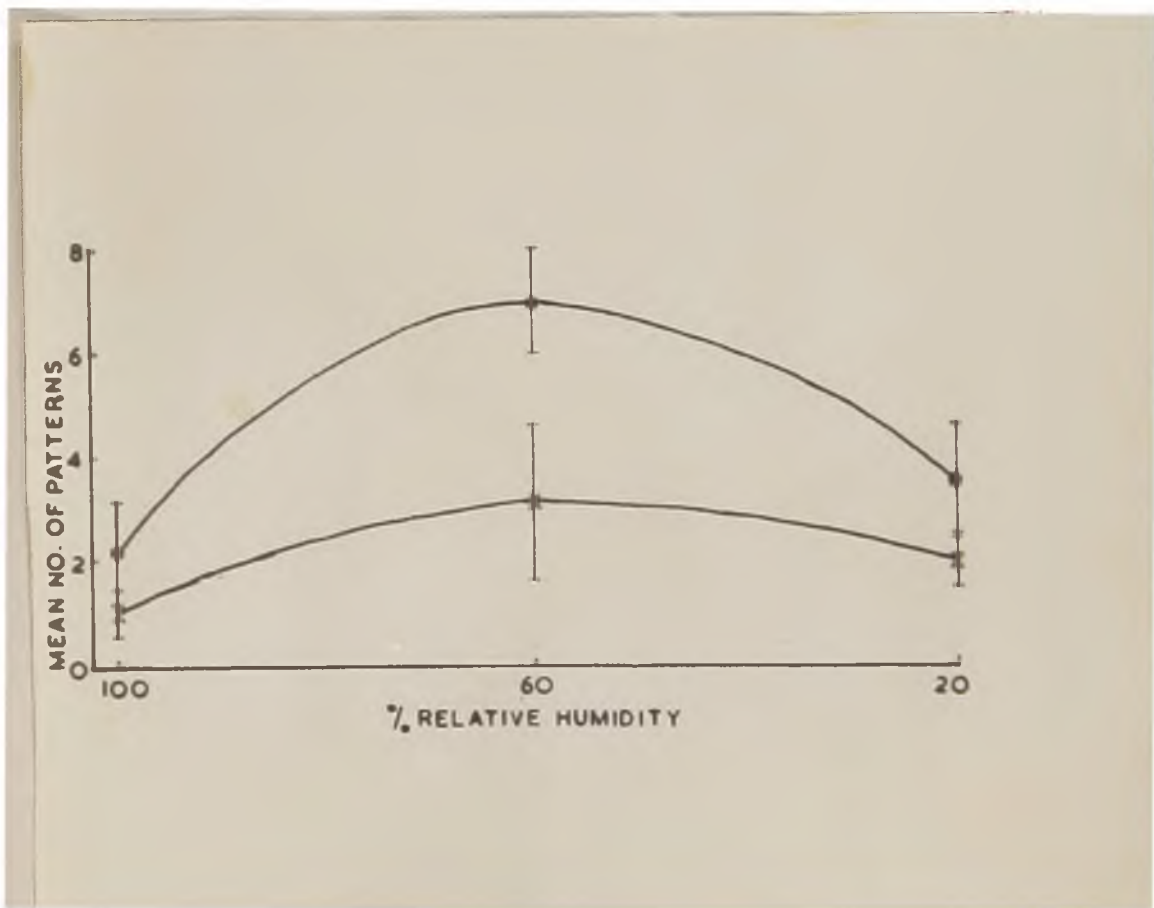


Fig. 9. 4. Limb movements.

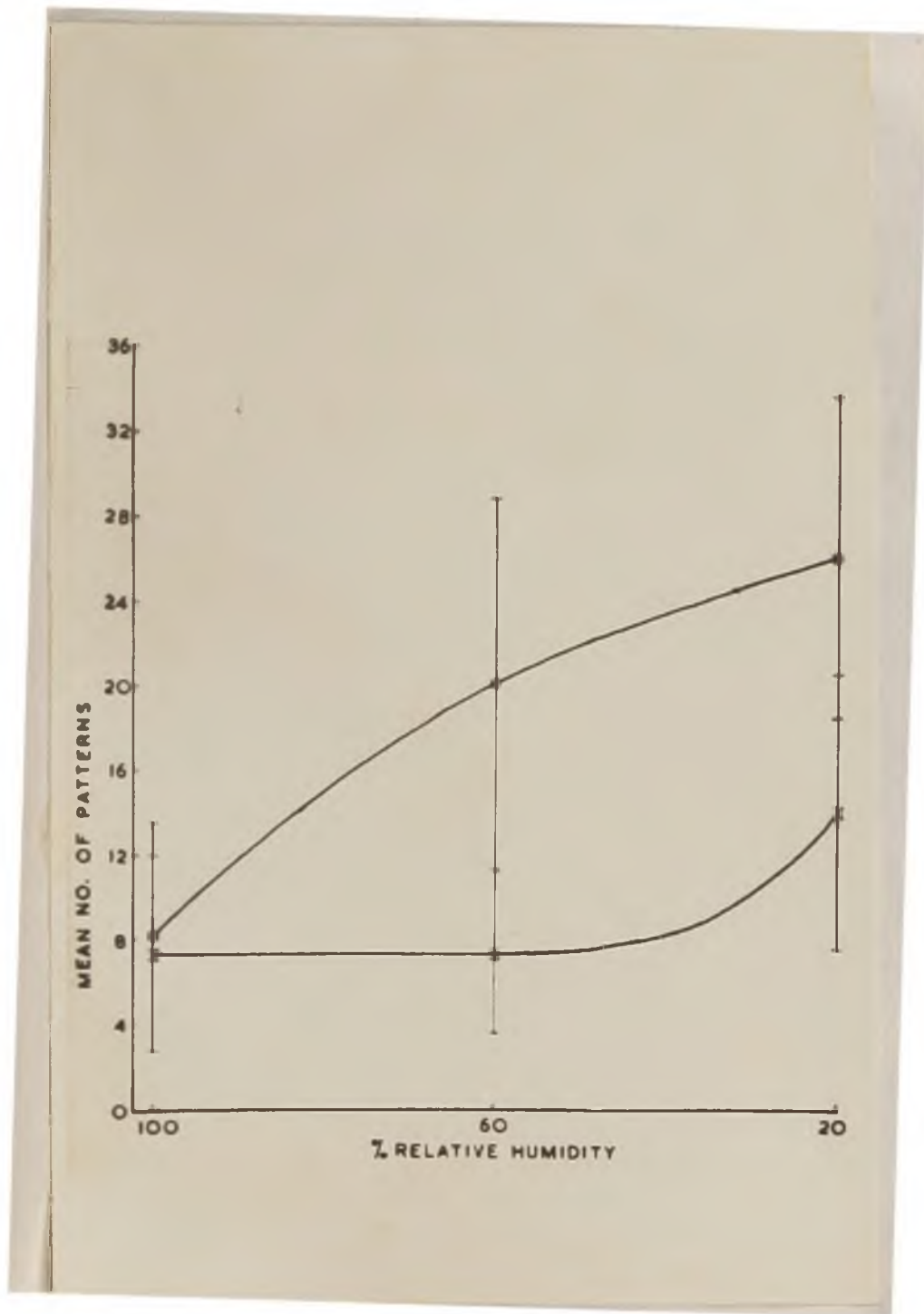


Fig. 9. 5. Combined patterns.

The data of Table 4 are expanded in Table 5 to show the behaviour patterns individually.

Table 5. As for Table 4, with the Behaviour Patterns shown individually.

	R.H.	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Bc	Tc
Still air	100%	40	15	208	80	0	164	326	0	17	0	50	12	3
	60%	133	102	326	373	137	254	489	7	49	7	115	39	7
	20%	205	141	643	785	264	290	688	1	24	4	147	55	7
Moving air	100%	48	30	183	108	4	90	208	5	8	0	48	9	2
	60%	60	15	110	192	2	88	170	6	12	13	45	13	1
	20%	122	60	248	443	3	160	308	23	16	0	75	32	5

H = Hop; C = Crawl; Eh = Escape Hop; Ec = Escape Crawl;
 N = Nosing; T = Turning; B = Bowing; Cr = Crouching;
 L = Leg Movement; D = Digging; Bt = Bow-turn; Bc = Bow-crawl;
 Tc = Turn-crawl.

Tables showing the full behaviour of each individual frog are given in Appendix I.

Histograms, to show variations in the frequency of the occurrence of the patterns of each group during the period of observation, are given in Fig. 10, 1 to 6. For the sake of clarity, the hour periods are only divided into three sections of 20 minutes each.

These results show a conspicuous difference between behaviour in still and moving air. The difference is most clearly shown in Fig. 9. The use of Fisher's t test in

Figure 10. 1-6. Histograms shownig the frequency of occurrence of the grouped behaviour patterns of Rana natalensis during 20-minute intervals in one-hour observation periods.

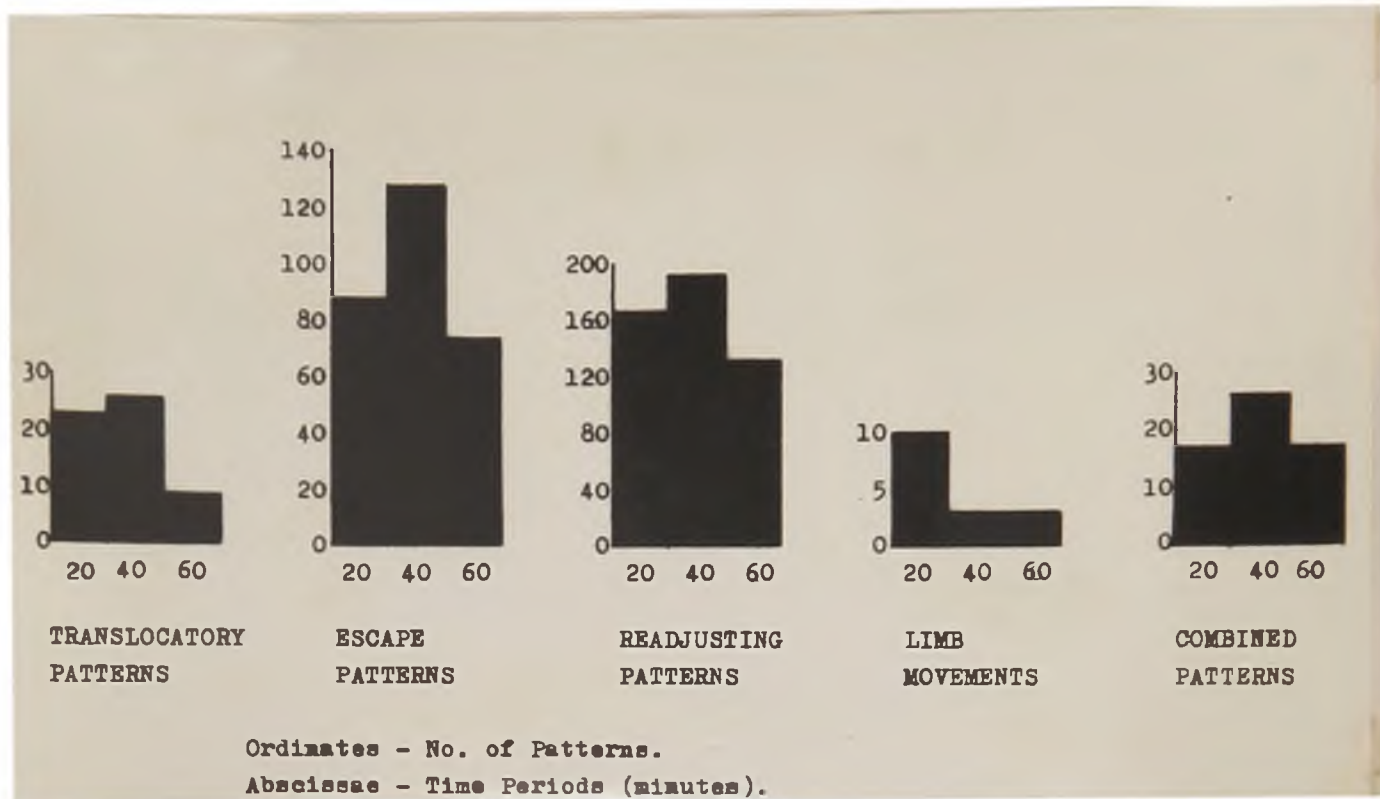


Fig. 10. 100% R.H., Still Air.

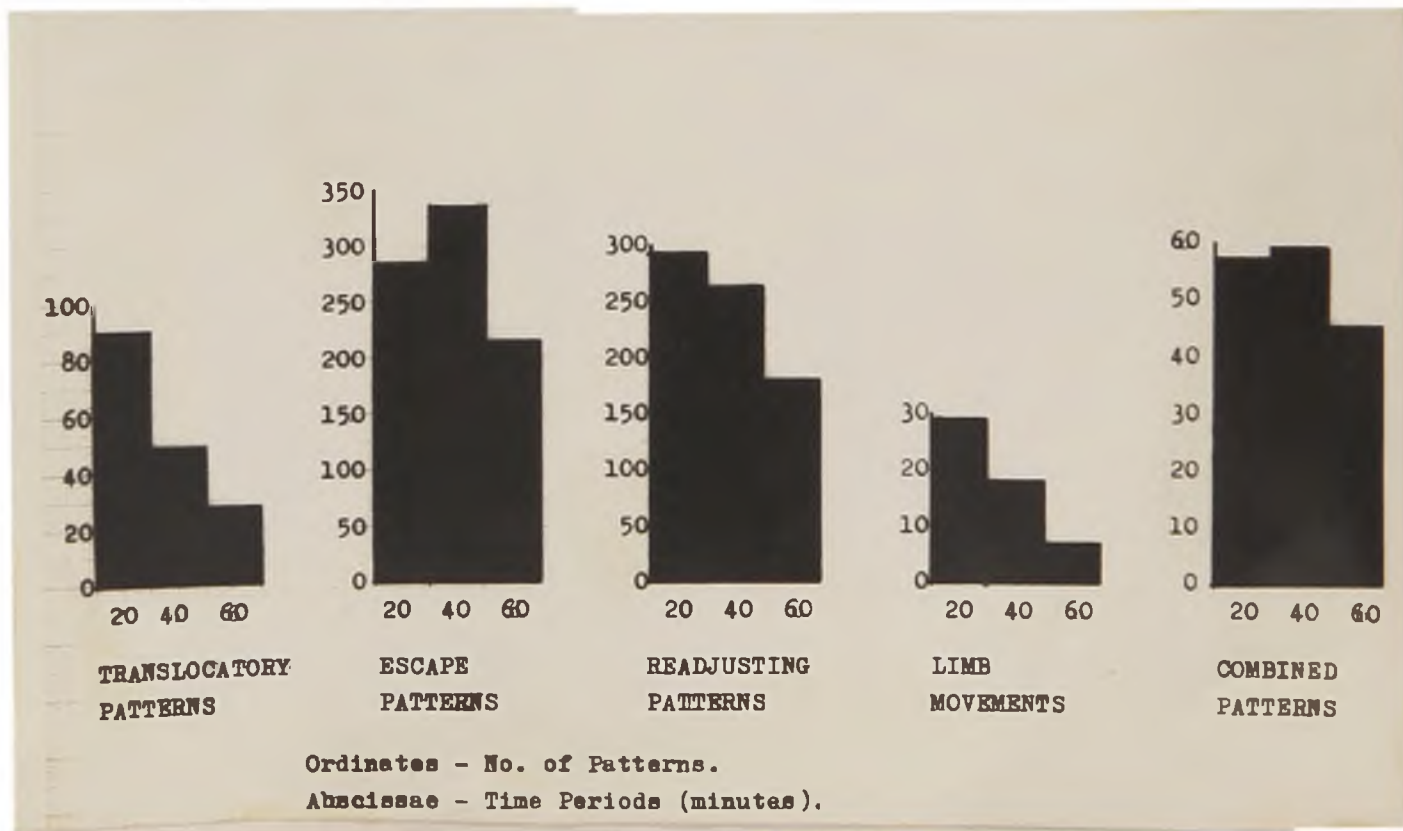


Fig. 10. 2. 60% R.H., Still Air.

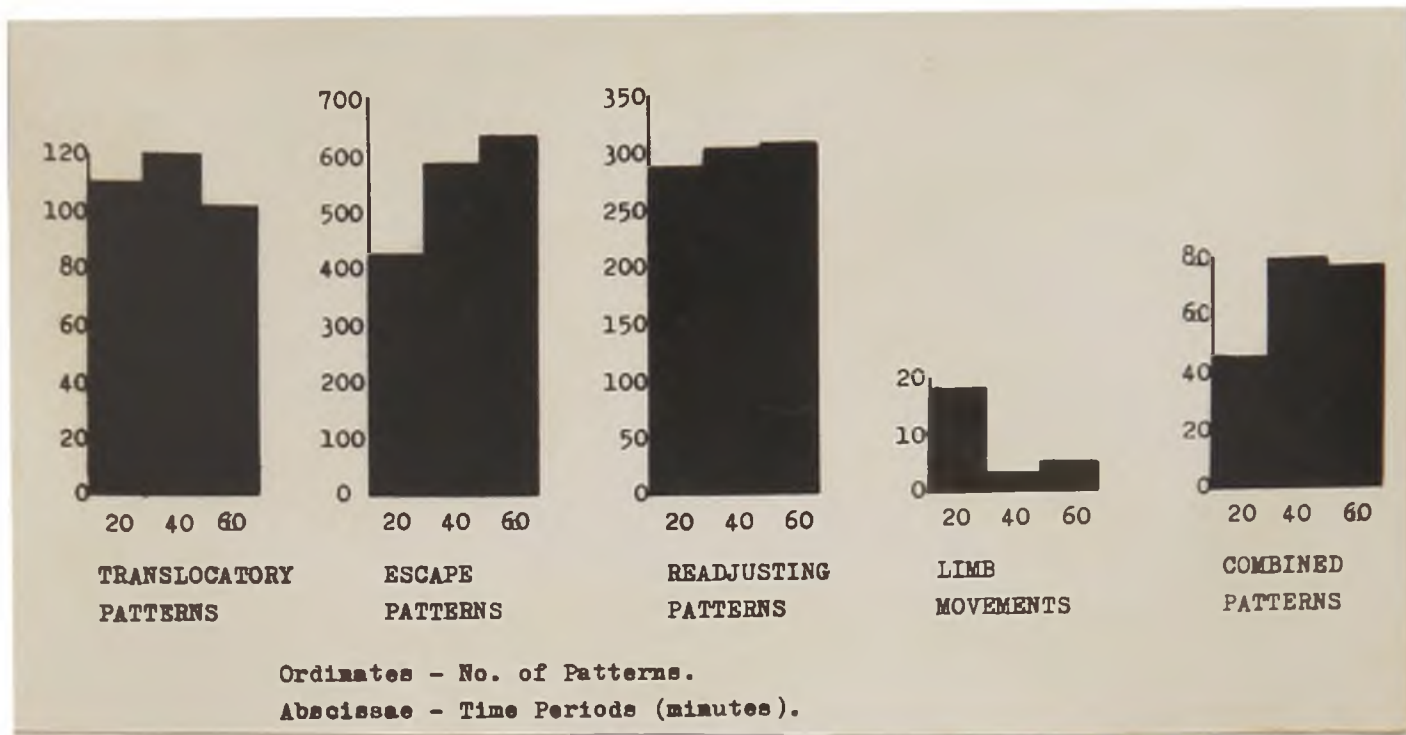


Fig. 10. 3. 20% R.H., Still Air.

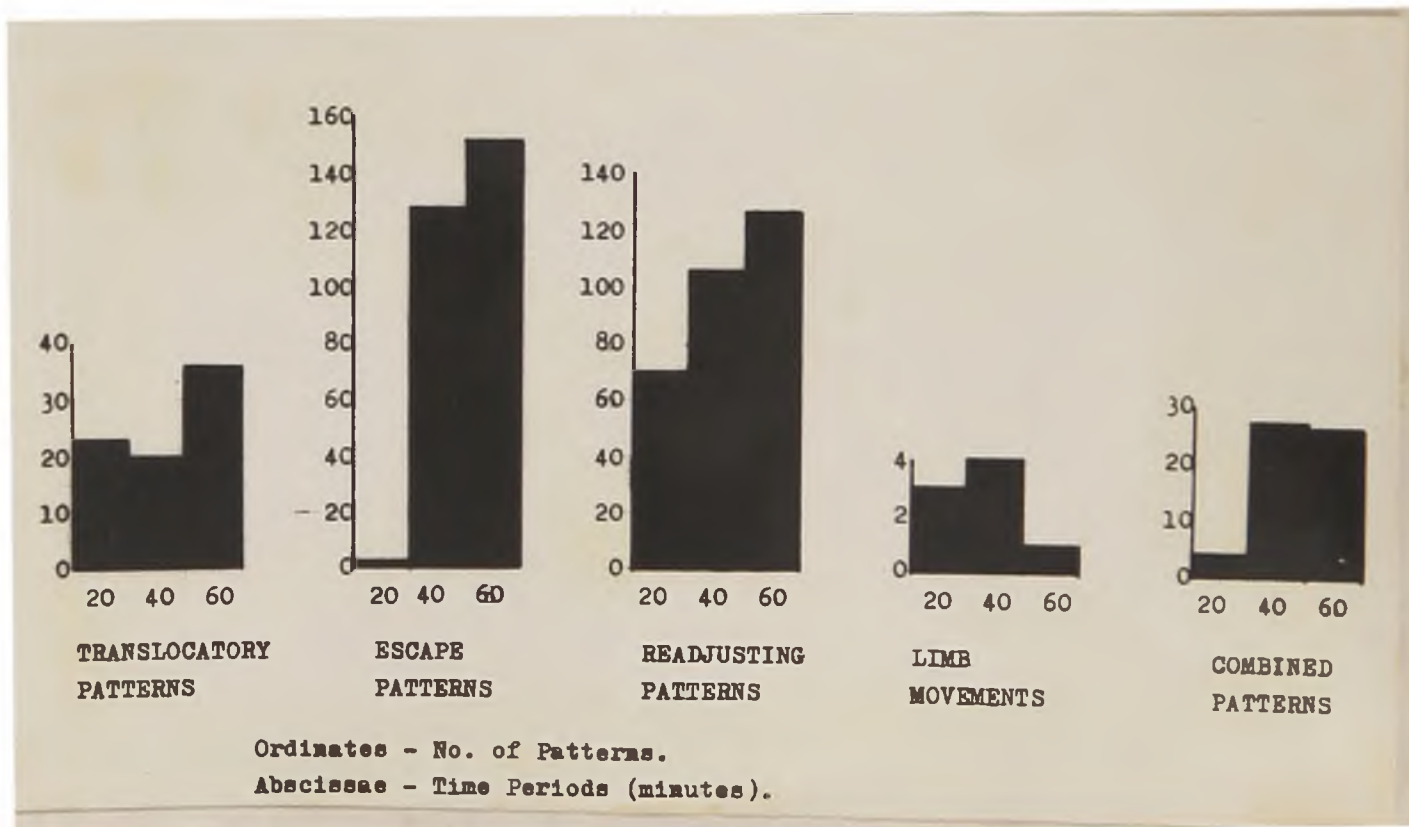


Fig. 10. 4. 100% R.H., Moving Air.

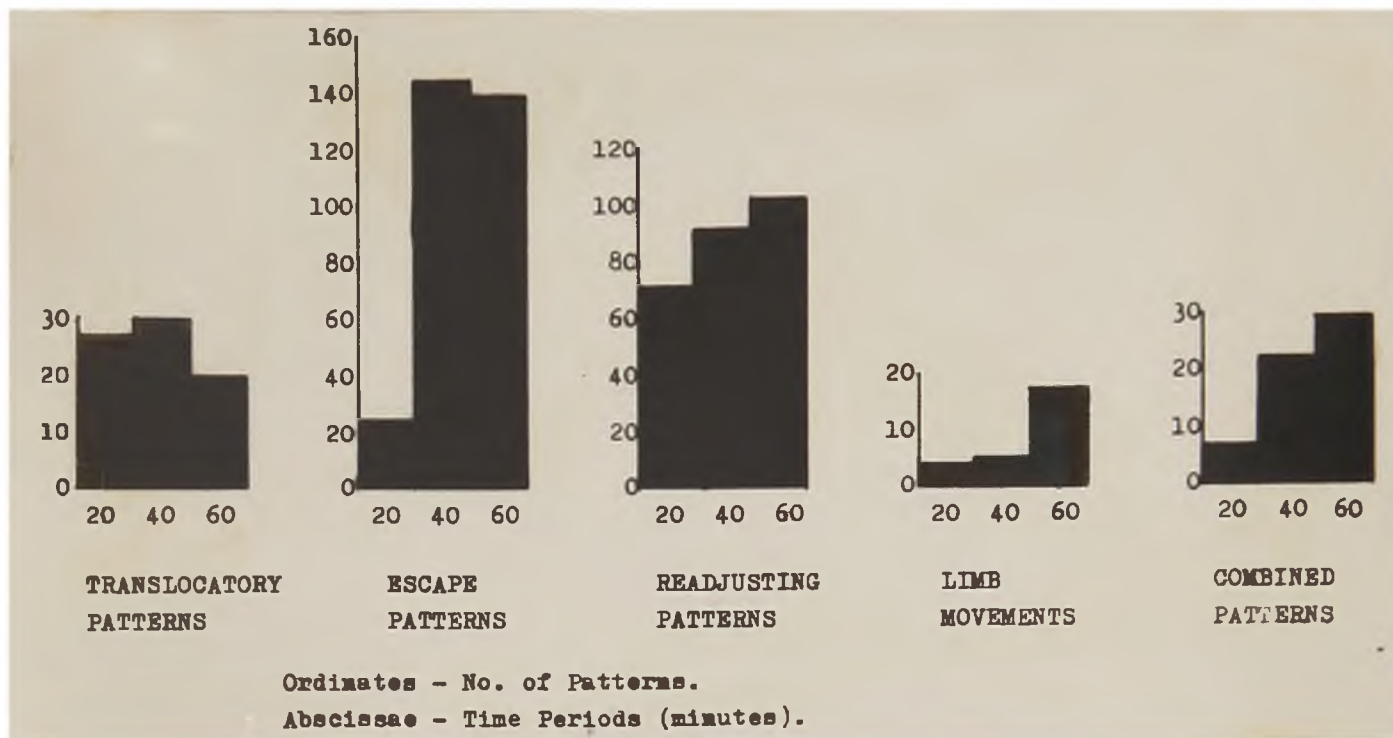


Fig. 10. 5. 60% R.H., Moving Air.

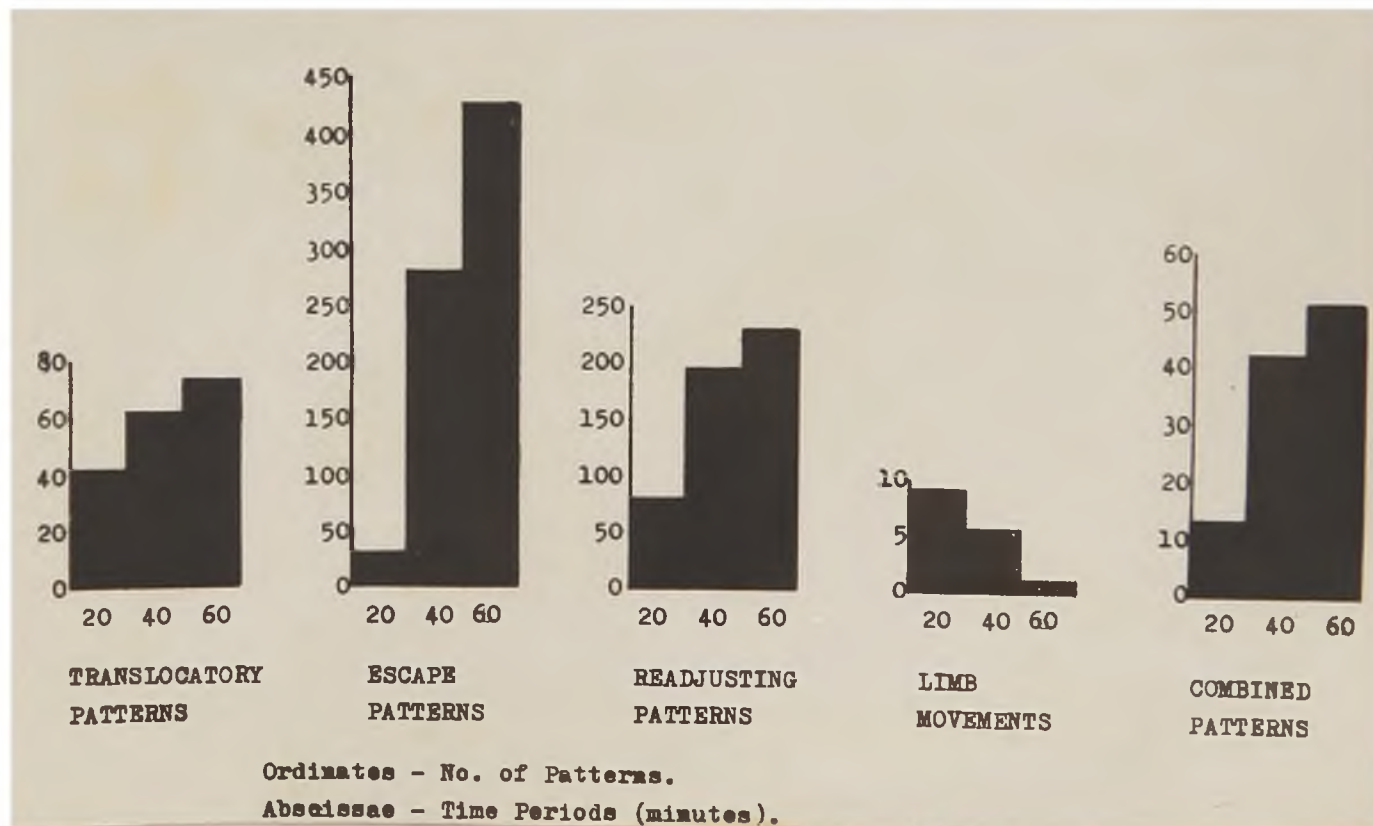


Fig. 10. 6. 20% R.H., Moving Air.

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testing for significance between behaviour in still and moving air at 60% and 20% R.H. gives the following values for P :-

60% R.H.	Patterns	
	Translocatory	$P > 0.05, < 0.1$
	Escape	$P > 0.2, < 0.3$
	Readjustment	$P > 0.1, < 0.2$
	Limb Movements	$P > 0.05, < 0.1$
	Combined	$P > 0.2, < 0.3$
20% R.H.		
	Translocatory	$P > 0.3, < 0.4$
	Escape	$P > 0.1, < 0.2$
	Readjustment	$P > 0.3, < 0.4$
	Limb Movements	$P \approx 0.3$
	Combined	$P > 0.3, < 0.4$

While none of these differences are in themselves significant, the consistent pattern shown in the graphs makes it unwise to assume that a greater number of observations would not produce a significant difference. Results obtained with different animals in the preliminary work supports this conclusion. A comparison of the behaviour of 18 frogs in still and moving air at 100% R.H., taken during two-hour observation periods, shows that even at this critical humidity, a considerable difference exists. These results are summarised in Table 5.

Table 5. The effect of Still and Moving Air on the Behaviour of 18 Frogs used in preliminary work at 100% R.H. during two-hour observation periods.

Patterns	No. of Occurrences of Patterns		P
	Still Air	Moving Air	
Translocatory	855	469	$\approx 0.01^*$
Escape	1,959	1,202	$> 0.1, < 0.2$
Readjusting	2,294	1,593	$> 0.1, < 0.2$
Limb Movements	191	115	$> 0.05, < 0.1$
Combined	212	163	$> 0.1, < 0.2$

* indicates significance

This table shows that there is a highly significant difference between translocatory movement in still and moving air, and that the other differences are not negligible.

The results therefore indicate that moving air depresses the amount of activity at all humidities. Furthermore, in addition to these quantitative differences between behaviour in still and moving air, the histograms show that a qualitative difference in behaviour exists as well. The escape behaviour in moving air is shown to be strongly inhibited at the beginning of the period in all humidities, an effect which is not found in still air. Another qualitative difference is to be seen in the consistently different shapes of the activity curves in still and moving

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air for all patterns excepting those included in limb movements (Fig. 9). In still air a tendency is shown towards a proportionate increase in activity with a lowering of the humidity from 100% R.H., while in moving air this tendency is delayed until the humidity falls below 60% R.H. (see Part 2).

The data therefore indicate that moving air influences the behaviour of Rana both qualitatively and quantitatively, under the conditions used. The value of using moving air in controlling the microclimate of the experimental animals therefore becomes questionable in view of its effect on the behaviour of these animals. This matter will receive further treatment in the next Section.

Part 2. The Humidity Reaction of Rana natalensis.

In Fig. 9 a consistent trend towards increased activity with lowered humidities may be seen in all behaviour patterns, except those included in limb movements, in spite of the complications of the effects of microclimates and moving air. Fisher's t tests comparing the activity of different humidities in still air give the following values of P :-

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Pattern Group	100% - 60% R.H.	60% - 20% R.H.	100% - 20% R.H.
Trans-locatory	$>0.01, <0.02^*$	$>0.5, <0.6$	$>0.05, <0.1$
Escape	≈ 0.1	≈ 0.2	$>0.02, <0.05^*$
Read-justing	$>0.3, <0.4$	$>0.6, <0.7$	$>0.3, <0.4$
Limb Movements	$\approx 0.01^*$	$>0.02, <0.05^*$	$>0.3, <0.4$
Combined	$>0.05, <0.1$	$>0.6, <0.7$	$>0.05, <0.1$

Similar tests for significance between the activity at 60% and 20% R.H. in moving air gave the following values of P :-

Pattern Group

Translocatory	$>0.05, <0.1$
Escape	$\approx 0.05^*$
Readjusting	$>0.1, <0.2$
Limb Movements	$>0.5, <0.6$
Combined	$>0.3, <0.4$

In each pattern group in still air, excepting the readjusting and combined groups, there is a significant difference in at least one of the comparisons, and in moving air, escape behaviour is significantly different in 60% and 20% R.H. Results obtained with different animals in the preliminary work confirms this difference. Using six frogs, in still air the combined patterns of the trans-locatory and escape groups showed the following differences

at different humidities, using Fisher's t test.

<u>Humidity</u>	<u>No. of occurrences of patterns</u>		
100% R.H.	746	} P > 0.2, < 0.3	} P < 0.01*
60% R.H.	1,558		
20% R.H.	4,398		

The behaviour of these animals differed significantly at 60% and 20%, and at 100% and 20% R.H. Another group of six frogs showed a highly significant difference between behaviour at 100% and 20% R.H. in still air. In the combined translocatory and escape groups of patterns, these patterns occurred 134 times at 100% R.H., and 2,909 times at 20% R.H. The t test gave $P < 0.01$. Indeed, comparing the behaviour of the final eight frogs with the behaviour of the frogs used in the preliminary work, the eight frogs were exceptional in that their humidity reaction was so poorly shown. Unfortunately, the behaviour of the animals used in the preliminary studies cannot be analysed closely, since their behaviour was recorded at the time when the behaviour patterns were still being worked out.

It may therefore be concluded from the results in this Part that the existence of a humidity reaction in Rana natalensis in still-air chambers is established, and this reaction was also shown in moving-air chambers when the humidity fell below 60% R.H. These results will be discussed in the next Section.

Part 3. General Characteristics of the Behaviour of Rana natalensis in Humidity Chambers.

The behaviour patterns of Rana did not all occur at the same relative frequency; some were very much more common than others. Table 6 gives the proportion in which the patterns of each group occurred out of the total number of patterns, and Table 7 gives these proportions for each individual pattern. The proportions are expressed as a percentage of the total number of patterns.

Table 6. The Relative Frequency of the occurrence of each Group of Behaviour Patterns of R. natalensis, expressed as a percentage of the total number of Behaviour Patterns

Condition		Pattern Groups				
		Trans-locatory	Escape	Read-justment	Limb Movements	Combined
Still air	100%	6.0	31.4	53.5	1.9	7.2
	60%	11.5	41.0	36.8	2.8	7.9
	20%	10.8	52.6	29.3	0.8	6.5
Moving air	100%	10.5	39.6	40.8	1.1	8.0
	60%	10.4	41.7	36.3	3.5	8.1
	20%	12.2	46.4	32.8	1.1	7.5

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R.H.		Trans-locatory	Escape	Read-justment	Limb Movements	Combined
Still air	100%	6.0	31.4	53.5	1.9	7.2
	60%	11.5	41.0	36.8	2.8	7.9
	20%	10.8	52.6	29.3	0.8	6.5
Moving air	100%	10.5	39.6	40.8	1.1	8.0
	60%	10.4	41.7	36.3	3.5	8.1
	20%	12.2	46.4	32.8	1.1	7.5

Table 7. As for Table 6, but showing individual Behaviour Patterns.

Condition	Patterns													
	R.H.	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Be	Tc
Still air	100%	4.4	1.6	22.7	8.7	0.0	17.9	35.6	0.0	1.9	0.0	5.5	1.4	0.3
	60%	6.5	5.0	16.0	18.3	6.7	12.5	24.0	0.3	2.4	0.4	5.6	1.9	0.4
	20%	6.4	4.4	20.0	24.4	8.2	7.8	21.4	0.1	0.7	0.1	4.6	1.7	0.2
Moving air	100%	6.5	4.0	24.6	14.5	0.5	12.1	28.0	0.7	1.1	0.0	6.5	1.2	0.3
	60%	8.3	2.1	15.1	26.3	0.3	12.1	23.4	0.8	1.7	1.8	6.2	1.8	0.1
	20%	8.2	4.0	16.6	29.6	0.2	10.7	20.6	1.5	1.1	0.0	5.0	2.2	0.3

H = Hop; C = Crawl; Eh = Escape Hop; Ec = Escape Crawl; N = Nosing;
 T = Turning; B = Bowing; Cr = Crouching; L = Leg movements;
 D = Digging; Bt = Bow-turn; Be = Bow-crawl; Tc = Turn-crawl.

Table 6 shows that in still air the proportion of escape patterns becomes greater as the R.H. is lowered, while the proportion of readjusting patterns decreases. The other patterns remain more or less unaffected by different humidities. In moving air the same tendency is shown, but is comparatively poorly marked - another instance of the effect that moving air has on the quality of the behaviour of Rana. Table 7 shows that escape crawling, and to a lesser extent, nosing, are the particular patterns which increase in frequency at lower humidities in still air, and that turning becomes correspondingly less frequent. In moving air, the increase in escape crawling

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with lowered humidity is apparently to some extent counterbalanced by a decrease in escape hops. Nosing is very infrequent in moving air at all humidities. The increase in escape crawling is also counterbalanced by a decrease in bowing in the lower humidities.

Out of the total number of recorded movements of all the eight frogs used in the final experiments, under all conditions, 98% were made within a distance of 4 cms. from the side of the chamber. The great bulk of the activity that was not included in the actual escape patterns themselves was therefore concerned with movement from one region of the side of the chamber to another, made by "hugging" the side; or else concerned with readjustments towards a single region of the side. The behaviour of the frogs in the humidity chambers may therefore be said to have been almost entirely concerned with attempts to get out of the chambers. No evidence was shown of a tendency for the proportion of the activity at the sides to increase at lower humidities; at 100% R.H. the animals still showed activity almost entirely at the side of the chambers.

Concerning activity in the central region, hopping was the most common pattern, constituting 29% out of the total number of patterns in this region, while crawling only accounted for 7% of the total number of patterns. 25% of

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the patterns were turns, and 17% were bows. Bowing and turning were often repeated many times in the centre of the chamber, without the animal leaving the same place. An extract from the behaviour of frog E in still air at 100% R.H. shows such behaviour.

Time interval (minutes)	Behaviour patterns (symbols as in Table 7).
32	H Bt T B B
34	T T B T T B T
36	T T
38	T
40	T
42	T Tc H

This behaviour could be interpreted as viewing the surroundings, or possibly as movements for sensing humidity changes or air currents. Repetitive bowing up and down also occurred in this "viewing behaviour" in the central area of the chamber. Frog G in still air at 100% R.H. gives an example of this up and down bowing.

Time interval	Behaviour patterns (<u>B</u> = bowing downwards; B = bowing upwards, other symbols as in Table 7).
12	B <u>B</u> B <u>B</u> Bc <u>B</u> B <u>B</u> B <u>B</u> B
14	B <u>B</u> B <u>B</u> B H....

The behaviour in the middle area of the chamber was thus largely concerned with movement from one part of the side of

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the chamber to another, mainly by hopping; and apparently with viewing the chamber as a whole.

Turning to behaviour at the side of the chamber, escape crawling and bowing were the most common patterns, together constituting nearly 50% of the total behaviour near the side, while escape hopping was the only other common pattern. These three patterns occurred in a characteristic organisation in the behaviour of individual animals. Typical behaviour at the side of the dish consisted of the alternation of escape hops and bows. An excerpt from frog E's behaviour in moving air at 100% R.H. shows this type of behaviour.

Time Interval	Behaviour Patterns (symbols as in Table 7).
34	Eh B Eh B Eh B Eh B Eh B Eh B Eh B Eh B
36	Eh B Eh Bt Eh Bt Eh B B Eh B Eh Bt Eh B Eh B Eh B Eh Eh B Eh B Eh B Bt Eh Bt Bt Bt Eh B
38	T Eh B B Eh B Eh Bt B Eh B Eh B Eh B Eh B Eh B Bt Eh B T Eh B Eh B Eh B

In some cases this alternating behaviour can completely dominate a frog's activity. In preliminary observations on one frog at 100% R.H., lasting two hours, these two patterns occurred 851 times out of the total number of 974 behaviour patterns. When alternating between escape hops and bowing, the frog ends an escape hop crouching on the floor, usually

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less frequently than the other escape patterns, constituting only 4.5% of the total activity. Like escape crawling, this pattern normally occurs repetitively. Frog G in still air at 20% R.H. gives an example of this behaviour

Time interval	Behaviour patterns (symbols as in Table 7).
16	Eh B T T Eh B Bt B T H Bc Bt T H H H T T N H H N N N
18	N N L H H N C B Bc Eh Eh L
20	N N N N N H H H E C C N N H
22	Bt T H H B Bt Eh B C C Eh H N N N N N
24	N N H N N H N N N N L L H H H N L C N N N H H H B B S T B

The appearance of nosing is quite different from that of escape hopping and escape crawling; the animal is attempting to push through the side of the chamber, not attempting to get over it. This nosing behaviour might be due to the translucence of the side of the chamber, but the side of the chamber had to be translucent to avoid complications due to shadows.

The only other individual patterns requiring comment are the crouching and digging patterns. The crouching attitude is typical of severely desiccated Rana, and it has been observed in Bufo in similar straits. Crouching is most common in moving air (Table 7), and it was found in the early part of the observation periods, its peak occurring

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in the 18 - 20 minute interval. Six of the eight frogs crouched in the first two-minute interval in moving air. Typical behaviour associated with crouching is shown in the following excerpt of frog A's behaviour in moving air at 20% R.H.

Time interval	Behaviour patterns (symbols as in Table 7)
2	Eh B Bt B Eh B Cr
4	L L
6	L H Cr
8	T Eh B
10	H Eh Bt T
12	Bt
14	Cr H

During this period the patterns were discharged at the normal intensity, but the intervening pauses were prolonged. Escape patterns are infrequent in this initial period, as can be seen in Fig. 10, and it seems that crouching is associated with the inhibition of the escape patterns at the beginning of the observation periods in moving air. The crouching behaviour cannot be said to be due to desiccation, because it occurs at the beginning of the observation periods, and so it must be a reaction to the new environment of the chamber. This reaction is very much more common in moving air (Table 5), and its occurrence gives another

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instance of moving air disturbing the behaviour of these animals.

Digging movements in the humidity chambers showed no apparent difference to these movements in the vivaria, and they typically occurred repetitively. An excerpt from the behaviour of frog H in moving air at 60% R.H. shows these movements.

Time interval	Behaviour patterns (symbols as in Table 7)
2	H T H
4	C
6	D D D D D D L
8	D D D D
10	D C D C

Such digging movements are normally made when a frog has its hind limbs in contact with the side of the chamber. It was anticipated that digging would be more frequent at the lowest humidity, since digging appears to be an adaptation for escaping from drying air, but Table 5 shows that this was not the case.

Of the combined patterns, as can be seen in Table 7, the pattern most closely associated with readjustment, the bow-turn, was much more common than the others, which contained a translocatory element as well as the readjustment element. The above excerpts from the behaviour of these frogs show that the bow-turn was freely interchangeable with

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bowing.

Considering the behaviour of the frogs in the humidity chambers as a whole, it may be said that practically all the activity was carried on near the side of the chambers, and that escape patterns were predominant, with the associated readjusting patterns typically alternating with individual or grouped escape patterns. Translocatory patterns were comparatively infrequent. Limb movements were not common, and digging did not increase with lower humidities. The combined patterns are essentially readjustment patterns.

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DISCUSSION

It is difficult to account for the depressing effect of moving air on the activity of Rana. If the function of the humidity reaction is to keep the frogs in the most humid region available, then it would be expected that the reaction would be all the stronger in moving air, where the probability of desiccation becomes greatly increased. In the absence of such an effect, moving air does not even raise the frequency of the occurrence of the protective patterns of digging or crouching to any marked degree. Nor was any evidence for the presence of an anemotaxis found when a jet of air was turned on localised parts of these frogs. More information from different sources is required before the effect of the moving-air chambers on the behaviour of Rana can be understood. For instance, it should be taken into consideration that the air passing through the baffle plates made a considerable noise. The effect of noise on behaviour in still air should be investigated by placing some sort of buzzer in the chamber. Indeed, since the air only passes the frog at 0.4 m.p.h. (see Apparatus and methods), it seems very surprising that such a slight air movement has such a pronounced effect, especially when the conditions in the field are considered. The

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possibilities of other forms of physical stimulation affecting the behaviour must be investigated very carefully before further work can proceed.

In view of the disturbing effect of the moving-air chambers on the behaviour of Rana, the position regarding the best rate of the flow of air entering the chamber must be reconsidered. In conducting the foregoing experiments, an air flow was used which was sufficient to reduce the microclimate to such an extent that its effect on the surrounding R.H. could not be measured accurately by the recording apparatus. If the data used to determine this air flow (see Apparatus and Methods) are plotted, the curve shown in Fig. 11 is obtained. It is evident from this curve that if more precise and extensive determinations were made, an exponential curve would be obtained. It follows that the present air flow in use could be enormously reduced without there being any serious increase in the extent of the microclimate. While it was as well to investigate the effects of a very high air flow on the behaviour of Rana in the humidity chambers in the preliminary work, in order to get as big a contrast as possible between the still-air and moving-air conditions, it seems that the most suitable rate of air flow to be used should be determined in a better way. Investigations must be carried out to see whether a gradual increase in the amount of air passing through the chamber

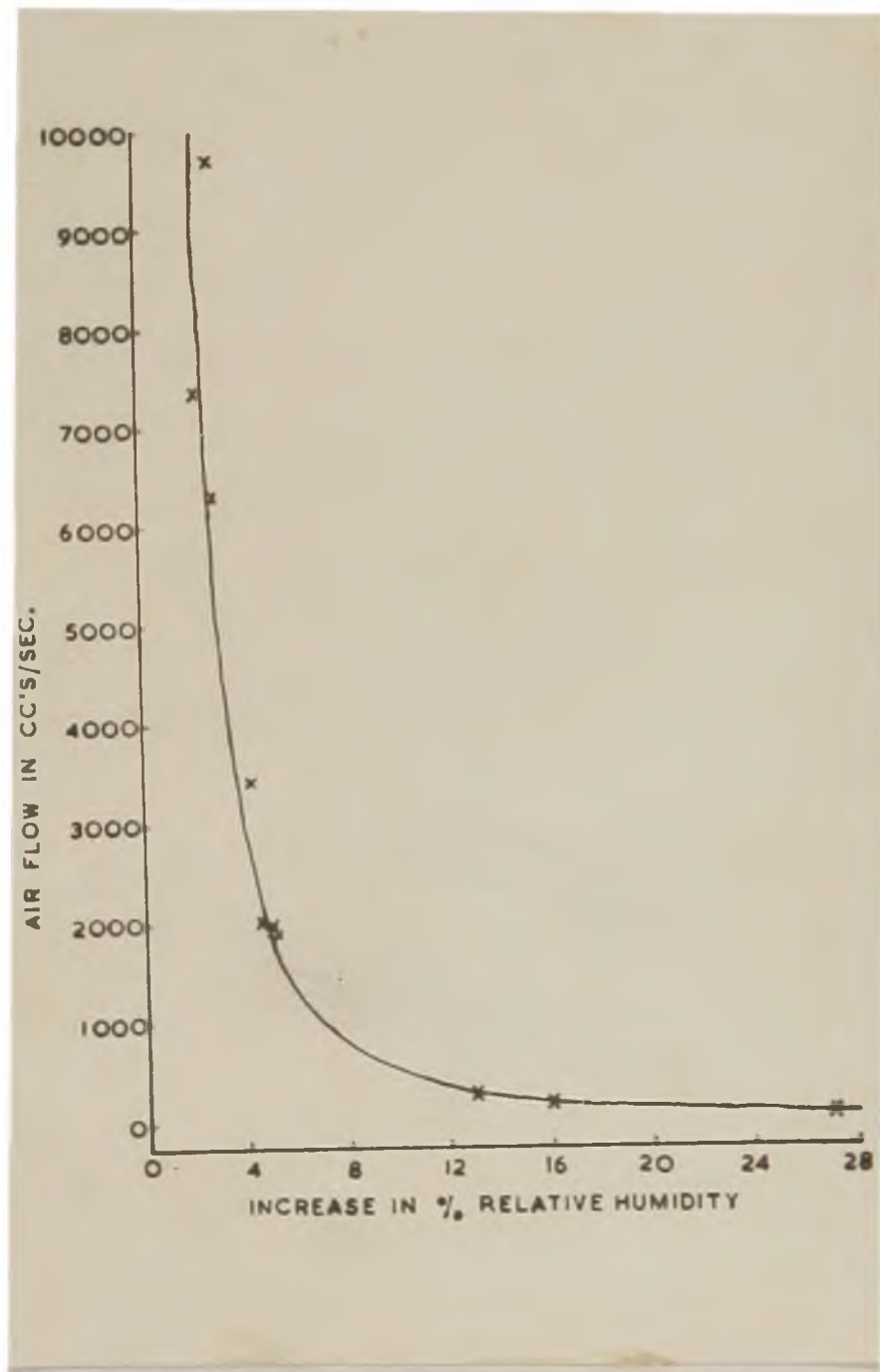


Fig. 11. The effect of moving air on the extent of the microclimate (increase in % R.H.) of Rana natalensis.

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brings about an increasingly clearly defined humidity reaction, due to the reduction in the microclimate, until a point is reached where the inhibiting effect of moving air begins to show its presence. If such a point can be determined, the conditions producing it will be of prime importance in deciding upon the air flow to be used. Reference should also be made to data collected on the effect of moving air on the extent of the microclimate (e.g. Fig. 11), rather than to the reliability of the recording apparatus being used, when deciding upon the correct air flow to be used in the behaviour experiments. In both of the conditions used in the present work, however, the presence of a humidity reaction has been shown, and there is therefore every reason to hope that a successful compromise between the two extremes will produce a condition in which the humidity reaction will be shown in a sufficiently well defined state to allow precise experiments to be carried out on it.

Turning to the nature of the humidity reaction, it may be seen from Fig. 9 that, excluding the limb movements, activity is raised by a lowering of the humidity. An increase in activity, whether it consists of an increase in the number of translocatory patterns, or of the patterns associated with escape, would raise the probability of a frog leaving an area in which it is more active, and would

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tend to confine it to an area in which it is less active. The humidity reaction would therefore tend to lead to an aggregation of frogs in the most humid surroundings available, through the effect of increasing the activity in all surroundings outside the most humid. The reaction is therefore a kinesis (Fraenkel and Gunn, 1940; Ewer and Bursell, 1950), in which the activity varies in response to variations in the intensity of stimulation, so that it is an undirected reaction. As it is brought about by changes in the level of activity, the reaction is a simple activity orthokinesis. Owing to the discontinuous behaviour of Rana, the speed orthokinesis and klinokinesis of the invertebrates has no parallel in these animals, for, while turning behaviour is common enough in Rana, it is equivalent to the "virtual inactivity" described by Gunn and Pielou (1940) in Tenebrio. The readjusting patterns and the limb movements could all be classified as "virtual inactivity" in the terminology of these authors, but the distinction of "activity" (roughly = translocatory behaviour) and "virtual inactivity" in the behaviour in Rana has no advantage over the classification developed in the present work. Since gradients were not used, it is not possible to say whether any directed reactions (taxes) are also a part of the humidity reaction. The histograms given in Fig. 10 give no indication of any adaptation taking place.

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From the information so far collected, it seems evident that the humidity reaction of Rana natalensis may be studied by using the same techniques that have been developed in work on the humidity reactions of invertebrates, and that the reaction is of the same elementary type as that which is found in the invertebrates. The complications that have arisen in the present work do not point to a reaction which is inherently different to the invertebrate reaction; they are the outcome of a quite different problem, which is the enormous microclimate that is produced by a moist-skinned amphibian.

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CONCLUSIONS

The work which has so far been done on the humidity reaction of Rana natalensis has possibly raised more problems than it has solved. An answer to the central problem has, however, been given. It has been found that a humidity reaction does exist in these animals, and a method for making a detailed study of the behaviour mechanisms involved in the reaction has been developed. As has been put forward in the previous Section, the technical difficulties so far encountered are not insurmountable, but require a fresh approach. Firstly, it must be made sure that moving air is not responsible for some indirect form of stimulation in the chamber which disturbs the behaviour of the experimental animals. Secondly, the rate of the air flow to be used must be determined by the rate producing the conditions in which the frog shows the best defined humidity reaction, or, this failing, the rate must be determined by data from the effect of different air flows on the extent of the microclimate, not by the properties of the apparatus used to record the extent of the microclimate.

It may be concluded that the indication, given at the end of this present work, as to how far studies of this

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nature can be carried in these animals, is that this field can carry extensive and detailed research, for it has been shown that the reaction does exist, and an experimental approach has been arrived at whereby this reaction can be effectively investigated.

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GENERAL CONCLUSIONS

It may be seen from the General Introduction that the investigations that were projected for the present work were essentially of an exploratory nature. The work was concerned with that part of the behaviour of the Amphibia which is specifically associated with the peculiar water relationships of this group. Two aspects of this behaviour were investigated; the water drive and the humidity reaction. In both cases, investigations got no further than to determine that there was a phenomenon to be investigated, or than to work round to what seems to be the best experimental approach to the problems. It was therefore work of a completely preliminary nature, which has at the best discovered no more than the bare essentials of the phenomena under investigation. But, at its conclusion, it appears that the feasibility and desirability of further investigation into these phenomena is beyond question.

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APPENDIX I.Tables showing the Full Behaviour of Individual Frogs (*Rana natalensis*) in Humidity Chambers.

Eight frogs were used in the final experiments to determine the effect of moving air in the behaviour of *Rana* (see Part II, Methods and Results). The behaviour of each of these animals is here shown in all the different humidities in still and moving air that were used.

Table 1. 100% R.H., Still Air.

Frog	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Be	To
A	1	0	1	0	0	2	0	0	0	0	0	0	0
B	1	0	4	0	0	6	0	0	3	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	1	0	0	0	0	0	0	0
E	18	2	69	0	0	67	90	0	3	0	18	1	2
F	0	0	0	0	0	1	0	0	1	0	0	0	0
G	20	11	134	80	0	87	236	0	9	0	32	11	1
H	0	2	0	0	0	0	0	0	1	0	0	0	0

H = Hop; C = Crawl; Eh = Escape Hop; Ec = Escape Crawl;

N = Nosing; T = Turn; B = Bow; Cr = Crouch; L = Leg Movement;

D = Digging; Bt = Bow-turn; Be = Bow-crawl; To = Turn-crawl.

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Table 2. 60% R.H., Still Air.

Frog	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Be	To
A	37	25	40	24	12	43	72	3	7	1	19	7	1
B	2	2	0	17	8	10	5	0	4	2	0	0	0
C	16	13	17	1	0	11	25	1	4	0	1	0	2
D	8	4	1	0	0	5	1	1	2	1	0	0	0
E	27	26	219	43	110	70	223	0	11	0	61	7	1
F	2	2	0	0	0	3	1	1	3	2	1	0	1
G	26	26	45	280	0	88	142	1	11	1	32	25	2
H	15	4	4	8	7	24	20	0	7	0	1	0	0

Table 3. 20% R.H., Still Air.

Frog	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Be	To
A	2	7	0	0	6	4	1	0	2	0	0	0	0
B	32	15	58	197	18	40	89	0	1	0	19	13	2
C	32	9	241	129	18	21	206	0	1	0	34	5	0
D	1	0	0	0	1	2	0	0	2	1	0	0	0
E	11	18	98	205	9	34	114	0	2	3	23	11	2
F	4	2	1	0	2	8	5	0	0	0	0	0	0
G	24	30	100	112	167	34	101	0	6	0	29	9	1
H	99	60	145	242	43	107	172	1	10	0	42	17	2

Table 4. 100% R.H., Moving Air.

Frog	H	C	Eh	Ec	N	T	B	Cr	L	D	Bt	Be	To
A	28	14	36	43	4	34	60	1	4	0	9	5	2
B	2	0	1	0	0	2	5	0	2	0	0	0	0
C	0	2	0	0	0	6	0	0	0	0	1	0	0
D	0	1	0	0	0	6	0	1	1	0	0	0	0
E	17	11	146	65	0	38	143	2	1	0	38	4	0
F	0	1	0	0	0	0	0	0	0	0	0	0	0
G	1	1	0	0	0	0	0	1	0	0	0	0	0
H	0	0	0	0	0	4	0	0	0	0	0	0	0

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Table 5. 60% R.H., Moving Air.

Frog	H	C	Eh	Eo	N	T	B	Cr	L	D	Bt	Bc	Te
A	1	1	0	0	0	2	0	1	2	0	0	0	0
B	2	0	0	3	0	11	7	2	0	0	2	0	0
C	3	0	0	0	0	3	4	0	1	0	0	0	0
D	34	4	61	90	2	28	78	2	2	1	21	4	0
E	13	6	43	78	0	33	67	0	0	0	19	8	0
F	0	0	0	0	0	1	0	0	0	0	0	0	0
G	5	1	6	21	0	9	14	1	6	0	3	1	1
H	2	3	0	0	0	1	0	0	1	12	0	0	0

Table 6. 20% R.H., Moving Air.

Frog	H	C	Eh	Eo	N	T	B	Cr	L	D	Bt	Bc	Te
A	20	3	49	41	0	13	41	8	5	0	10	1	0
B	29	6	34	40	0	32	33	1	0	0	4	0	2
C	10	1	0	0	0	7	4	3	2	0	0	0	1
D	24	6	78	89	0	17	55	2	1	0	15	7	1
E	17	16	40	72	0	25	67	5	1	0	8	2	0
F	3	2	27	33	0	22	8	2	3	0	2	0	0
G	19	26	20	168	3	44	100	2	2	0	36	22	1
H	0	0	0	0	0	0	0	0	2	0	0	0	0

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APPENDIX II.The Desiccation Rate of *Rana natalensis* in Humidity Chambers of difference Conditions.

In the experiments on the final group of eight frogs (see Part II, Methods), the weight of each frog was recorded before and after it was placed in a humidity chamber for an hour-long observation period. The percentage loss in weight during the hour period was calculated, and the mean percentage loss for all eight frogs in the different conditions is given below, with the standard errors.

	<u>100% R.H.</u>	<u>60% R.H.</u>	<u>20% R.H.</u>
Still Air	2.26 ± 0.44	5.24 ± 0.35	9.23 ± 0.75
Moving Air	2.20 ± 0.25	6.51 ± 0.91	10.86 ± 0.97

Some loss is shown at 100% R.H., which, according to Adolph (1932), is due to the heat produced by metabolism raising the saturation deficiency of the surrounding air. As is to be expected, the rate of water loss increases with lowered humidity, but it is surprising that no significant difference is shown between the loss in still and moving air. These results therefore show that no complications are involved in the use of moving-air chambers as far as desiccation is concerned.

REFERENCE.

Adolph, E.F. (1932). The vapour tension relations of frogs. Biol. Bull. Wood's Hole 62, 112-125.

APPENDIX III.The Effect of Pituitrin, Gonadotropin and Sex Hormones on the Frequency of Moulting in *Bufo carens*.

A record was kept of the frequency of moulting in the toads used in the water drive experiments. Each toad was marked with Gestetner Correcting Fluid, and since this mark came away with the cast at each moult, the frequency of moulting could be recorded (Bouwer, Ewer and Shiff, 1953). The mean duration of the intermoult periods of each experimental group of toads was calculated, and the results are set out below. N stands for the number of animals of each group.

Treatment	N	Mean Duration of Intermoult (Days)	Standard Error
Control	22	4.9	0.35
Injections of Ringer	8	5.0	0.43
" " Pituitrin	6	4.6	0.86
" " Chorionic Gonadotropin	6	2.9	0.20
" " Testosterone	8	6.7	1.33
" " Estrogen	4	5.6	1.02

The length of the control intermoult does not compare well with the intermoult period for *B. carens* given by Bouwer et al. (4.3 ± 0.1 days). This difference might be accounted for by the fact that it was not possible to commence observations on

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the moulting of each animal at the beginning of a moult, owing to the requirements of the main experiment for which they were being used. Consequently the exact number of days between the intermoult of each animal could not always be recorded. In addition, the animals used in this work were under observation for less than half the time that was taken by Bouwer et al.

The Ringer and Pituitrin results do not differ significantly from the control value. It is difficult to interpret the remaining results. Chorionic gonadotropin markedly depresses the intermoult period, and therefore it would be expected that the sex hormones would induce a shorter intermoult period as well, if they showed any effect at all. But in point of fact both sex hormones tended to lengthen the intermoult period. More work on these effects is desirable, since a definite problem appears to exist.

REFERENCE.

- Bouwer, S., Ewer, D.W. and Shiff, C. (1953). Frequency of moulting in anura. Nature, Lond. 172, 408.

APPENDIX IV.A Note on the Distribution of Bufo carens and B. regularis.

At the time when this work was commenced, it was noted that B. carens and B. regularis were found in separate localities in Pietermaritzburg. It was therefore intended that the water drive of these two species should be compared, in order to see if differences in the water drive could be held to be the cause of the segregation of these two species. Time did not permit any investigations into the water drive of B. regularis to be carried out, but a preliminary survey was conducted during the latter half of the summer of 1953 to see how far these two species were restricted to different localities. While the survey was by no means extensive, it was sufficient to show that areas do occur in which one species occurs to the almost complete exclusion of the other. B. carens was found to occur in very great numbers in the Oribi Government Village, the B. carens : B. regularis ratio being about 20 ; 1 in a count of 153 toads. Between this area and the suburbs of Scottsville and Pentrich there was a belt, varying from about one half to two miles in width, in which the species were found in roughly equal proportions. Outside this zone, B. regularis was common, and B. carens was very rare. In Pretoria these two species again show a well-marked segregation into different localities.

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W. Pople (personal communication) has recorded that the two species are found in an approximately 1 : 1 ratio in one low-lying area in Durban (Wentworth), while at a locality on higher ground (Montclair), B. regularis greatly outnumbers B. carens.

There is no indication that the factors determining the occurrence of one or other of the two species lies in the degree to which the area has been built upon or cultivated, and topographical, climatic, edaphic and botanical features appear to be without significance in the distribution of these two species. The problem is complicated by the fact that R. Singh (personal communication) has noted that an area (Raisethorpe, Pietermaritzburg) that was once occupied predominantly by B. carens, is, at the present time of writing, occupied by B. regularis. No explanation of the differential localisation of the two species appears to be available from the present amount of information.