THE VIRGINIA JOURNAL OF SCIENCE

A JOURNAL ISSUED QUARTERLY BY THE
VIRGINIA ACADEMY OF SCIENCE

Vol. 12, New Series **January, 1961** **No. 1**

VOL. 12, NEW SERIES No. 1

JANUARY, 1961

THE VIRGINIA JOURNAL OF SCIENCE

PUBLISHED FOUR TIMES A YEAR IN JANUARY, APRIL, JULY, AND
SEPTEMBER, BY THE VIRGINIA ACADEMY OF SCIENCE

Printed by *The Bassett Printing Corporation*, Bassett, Virginia

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Entered as second-class matter, at the post office at Bassett, Virginia, and Blacksburg, Virginia, under the Act of March 3, 1897. Subscription $3.00 *annually. Published four times a year: in January, April, July, and September, by the Virginia Academy of Science at Blacksburg, Va.*

Mailed June 16, 1961

THE VIRGINIA
JOURNAL OF SCIENCE

VOL. 12, NEW SERIES JANUARY, 1961 NO. 1

RESPIRATION AND HEMOLYSIS OF ULTRAVIOLET IRRADIATED FROG ERYTHROCYTES[1]

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INTRODUCTION

Ionizing radiations have been known for some time to cause hemolysis in mammalian erythrocytes. Early experiments indicated that X-irradiation-induced hemolysis of human and beef erythrocytes was preceded by slow swelling of the cells (Ting and Zirkle, '40). In the same report, these authors suggested that hemolysis and swelling were dependent on an increased permeability to sodium and potassium ions. Subsequent analysis by Sheppard and Beyl ('51) confirmed the hypothesis that the cell was more permeable to sodium and potassium after X-irradiation; they further showed that these ions exchange quantitatively. Non-ionizing radiations have also been shown to cause hemolysis (Sonne, '29; Cook, '56; Green, '56). The hemolytic effects of ultraviolet light as reported by Cook ('56) and Green ('56), are, in general, in agreement with those produced by X-rays.

It has been demonstrated that the energy for maintaining the balance of potassium and sodium in non-nucleate erythrocytes is supplied by glycolysis (Maizels, '51; Weller and Taylor, '51; Solomon, '52). Green ('56) has shown, however, that ultraviolet irradiation of human red cells causes an acceleration in the loss of potassium and gain of sodium ions without inhibiting glycolysis. Sheppard and Stewart ('52) obtained similar results with human red cells exposed to X-rays. This suggests that, in addition to metabolic energy, other factors are important in regulating the exchange of these cations in non-nucleated red cells. One obvious structure for such regulation is the cell membrane and, indeed, gross changes in the mammalian erythrocyte membrane

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[1] This paper was presented to the Virginia Academy of Science on May 8, 1959 at the University of Virginia. An abstract has been published in the Virginia Journal of Science, Sept. 1959, 10(4): 263-264.

have been observed following X-irradiation (Zacek and Rosenberg, '50). It seems clear, then, that radiation-induced hemolysis in the mammalian erythrocyte is preceded by a change in ion permeability which is not dependent on an alteration in the energy supply of the cell, i.e., glycolysis.

Most of the investigations on erythrocytes have been concerned with mammalian cells; comparatively few studies on nucleated erythrocytes are available. Hunter and Hunter ('57) have shown that the oxygen consumption of nucleated erythrocytes is significantly higher than that of non-nucleated erythrocytes when compared at the same temperature. Evidence for an active, oxidative metabolism in nucleated red cells of the chicken may be found in the work of Rubinstein and Denstedt ('53), and Defendi and Pearson ('55). Ion transport in nucleated erythrocytes (chicken, grass-snake) depends on this aerobic respiration (Maizels, '54). The problem, then, arises as to the effect of hemolytic doses of radiation on the aerobic metabolism of the nucleated red cell.

Martin and her co-workers ('56), in studying bullfrog erythrocytes, observed an inhibition in oxygen consumption of these cells following X-irradiation. The present work has been undertaken to study the relation between ultraviolet-induced hemolysis and respiration following ultraviolet radiation in nucleate erythrocytes of the leopard frog, *Rana pipiens*. The results indicate that this radiation increases hemolysis without inhibiting respiration.

METHODS

Frogs used in this investigation were kept in covered porcelain pans in about an inch of water, the temperature of which varied between 20°C and 22°C.

Blood from the aorta of one or two pithed frogs was drawn into a fine glass pipette, the tip of which had an inside diameter of 0.7 mm, and mixed with phosphate buffered Ringer's (0.11M $NaCl$, 0.02M KCl, 0.01M $CaCl_2$, 0.0009M Na_2HPO_4, and NaH_2PO_4; pH 7.4) in which a few crystals of sodium citrate had been dissolved as an anticoagulant. Just before drawing up the blood, the pipette was rinsed with some of this solution in order to prevent clotting of the blood on contact with the inside wall of the pipette. Erythrocytes harvested from this blood by centrifugation were washed twice and finally suspended in phosphate buffered Ringer's solution. Cell concentration, determined from hemacytometer counts, ranged from 70,000 to 150,000 per cubic mm.

The cell suspension was divided into two 3.5 ml aliquots, each of which was placed in a 50 ml round bottom quartz flask and rotated at five rpm to keep the cells in suspension. One aliquot was irradiated with a general Electric G15T8 germicidal lamp, the times of irradiation ranging from 10 minutes to 60 minutes. The distance of the surface of the cell suspension from the lamp was 4.5 cm. All experiments were carried out at room temperature (approximately 25°C). The second aliquot, which was treated in the same way as the first except that it received no ultraviolet radiation, acted as the control. Respiration of both irradiated and non-irradiated cells was measured using a standard Warburg technique, following the procedures described by Umbreit *et al* ('57). Flasks containing two ml of cell suspension, to which was added glucose in a final concentration of 50 mg %, were placed in a water bath at 27.3°C and oscillated 100 times per minute through a five cm stroke. Since cell number varied with each experiment, oxygen consumption was recalculated on the basis of 100,000 cells.

Separate flasks containing about one ml of cells were oscillated in the water bath. For hemolysis determination, samples were taken from these flasks, diluted 1:10 with Ringer's, and placed on glass slides for counts of hemolysed cells. Percent hemolysis was calculated as the number of hemolysed cells per total cells counted (total cell counts between 400 and 500).

RESULTS

Non-hemolysed cells had an opaque, yellowish appearance, with the nucleus clearly discernable. Dark bands running at right angles to the long axis of the cell were apparent. These have been described by Trotter ('56) who attributed them to the residual alkali in the glass slide. As the cell swelled following irradiation, the bands disappeared, the cell became subspherical and at hemolysis rapidly faded with the loss of hemoglobin to a "ghost." The appearance of the "ghost" was the end point in these experiments.

Cumulative oxygen consumption increased as a direct linear function of time for about the first six hours from the beginning of the manometric measurements. A typical experiment (30 minutes ultraviolet radiation) is presented in Figure 1. It should be noted that there was no significant difference in the oxygen consumption of irradiated and non-irradiated cells.

Hemolysis of irradiated erythrocytes increased in a similar manner for about the first four hours. This was followed by a leveling of the hemolysis curve. This "plateau" in the curve lasted three or four hours after which it rose sharply to 100%. Time required for complete hemolysis was approximately twelve hours. Non-irradiated cells showed no hemolysis.

The initial amount of hemolysis after irradiation varied according

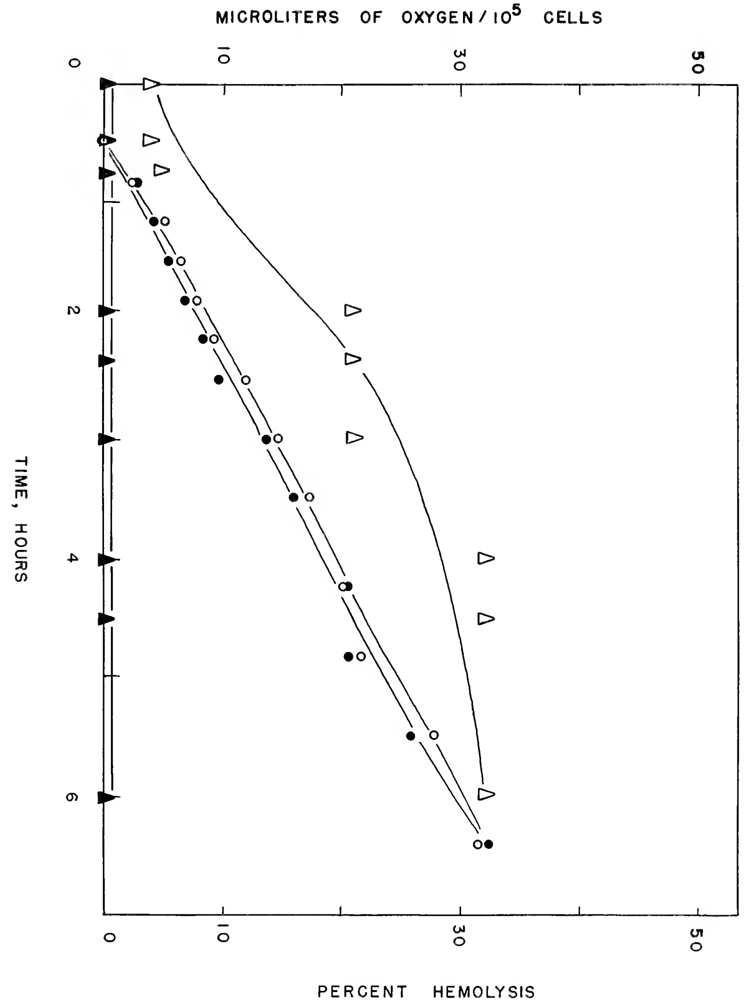

FIGURE 1

Oxygen consumption and percent hemolysis of ultraviolet irradiated and non-irradiated frog red cells (open circles = respiration, ultraviolet; closed circles = respiration, control; open triangles = hemolysis, ultraviolet; closed triangles = hemolysis, control.) Ultraviolet dose = 30 minutes.

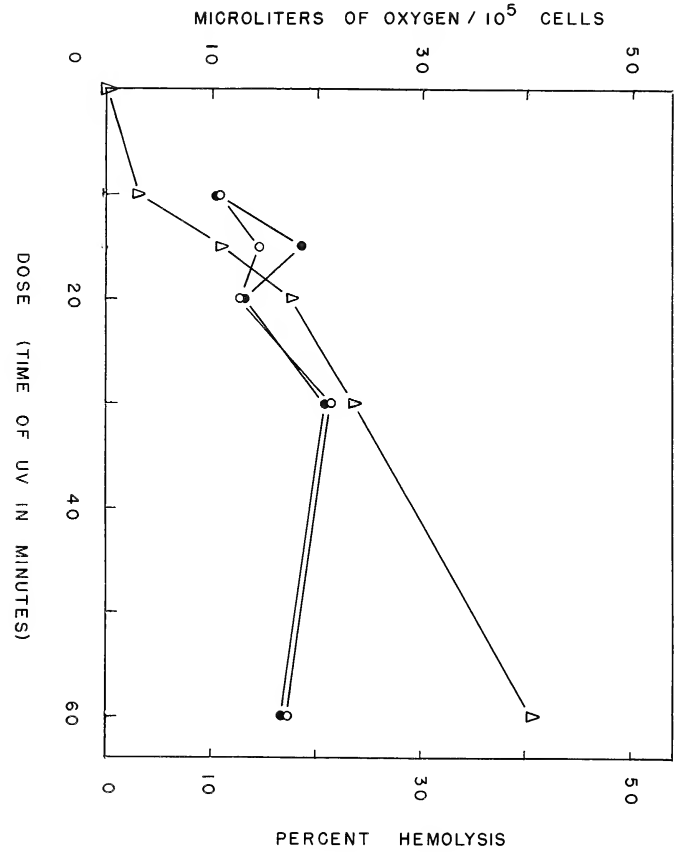

FIGURE 2

Respiration rates and per cent hemolysis values of ultraviolet irradiated and non-irradiated frog red cells four hours after exposure to radiation (open circles = respiration, ultraviolet; closed circles = respiration, control; triangles = hemolysis). The control respiration rate is included for each ultraviolet dose to better point up the fact that there is no significant difference in respiration rate between irradiated and non-irradiated cells.

to the dose used. At a 10 minute dose, no hemolysis was observed in the irradiated cells until three or four hours following irradiation. At a 15 minute dose, hemolysis began about one hour after irradiation, while for a 20 minute dose, hemolysis began immediately following irradiation. At 30 and 60 minute doses, 4 and 10 percent, respectively, of the cells were hemolysed in the first count following irradiation.

Respiration rate for the first four hours and the per cent hemolysis at four hours following irradiation are presented as functions of ultraviolet dose in Figure 2. The per cent hemolysis increased with the ultraviolet dose while the respiration rate was not significantly different between the irridiated and non-irradiated cells.

DISCUSSION

The first apparent change following ultraviolet radiation was a swelling of the frog red cell. A reasonable explanation for this swelling involves the phenomenon of colloid osmotic pressure which consists essentially of two phases (Ponder, '48): (1) a lytic agent alters the cell membrane's selective permeability to cations; (2) as potassium and sodium ions diffuse to equilibrium across the membrane, water enters the cell under the influence of the colloid osmotic pressure of the hemoglobin. This penetration of water results in swelling of the cell to a critical volume at which hemoglobin is released to the environment. The phenomenon of colloid osmotic pressure has been suggested to account for hemolysis following both ultraviolet (Leu *et al*, '42; Cook, '56) and X-radiation (Ting and Zirkle, '40) in mammalian red cells. The inhibition of ultraviolet-induced hemolysis of frog red cells by sucrose (Maroney, '59) lends support to the colloid osmotic hemolysis theory.

For the colloid osmotic pressure of hemoglobin to be effective in causing hemolysis, the ions must be free to diffuse to equilibrium across the cell membrane. Energy is used to prevent the potassium and sodium ions from reaching diffusion equilibrium in the intact cell (Harris, '41; Danowski, '41; Maizels, '54). Inhibition of metabolism is, then, one means for allowing ions to reach diffusion equilibrium. An example of this may be found in mammalian red cells kept at low temperatures (5°C). Potassium is lost from these cells and regained upon returning the cells to physiological temperature, provided that glucose is present (Harris, '41). The results of the present study show that ultraviolet-irradiated frog erythrocytes respire at the same rate as non-irradiated erythrocytes; yet cells exposed to ultraviolet radiation exhibited hemolysis while non-irradiated cells did not. It seems apparent that the cell's aerobic respiration is intact and an explanation other than the inhibition of energy metabolism must be sought to explain hemolysis in the frog red cell.

The concept that metabolic energy is solely responsible for maintaining cation gradients has been criticized by Parpart and Green ('51). Rabbit red cells, when treated with n-butyl alcohol, gain sodium and lose potassium at comparable rates. Further, the recovery of potassium and sodium exchange after removal of the alcohol was much more rapid than would be expected if metabolism had been interfered with. They point out that it is more likely that the butyl alcohol has an effect on the membrane alone, permitting a rapid exchange of potassium and sodium.

It is suggested, then, that ultraviolet radiation, in the case of the frog red cell, altered the cell membrane allowing potassium and sodium to diffuse freely. The cell then swelled because of the excess intracellular osmotic pressure. In this regard, frog erythrocytes were acting as did the mammalian red cell following ultraviolet radiation (Green, '56). It should be noted that the respiratory rates of irradiated and non-irradiated cells were the same in spite of the fact that up to 40 per cent of the irradiated cells had hemolysed. This would indicate that the respiratory apparatus of the hemolysed cell was intact and functioning normally although further study of this phenomenon is desirable.

SUMMARY

Frog erythrocytes washed in phosphate buffered Ringer's solution were exposed to ultraviolet light for various lengths of time. Respiration and hemolysis of both irradiated and non-irradiated cells were measured for about six hours following exposure to ultraviolet radiation.

It was found that this radiation increases hemolysis without affecting oxygen consumption of these cells.

The similarity of the mechanism of hemolysis in frog erythrocytes to the proposed mechanism of hemolysis in mammalian erythrocytes is discussed.

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BALLOON-WING, A NEW MUTATION IN BLATTELLA GERMANICA (L.)

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INTRODUCTION

The study of spontaneous and induced mutations in such insects as *Drosophila*, *Habrobracon*, and several others, has been of great significance in the development of formal genetics. In fact, were it not for *Drosophila*, genetics might have progressed at a much slower rate. With regard to inheritance in the myriad of other insect species, however, there is still very little information available. For example, the mutation reported in the present paper is, to the writers' knowledge, the first genetically controlled modification of the wing to be described in the ubiquitous German cockroach, *Blattella germanica* (L.). Although it might be argued that this is an extremely stable species and that mutations would, therefore, be uncommon, the facts do not seem to bear this out. During the course of the present investigation several other spontaneously occurring variants have been found in the same inbred stock. Some of these traits are now under study, and the results will be published elsewhere.

In *Drosophila* it is known that approximately 150 genes influence or modify the development of the wing (Emerson, 1955: 648). Mutations at several of these loci show some similarity to the one described here. In their compilation of known mutations in *D. melanogaster*, Bridges and Brehme (1944: 20, 23, 29, 26, 152, 95) listed the following traits: balloon (ba), Bubble (Bb), blister (bs), bloated (blo), puff (puf), inflated (if) and vesiculated (vs). Mutations of this type are also known in other species of *Drosophila* (Gordon *et al.*, 1939: 51; Metz *et al.*, 1923: 16) and in *Musca domestica* (Sullivan and Hiroyoshi, 1960: 214; Milani, 1956: 225). These mutations are all characterized by swellings on the wing, usually filled with fluid.

The name "balloon" was chosen for the present mutation because the character seems to resemble most closely balloon-wing as described in the cheese skipper, *Piophila casei*, (Painter, 1917: 306) and, to a lesser degree, ballon-wing in *D. melanogaster*. Balloon in *P. casei* and in *D. melanogaster* is recessive and is not sex-linked. As will be shown, this is also true in *B. germanica*.

DESCRIPTION OF CHARACTER

Balloon-wing roaches are characterized by a single, bubble-like swelling on each forewing and, usually, in each hindwing. During the course of this study 991 balloon-wing specimens were examined. On the forewings the swellings are oval or pear-shaped, usually from 0.24-0.28 cm. in length and 0.15-0.17 cm. in width. In 85% of the specimens examined the anterior ends of the swellings occurred opposite or proximal to the terminus of the first anal vein (1 A), but not within the anal area. This usual positioning of the swellings is shown in Pl. I, A and B. A few swellings occurred within the anal area, and these were frequently associated with a minute swelling in a terminal or subterminal position (Pl. I, D). In certain crosses there was a tendency for the swellings to be more distal in position than usual, as shown in Pl. I, C. Four specimens occurred with a swelling on one forewing only.

A number of other variants of the forewing occurred with some frequency in certain crosses. Some of these are: divergent wings, wings with branching anal veins, wings shriveled or unfolded, dwarfed wings, and wings with one or more minute blisters. A swelling, filled with hemolymph, was also present on one or both sides of the pronotum in some balloon-wing roaches, but this character does not appear to be confined to particular crosses.

On the hindwings the swellings are elongate, usually from 0.20-0.26 cm. in length and 0.10-.12 cm. in width. Sometimes the entire wing, with the exception of the anal area, is inflated. Hindwings of 869 balloon-wing roaches were examined. Of these, 818 had swellings on one or both wings. In 83% of the specimens the swellings occurred within the proximal two-thirds of the wing, but not within the anal area, as shown in Pl. II, A and B. Swellings within the anal area (Pl. II, B) or in a terminal or subterminal position (Pl. II, C) were relatively rare, and confined to a few crosses. There were 103 roaches with one normal-appearing hindwing (98♂ and 5♀), and approximately half this number (51, all ♂) which had no swellings on either hindwing.

Several variants of the hindwing occurred which may be of genetic interest. As mentioned in the preceeding paragraph, two types of bubble-like swellings other than the usual balloon-wing were found. In addition, there were a number of tiny, blister-like swellings, a broken or incomplete cubitus (Pl. II, C) and several other aberrations of the venation.

GENETICS

The balloon-wing roaches used for crossing came from a colony which apparently was breeding true for this trait. All individuals from the colony which were examined showed the balloon wing. They were derived from those few mutants originally observed in a normal-appearing inbred stock. Those roaches involved in the crosses had the balloon in the usual position (Pl. I, A and B) with a few in Pl. I, C. The wild type (normal) roaches came from a different stock. The following series of crosses were made:

Series A. — Twenty-one crosses involving balloon-wing males and females.

Series B. — Twelve crosses reciprocally mating balloon-wing specimens with normal roaches.

Series C. — Twenty-four crosses reciprocally mating balloon-wing specimens and normal-appearing hybrids.

Series D. — Nineteen crosses involving hybrid males and females obtained from Series B.

Series E. — Nine crosses reciprocally mating normal-appearing hybrids and roaches from the normal stock.

Result of Crosses

The results obtained from the five series of crosses listed above are presented in table 1. The reciprocal matings showed no significant difference and are not listed separately in the table. The results indicate that the mutation under study is a simple Mendelian recessive. As would be expected, balloon x balloon (ser. A) produced all balloon offspring; balloon x normal (ser. B) produced all normal-appearing offspring; balloon x hybrid (ser. C) gave results which closely approximate a ratio of one balloon to one normal; hybrid x hybrid (ser. D) produced offspring in a ratio of three normal to one balloon; and hybrid x normal (ser. E) gave all normal appearing offspring. The results from series C and D were analyzed statistically by the chi square goodness of fit test. It was found that the chi square values were very low. This indicates that the deviations from a perfect 3:1 or 1:1 ratio are minor and are well within the range of deviations expected by chance alone.

The four specimens mentioned in the preceding section which had swellings on only one forewing are believed to be the result of reduced expression of the trait. In order to test this hypothesis two of the specimens were successfully crossed, one with a normal and the other with a balloon-wing roach. In the F_1 of the former, all offspring were of normal appearance. In the F_2 there were 190 normal-appearing roaches and 65 roaches with swellings on both forewings. This is also well within the expected chance deviations from a 3:1 ratio. In the second cross, there were 26 males and 32 females, all with the usual balloon-wing swelling on both forewings. Thus it would appear that in these two individuals, at least, the absence of a swelling on one forewing was a phenotypic variation.

In *B. germanica,* as in other Orthoptera, the sex determination mechanism is of the XO type. If it is assumed that approximately half of the sperm carry the X chromosome, the sex ratio should be 1:1. In the present study an opportunity for checking this ratio was present. The results gave a total of 2132 males and 2085 females from 85 matings. This is well within the expected range of variation for a 1:1 ratio.

DISCUSSION

In the present paper the character "balloon-wing" has been described and its inheritance elucidated. The associated problem of how the gene responsible for this trait causes the swelling to develop has not been investigated. However, the writers have observed the development of the swelling in several newly emerged adult roaches and found that it first shows as one, two or three tiny blisters which appear suddenly when the wing is partially unfolded. The blister or blisters enlarge quickly to form a swelling of the usual size by the time unfolding is complete. Normal-appearing veins are present before the appearance of the blisters and after formation of the swelling are still discernable on the lower, if not the upper, surface of the swelling. The only explanation for the trait which can be offered at this time is the speculative one that perhaps it is an inheritable weakness of the middle membrane of the wing.

Along these same lines, it is interesting to note that the character (balloon-wing) does not always express itself fully in roaches. This is probably the result of variable expressivity as defined by Srb and Owen (1953:303). Evidence on this point comes from the two crosses reported in the previous section where the roaches showed a swelling on only one forewing. In both cases they were genetically of the balloon type. If it is assumed that this is the case, then a calculation of the per cent expression can be made. For this purpose it will be considered that complete expression prevails in all those specimens in which a balloon appears on both wings of a pair. Accordingly, full expression of the trait in forewings is greater than 99 per cent, while in hindwings it is approximately 80 per cent. This difference in expressivity in fore- and hind-wings has not been studied, but it may be genetically controlled. On the other hand, there is an explanation which does not require any genetic involvement. The hindwings normally lie in a folded condition between the dorsal body wall and the forewings. It is possible that the folds and the confined position confer enough structural reinforcement to the hindwings that the swellings fail to appear in a higher percentage of hindwings than forewings.

As adults, balloon-wing *Drosophila* lose the characteristic swelling and are best recognized by divergent wings and an extra plexus of veins (Waddington, 1940: 128). Balloon-wing roaches seldom have divergent wings and show no extra plexus of veins. The swelling on the wings are the only constant phenotypic expression of the mutation.

SUMMARY

A new mutation has been described for the German cockroach, *Blattella germanica* (L.). Based on analogy with similar traits in other insects, it has been named "balloon-wing." The character has its phenotypic expression as a swelling on each of the four wings of this insect. The swellings are relatively large, and on the forewings usually occur within the proximal half or near the center of the wing. On the hindwings the usual expression is within the proximal two-thirds of the wing. In a small percentage of cases the swellings are unusually located. A number of other unusual variants, some of which are now under study, have also been described.

In order to determine the mechanism by which this trait is inherited, a series of crosses involving the balloon-wing stock and a normal stock have been made. The results showed that the swellings are the phenotypic expression of a simple Mendelian recessive gene. Four specimens ($<1\%$ of total) were found with a swelling on only one forewing. A number of specimens (approx. 20%), usually males, were found which lacked swellings on one or both hindwings. This is probably the result of variable expressivity, and, if so, the per cent of full expression of the trait is considerably lower in the hindwings than in the forewings. A possible explanation for this difference together with a discussion of several other points is included.

ACKNOWLEDGEMENTS

This work was partially supported by a grant (No. RG-6365) from the U. S. Public Health Service, Division of General Medical Sciences.

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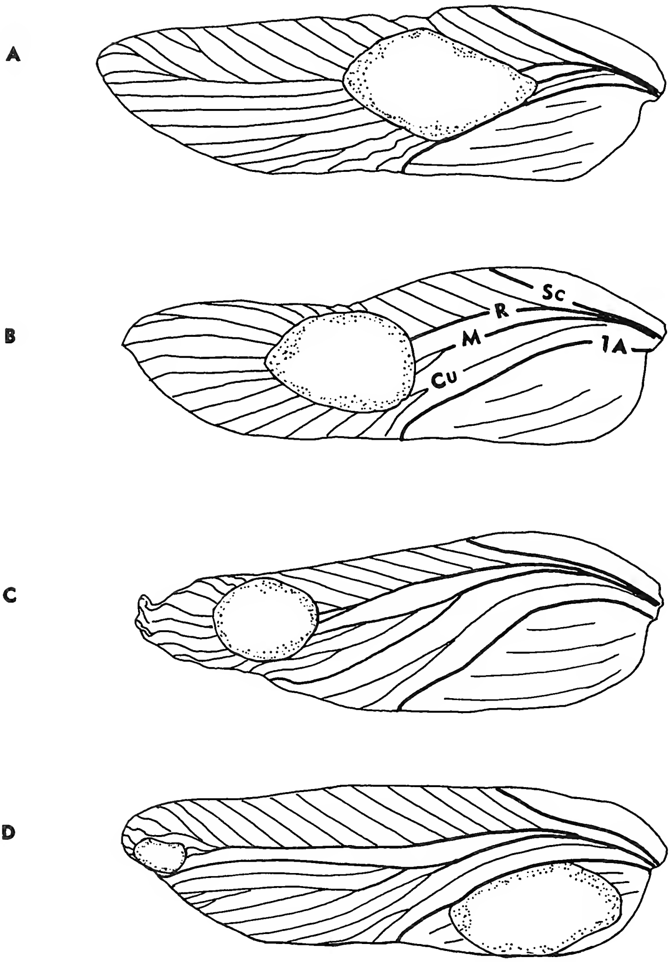
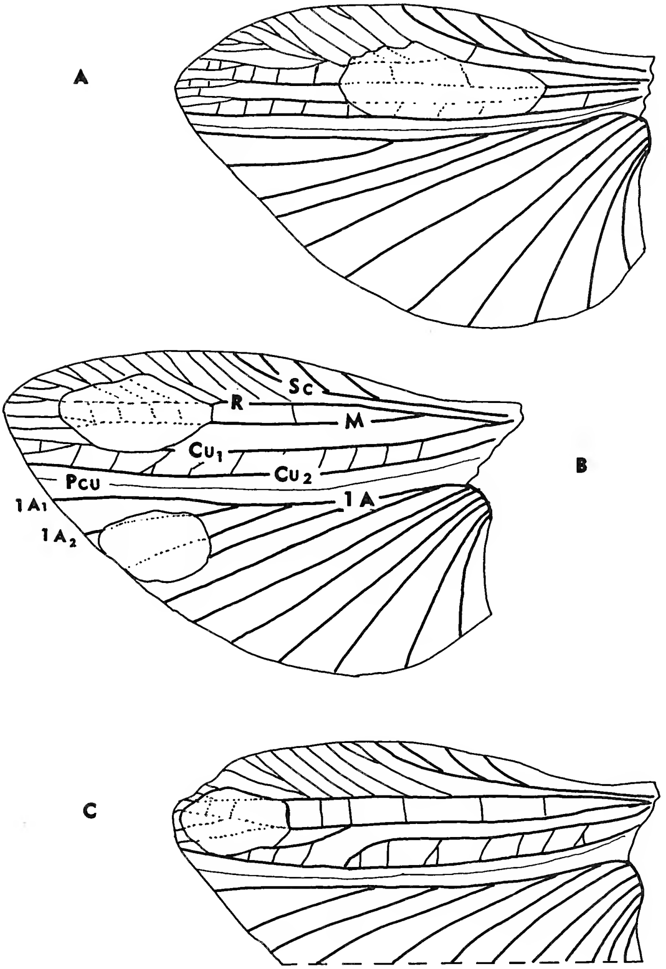
Table 1. — Results from a series of crosses involving a balloon-wing and a normal stock of *B. germanica.*

Series	No. of normal-appearing offspring ♂	♀	total	No. of balloon-wing offspring ♂	♀	total
A (balloon x balloon)	0	0	0	456	424	880
B (balloon x normal)	417	423	840	0	0	0
C (balloon x hybrid)	255	247	502	279	236	515
D (hybrid x hybrid)	433	440	873	140	140	280
E (hybrid x normal)	152	175	327	0	0	0



Explanation of Plate I

Plate I. The drawings represent left forewings of *B. germanica,* and show the various expressions of the "balloon-wing" mutation. A and B, balloon in usual location on wing; C, balloon more distal than usual; D a small terminal swelling plus a balloon in the anal area.



Explanation of Plate II

Plate II. Similar to Plate I. Drawings of left hindwings. A, balloon in usual position; B. balloon in the usual position plus a balloon in the anal area; C, balloon more distal than usual plus an interrupted cubitus vein.

THE NEGATIVE BINOMIAL DISTRIBUTION: A REVIEW OF PROPERTIES AND APPLICATIONS*

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1. INTRODUCTION

The negative binomial distribution (NBD) was discussed by Pascal and Fermat (Todhunter, 1865). However, the earliest known derivation and publication is due to Montmort in 1714. A particular form of the negative binomial, the inverse binomial sampling form, is sometimes referred to as the Pascal distribution.

Many forms of the NBD appear throughout the literature. The most frequently occurring forms, as given in Table I, are discussed in this paper.

TABLE I

Forms of the NBD	Parameters	Comment
(1) $\binom{x+r-1}{x} p^r q^x$	r, p	Pascal form; p+q=1, r is an interger.
(2) $\binom{n-1}{r-1} p^r q^x$	r, p	Pascal form; n=r+x.
(3) $\binom{x+k-1}{x} p^x q^{-x-k}$	k, p	q=1+p; k not necessarily an integer.

The NBD, a two parameter distribution, has the same sample space (0, 1, 2, . . .) as the Poisson. In particular, the Pascal form (a term reserved specifically for the case where r is an integer) is a distribution over the non-negative integers.

In more recent years NBD applications have increased, particularly in health and accident statistics, binomial sampling, population counts, data of contagious distributions and communications.

2. ANALYTIC PROPERTIES

Although many forms of the NBD are utilized, the Pascal form is of major importance. Its primary analytic properties follow.

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*Research supported by the National Institutes of Health and the Office of Ordnance Research, U.S. Army.

The characteristic function of the Pascal form is $\phi_x(t) = p^r[(1 - qe^{it})^{-r}]$. Accordingly, the mean is rqp^{-1} and the variance rqp^{-2}. In this distribution the variance is larger than the mean. From the form of $\phi_x(t)$ it is easy to see that the sum of two independent negative binomial variates $x_1 + x_2$ with the same p is also a negative binomial variate.

The cumulant generating function is

$$\begin{aligned}\kappa_x(t) &\equiv \ell n\ \phi_x(t)\\ &= -r\ \ell n[1 - q(e^{it} - 1)/p] .\end{aligned}$$

From the definition i.e., $\mu'_{[k]} = \mathcal{E}[x(x-1)\ldots(x-k+1)]$, the factorial moments are given by $\mu'_{[k]} = r(r+1)\ldots(r+k-1)p^{-k}q^k$.

A very interesting and convenient property of the Pascal form is that its c.d.f.

$$F(y) = \sum_{x=0}^{y} \binom{x+r-1}{x} p^r q^x = I_p(r,\ y+1) \tag{4}$$

can be computed by entering tables of the incomplete Beta function as was pointed out by Pearson and Fieller (1933). A presentation of their result follows.

The remainder after y terms of the Maclaurin series in the integral form for a function g(x) is

$$R = \frac{1}{\Gamma(y)} \int_0^x g^{(y)}(x-t)t^{y-1}dt .$$

Writing the Pascal form (1) as $p^r[(1-q)^{-r}]$ and taking $g(q) = (1-q)^{-r}$ we have

$$R = \frac{\Gamma(r+y)}{\Gamma(r)\Gamma(y)} \int_0^q (1-q+t)^{-r-y}t^{y-1}dt . \tag{5}$$

Let $(1-u) = (1-q)/(1-q+t)$; (5) with the factor p^r included then becomes

$$\frac{p^r\Gamma(r+y)}{\Gamma(r)\Gamma(y)} \int_0^q (1-u)^{r-1}u^{y-1}du(1-q)^{-r}$$

and finally $R = I_q(y, r)$,

where $I_x(a, b)$ is the standard notation for the incomplete Beta function

$$\int_0^x t^{a-1}(1-t)^{b-1}dt/\beta(a,b).$$

Therefore,

$$\sum_{x=y}^{\infty} \binom{x+r-1}{x} p^r q^x = I_q(y,r)$$

and

$$F(y) = 1 - I_q(y+1, r) = I_p(r, y+1) . \tag{6}$$

It is interesting to note that the use of incomplete Beta tables for the evaluation of this c.d.f. has been rediscovered by Patil (1960).

3. LIMITING FORMS

Three limiting forms of the NBD are discussed in this section.

3.1 The Poisson Distribution.

Assume the Pascal form (1) whose moment generating function (m.g.f.) is $p^r/(1-qe^\theta)^r$. Let λ be fixed such that $q = \lambda/r$, and let $p \to 1$, $q \to 0$ then

$$\lim_{r \to \infty} \left(\frac{p}{1-qe^\theta}\right)^r = e^{\lambda(e^\theta-1)} , \tag{7}$$

the m.g.f. of the Poisson distribution, $e^{-\lambda}\lambda^x/x!$, where x is the number of failures before the desired number of successes r.

3.2 The Gamma Distribution.

Consider the Pascal form (2) giving the distribution of sample size n as

$$f(n) = \frac{(n-1)!}{(n-r)!(r-1)!} p^r q^{n-r} , \qquad n = r, r+1, \ldots$$

Suppose each trial takes time Δt and that the probability of success p is proportional to Δt, i.e., $p = \lambda\Delta t$. then time $t = n\Delta t$ required to achieve r successes has the d.f. f(t) given by

$$f(t)\Delta t = \frac{(\frac{t}{\Delta t} - 1)!.}{(\frac{t}{\Delta t} - r)!(r-1)!} (\lambda\Delta t)^r (1 - \lambda\Delta t)^{\frac{t}{\Delta t} - r}$$

or

$$f(t) = \frac{(t - \Delta t)\ldots(t - \overline{r-1}\Delta t)\lambda^r(1-\lambda\Delta t)^{\frac{t}{\Delta t} - r}}{(r - 1)!} .$$

Let $\Delta t \to 0$; then f(t) becomes the Gamma density

$$f(t) = \frac{\lambda^r \, t^{r-1} \, e^{-\lambda t}}{\Gamma(r)} \tag{8}$$

which may be interpreted as the "holding time" before the rth occurrence of a rare event.

3.3 The Logarithmic Distribution.

In many applications of the NBD

$$P(i) = \frac{(k+i-1)!p^i}{(k-1)!i!(1+p)^{k+i}} , \qquad i = 0, 1, 2, \ldots \tag{9}$$

the number i observed in any sample may have all integral values including zero. However, in some cases the value i = 0 will not be observed. For example in the application of the NBD to the number of representatives of different species of butterflies obtained in a collection, only frequencies of numbers greater than zero will be observable, since by itself the collection gives no indication of the number of species which are not found in it.

The following development of the logarithmic distribution is due to Fisher (1943). In nature the abundance of different species of the same group generally varies very greatly. Fisher found in a study of Malayan butterflies that the NBD which often fits such data well has a value of k so small as to be almost indistinguishable from zero. That it is not really zero for collections of wild species follows from the fact that the total number of species and therefore the total number not included in the collection is really finite. The real situation in which a large number of species are so rare that their chance of inclusion is small, is well represented by the limiting form of the NBD when k tends to zero.

The limiting value k = 0 cannot occur in cases where the frequency at zero is observable, for the distribution would then consist wholly of such cases. If, however, we put k = 0 in (9), write x for p/(1+p), so that x is a positive number less than unity varying with the size of the sample and replace the constant factor (k-1)! in the denominator by a new constant factor α in the numerator, we have an expression for the expected number of species with i individuals, where i now cannot be zero,

$$\alpha x^i/i.$$

The total number of species expected is consequently

$$\sum_{i=1}^{\infty} \alpha x^i/i = -\alpha \ln(1-x) \tag{10}$$

the logarithmic distribution. The total number of individuals expected is

$$\sum_{i=1}^{\infty} \alpha x^i = \alpha x/(1-\alpha). \tag{11}$$

Thus the two relationships (10) and (11) enable the series to be fitted to observational data, for if S is the number of species observed, and N the number of individuals the two equations

$$S = -\alpha \ln(1-x), \qquad N = \alpha x/(1-x), \tag{12}$$

are sufficient to determine the values of α and x. Tables which facilitate the solution of these equations and an example can also be found in Fisher (1943).

4. TRUNCATED NBD

In fitting data where the zero class is not observed it may be the case that a truncated NBD gives a better fit than the logarithmic distribution. Sampford (1955) considers the truncated NBD and gives procedures and tables to obtain iteratively either the moment or maximum likelihood estimates of the parameters. Included also is a discussion of the efficiency of these procedures.

Simplified methods for the estimation of the parameters of the truncated distribution by a modified method of moments and by a modification of maximum likelihood procedures, due to Brass (1958) are discussed below. Brass also discusses the efficiency of these two procedures.

Consider the NBD of the form (3)

$$P(i) = \frac{(k+i-1)!p^i}{(k-1)!i!(1+p)^{k+i}}, \qquad i = 0, 1, 2, \ldots \tag{13}$$

so that $P(0) = 1/(1+p)^k$. To obtain the corresponding probabilities (13) must be divided by $[1 - P(0)]$; writing $\omega = 1/(1+p)$

and $\eta = 1 - \omega$ it follows that

$$P_T(i) = \frac{\omega^k (k+i-1)!\eta^i}{(1-\omega^k)(k-1)!i!}\,, \qquad i = 1, 2, \ldots$$

where the subscript T denotes the truncated distribution.

By considering the proportion in the first class of the truncated distribution and its mean and variance found by use of factorial moments Brass develops simple estimates for ω, k and also for M, the mean of the complete NBD. The value M enters in his discussion of efficiency. Aside from M, the estimates are

$$\bar{\omega} = m(1 - n_1/n)/s^2$$

$$\text{(14)} \qquad \bar{k} = \frac{\bar{\omega}m - n_1/n}{1 - \bar{\omega}}\,,$$

where the bars denote the estimates. n_i is the number of sample observations with measurement i and n is the total sample size. m and s^2 are the unbiased estimates of μ and σ^2, that is

$$m = \sum_{i=1}^{\infty} \frac{in_i}{n}\,, \qquad s^2 = \sum_{i=1}^{\infty} \frac{n_i(i-m)^2}{n-1}\,.$$

Considering the log likelihood

$$\ell n\, L = \sum_{i=1}^{\infty} n_i \ell n\left[\frac{\omega^k (k+i-1)!(1-\omega)^i}{(1-\omega^k)(k-1)!i!}\right].$$

Brass obtains the following modified maximum likelihood expressions.

$$\text{(15)} \qquad \bar{\omega} = \frac{\bar{k} + n_1/n}{\bar{k} + m}$$

$$\text{(16)} \qquad \frac{m(\bar{k} + n_1/n)}{\bar{k}(m - n_1/n)} \ell n \left(\frac{\bar{k} + n_1/n}{\bar{k} + m}\right) + \frac{1}{n} \sum_{j=1}^{R} (\bar{k} + j - 1)^{-1} \sum_{i=j}^{R} n_i = 0$$

where R is the largest observed value of i. The estimate from (14) gives a convenient first entry for $\bar{k}$ in the iterative procedure (16). ω then follows immediately from (15).

The above methods of fitting are illustrated on the data below also from Brass. The observations are the number of children ever born to a sample of mothers over forty years of age.

	No. of Children Per Mother												
i	1	2	3	4	5	6	7	8	9	10	11	12	Total
No. of Mothers n_i	49	56	73	41	43	23	18	18	7	7	3	2	340

$m = 3.9912$, $s^2 = 5.9734$, $n_1/n = 49/340 = 0.1441$.

Then from (14) $\bar{\omega} = 0.572$ and $\bar{k} = 5.00$.

For the maximum likelihood solutions write (16) as

$$\phi = \frac{m}{m-n_1/n}\left(1 + \frac{n_1/n}{k}\right)\ell n\left(1 - \frac{m-n_1/n}{k+m}\right) + \frac{1}{n}\sum_{j=1}^{R}(k+j-1)^{-1}\sum_{i=j}^{R} n_i \; .$$

Then

$$\frac{d\phi}{dk} = \phi' = \frac{m}{k(k+m)} - \frac{mn_1/n}{k^2(m-n_1/n)}\,\ell n\left(1 - \frac{m-n_1/n}{k+m}\right) - \frac{1}{n}\sum_{j=1}^{R}(k+j-1)^{-2}\sum_{i=j}^{R} n_i \; .$$

The iterative procedure is to calculate ϕ and ϕ' for some value of k; a second value of k which will make ϕ closer to zero is then found by the aid of ϕ' on the assumption that the relation between ϕ and k is roughly linear over a short range. Further improved approximations are obtained by linear interpolation from the values of ϕ for the preceding two estimates of k.

k	ϕ	ϕ'
5.00	−0.00064	−0.00258
4.75	+0.00008	–
4.78	0.00000	–

The first approximation to k is taken as 5.00 from the estimate by (14) The second approximation to k is

$$5.00 - \frac{-0.00064}{-0.00258} = 4.75.$$

One linear interpolation between 5.00 and 4.75 gives 4.78. From $\bar{k} = 4.78$, $\bar{\omega}$ can be found directly from (15) giving $\bar{\omega} = 0.561$. The estimates again from (14) are $\bar{k} = 5.00$ and $\bar{\omega} = 0.572$.

The estimates by the two procedures differ little. Brass gives the efficiencies for both methods at about 95%. These efficiency values are read from tables which appear in his paper. The tables are based on estimates of k and M. In general the modified moments method of estimation is more valuable in that slight labor is involved and efficiency is high in many cases met in practice.

5. OCCURRENCE

Inverse binomial sampling is of primary importance in the consideration of the NBD. It occurs in the following general type of situation. If a proportion p of individuals in a population possesses a certain characteristic, the number of observations in excess of r which must be taken to obtain exactly r individuals with the characteristic has the Pascal form of the NBD.

Greenwood and Yule (1920) study the NBD in connection with a Poisson distribution with varying mean.

Yule (1924), Furry (1939) and Kendall (1949) consider the distribution in connection with birth-death processes. Yule is concerned with the mathematical theory of evolution; Furry with cosmic radiation and Kendall with the process in general.

McKendrick (1914) and Quenouille (1949) discuss contagious distributions. Irwin (1941) and Lüders (1934) are concerned with the NBD as it occurs in multiple accident theory.

The first of these situations, inverse binomial sampling, is the simplest mathematically and is the only one where the mathematical model is likely to hold exactly in practice.

6. COMPUTATIONAL PROCEDURES ON THE PASCAL FORM

6.1 Point Estimate of p.

Occasions may arise in which the frequency of an attribute p is unknown. For the Pascal form the estimation of p from the sample data is a simple matter.

Haldane (1943) considers the NBD (2) and derives an unbiased estimate of p, viz.

$$(17) \qquad \frac{r-1}{n-1}$$

where n is the total number of items sampled in order to recover the predetermined number r. Haldane also develops an expression for the variance of this estimate. It is, however, approximate. Finney (1949) derives an exact expression for the variance and also obtains an unbiased estimate of the variance.

6.2 Interval Estimate of p.

It may not have been generally realized that methods and tables for determining exact confidence limits on p in direct or binomial sampling may be adapted very easily to inverse binomial sampling. The proof which follows is due to Finney (1949).

If p_U is the upper $\frac{1}{2}\alpha$ confidence limit for a value of p estimated from inverse sampling, by definition it is the value of p for which the probability of obtaining the quota r in n or more trials is exactly $\frac{1}{2}\alpha$. This value p_U is the same value of p which would give r-1 or less with the attribute in a sample of predetermined size n-1 in direct sampling. Likewise p_L, the corresponding lower limit, is the value of p which would give the quota in n trials or less with a probability of $\frac{1}{2}\alpha$, and is, therefore, the same as the value of p which in direct sampling would give r or more with the attribute in a sample of size n. Thus, the rules for adapting Table 41 of *Bometrika Tables for Statisticians* which gives confidence limits for the direct binomial can be stated:

(i) For the upper confidence limit on p enter the table with $c = r\text{-}1$, $n = n\text{-}1$.

(ii) For the lower confidence limit enter the table with $c = r$, $n = n$.

As an example of the use of these rules consider the following situation. An experimenter has concluded an inverse sampling plan in which he obtained four desired attributes in ten trials. Here $r = 4$, $n = 10$. To find the upper confidence limit on p enter the table with $c = 3$, $n = 9$. For the lower confidence limit enter with $c = 4$, $n = 10$. Consequently, the two sided 95% confidence interval for p is $0.11 \leq p \leq 0.70$. The point estimate of p from (17) is 1/3.

6.3 An Example of Inverse Binomial Sampling.

Chapman (1952) considers an inverse binomial sampling plan in which it is desired to estimate the size N of a population. In Chapman's plan a number t of individuals are taken from this population. They are tagged and returned. In the second sample, n individuals are sampled in order to recover a predetermined number r of the tagged ones.

If sampling is with replacement P(n) the probability of having to sample n individuals to obtain r tagged ones is given by

$$P(n) = \binom{n-1}{r-1}\left(\frac{t}{N}\right)^{r}\left(1 - \frac{t}{N}\right)^{n-r},$$

where r is the fixed parameter and n the random variable. $\mathcal{E}(n) = rN/t$ and nt/r is an unbiased estimate of N. Chapman considers a normal approximation and a χ^2 approximation for confidence limits on N. However, a more expedient method is to use the rules stated in (6.2) and *Biometrika Table* 41. Let $t/N = p$, replacing N by nt/r, p becomes r/n. Then p_U and p_L can be determined such that $P(p_L \leq p \leq p_U) \geq 1-\alpha$.

On replacing p by t/N the confidence statement
$P(t/p_U \leq N \leq t/p_L) \geq 1-\alpha$ is obtained.

7. ESTIMATION OF PARAMETERS

The estimation of parameters of other forms of the NBD is not as straightforward as it is in the case of the Pascal form. Parameter estimation in these cases is usually accomplished by the method of moments. However, when this method is not satisfactorily efficient, the method of maximum likelihood is commonly used.

Consider first the method of moments. Fisher (1941) discusses the NBD (3)

$$f(x) = \frac{(k+x-1)!p^x}{(k-1)!x!(1+p)^{k+x}}$$

and finds the estimates of p and k by the method of the moments. These estimates are

$$p^* = (s^2 - \bar{x})/\bar{x}$$

(18) and

$$k^* = \bar{x}^2/(s^2 - \bar{x}) .$$

Fisher also compares the determinants of the covariance matrices for large-sample estimates found by moments and by maximum likelihood obtaining an expression for the reciprocal of the efficiency. This further yields an expression, viz.

(19) $(1 + 1/p)\ (k + 2)$

which indicates when moment estimates are satisfactorily efficient, i.e., > 90%. This is the case when (19) exceeds 20 using the values of p and k found from (18). If the guide (19) indicates that moment estimates are not satisfactory then another method of estimation must be employed.

Estimation by maximum likelihood is efficient. Haldane (1941) considers the estimates of p and k by maximum likelihood. The estimate of p is

$$(20) \qquad p = \bar{x}/k$$

where k is obtained iteratively from the following expression

$$(21) \qquad N[\ell n(k+\bar{x})-\ell n(k)] = \frac{n_1+n_2+\ldots+n_R}{k} + \frac{n_2+\ldots+n_R}{k+1} + \ldots + \frac{n_R}{k+R-1} \ .$$

n_i is the observed frequency of x_i , R is the the maximum value of x_i such that $i = 0, 1, \ldots, R.$ $N = \sum_{i=0}^{R} n_i,$ the total number of observations and $\bar{x} = \frac{1}{N} \sum_{i=0}^{R} x_i n_i$.

As an illustration of the two methods of estimation, consider the following data from Fisher (1941). The table gives a sample of sheep classified according to the number of ticks found on each.

i	0	1	2	3	4	5	6	7	8	9	10	
number of ticks x_i	0	1	2	3	4	5	6	7	8	9	10	
number of sheep n_i	7	9	8	13	8	5	4	3	0	1	2	N = 60

$\bar{x} = 3.25 \qquad s^2 = 5.9195.$

From (18) $k = 3.75$ and $p = 0.821$. With these values (19) is 13.2 so that maximum likelihood estimates are the desirable ones. By (21) $k = 3.75$. $p = 0.867$ follows directly from (20).

8. HYPOTHESIS TESTING

Barnard's (1946) method for testing the hypothesis H_0: $p_1 = p_2$ in inverse binomial sampling is presented below.

Consider the situation in which an instrument has been modified in the hope of removing a cause of failure. The sampling procedure consists in arranging to try the modified one, under similar conditions, and to continue to try each until a predetermined number r_1 of failures of the modified instrument and a predetermined number r_2 of failues of the unmodified instrument are observed (inverse binomial sampling). Then if p_1 refers to the modified instrument and p_2 to the unmodified one, and n_1, n_2 are the number of observations made in the two cases, the probability of the pair (n_1, n_2) is

$$\binom{n_1-1}{r_1-1}p_1^{r_1}q_1^{n_1-r_1}\binom{n_2-1}{r_2-1}p_2^{r_2}q_2^{n_2-r_2} .$$

If $p_1 = p_2 = p$, the probability of obtaining some pair of results having the same total $n_1 + n_2 = N$ is

$$\binom{N-1}{r_1+r_2-1}p^{r_1+r_2}q^{N-(r_1+r_2)} .$$

Then the relative probability on the null hypothesis $p_1 = p_2$ of obtaining the pair (n_1, n_2) out of all results with he same total N is

$$\frac{[N-(r_1+r_2)]!(r_1+r_2-1)!(n_1-1)!(n_2-1)!}{(N-1)!(r_1-1)!(r_2-1)!(n_1-r_1)!(n_2-r_2)!} \tag{22}$$

which is independent of p. Hence, if we sum over more extreme pairs having the same N, r_1 and r_2 we shall obtain the significance level of our result.

For example, with $r_1 = 1$, $r_2 = 2$ if we find $n_1 = 10$, $n_2=4$ we have the significance level

$$\frac{2!11!}{13!0!1!}\left(\frac{9!3!}{9!2!} + \frac{10!2!}{10!1!} + \frac{11!1!}{11!0!}\right) = 1/13$$

which is non-significant at the 5% level.

Barnard also develops an approximation procedure when p_1 and p_2 are small and n_1 and n_2 are large. The rule is to calculate $R = (n_1-r_1/2)r_2/(n_2-r_2/2)r_1$ and to enter the F tables with this value of R for $(2r_1, 2r_2)$ degrees of freedom.

For the case of a $2x\ell$ trial, provided p_1, p_2 . . ., p_ℓ are all small, Bartlett's or Hartley's test can be used. Barnard did not elaborate this point. It seems worth-while, therefore, to examine how this situation can be referred to Bartlett's test.

Note that $2(n_1 - r_1/2)/k_1 \frown \chi^2_{2r_1}$, and

$(n_1 - r_1/2)/r_1 \sim (\chi^2_{2r_1})k_1/2r_1$. Identify $k_1 = \sigma^2_1$ and

$(n_1 - r_1/2)/r_1 = s^2_{2r_1}$, i.e., s^2 with $2r_1$ degrees of freedom. Bartlett's test, $M = (N' - \ell)\ell n\ s^2_p - \Sigma[(n'_i - 1)\ell n\ s^2_i]$ where N' = total number of observation, n'_i = number of observations in the ith sample, ℓ = number of samples, then becomes in our notation

$$M = 2\Sigma r_i \ell n\ s^2_p - 2\Sigma r_i \ell n\ s^2_i \qquad (23)$$

where $s^2_i = (n_i - r_i/2)/r_i$, $i = 1, 2, \ldots, \ell$

and $s^2_p = \Sigma(n_i - r_i/2)/\Sigma r_i$.

To perform the test refer M to $\chi^2_{\ell - 1}$.

If $r_1 = r_2 = \cdots = r_\ell$ perform Hartley's test, max s^2/min s^2, and refer to *Biometrika Table* 31.

9. χ^2 -TESTS FOR CONTINGENCY TABLES OF NEGATIVE BINOMIAL TYPES

Steyn (1959) discusses the problem of the analysis of a contingency table where the frequency of one of the cells is predetermined. A summary of his work on this problem follows.

Suppose sampling with replacement is performed in a population consisting of categories FE, $\bar{\text{F}}$E, F$\bar{\text{E}}$, and $\overline{\text{FE}}$ in proportions p_{11}, p_{01}, p_{10} and p_{00} respectively. Individuals are drawn at random until r $\overline{\text{FE}}$'s are found. Obviously the sample size is a random variable.

The following table shows the observed frequencies for the negative fourfold table. This table is obtained when two events E and F can occur or fail either alone or together and sampling is continued until the rth joint failure has occurred, thereby fixing a priori the frequency in one cell but not the total in the sample.

Frequencies

	$\bar{E}$	E	
$\bar{F}$	r	f_{01}	$r + f_{01}$
F	f_{10}	f_{11}	$f_{10} + f_{11} = y$
	$r + f_{10}$	$f_{01} + f_{11} = x$	

Steyn develops the χ^2 expression for the test on this table. The expression with one degree of freedom is

$$(24) \quad \chi^2 = \left[f_{01} - r\left(\frac{\hat{p}'}{q'}\right) \right]^2 \left[\frac{1}{r\left(\frac{\hat{p}'}{q'}\right)} + \frac{1}{r\left(\frac{\hat{p}}{q}\right)} + \frac{1}{r\left(\frac{\hat{p}}{q}\right)\left(\frac{\hat{p}'}{q'}\right)} - \frac{1}{r\left(\frac{\hat{1}}{qq'}\right)} \right]$$

where $(\hat{p}'/q') = [-(r-x+y) + \sqrt{(r+x+y)^2 - 4xy}]/2r$,

$(\hat{p}/q) = [-(r+x-y) + \sqrt{(r+x+y)^2 - 4xy}]/2r$

and $(\hat{1}/qq') = 1 + (\hat{p}'/q') + (\hat{p}/q) + (\hat{p}/q)(\hat{p}'/q')$.

The extension to the case where there are h possibilities for the event E and ℓ possibilities for F is also discussed.

SUMMARY

This paper is a review of the major literature dealing with the negative binomial distribution (NBD). An account of the forms of the distribution is presented. This includes limiting and truncated forms. Parameter estimation is treated in a few specific cases. The problem of testing $p_1 = p_2$, also that of testing $p_1 = p_2 = \dots = p_\ell$ along with a discussion on contingency tables of negative binomial types is presented in some detail.

The inverse binomial sampling form is emphasized throughout the paper. In the discussion of this form several extremely useful relations are discussed. Among these is a discussion of the use of the *Tables of the Incomplete Beta Function* for the evaluation of the cumulative distribution function (c.d.f) Also discussed is the method of adapting Table 41 of *Biometrika Tables for Satisticians,* giving confidence limits on p in the positive binomial, to the inverse binomial sampling case.

ACKNOWLEDGEMENTS

I should like to thank Dr. Herbert A. David for his valuable suggestions and advice and Dr. Boyd Harshbarger for his encouragement.

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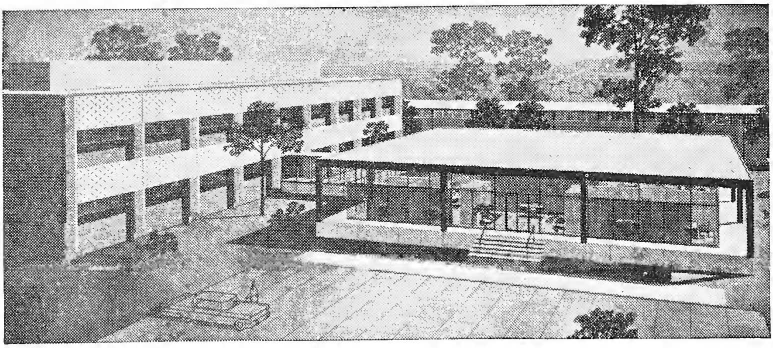


NEWS AND NOTES

(*Editor's Notes — News contributions should be sent to the person whose name appears at the end of the appropriate sections.*)

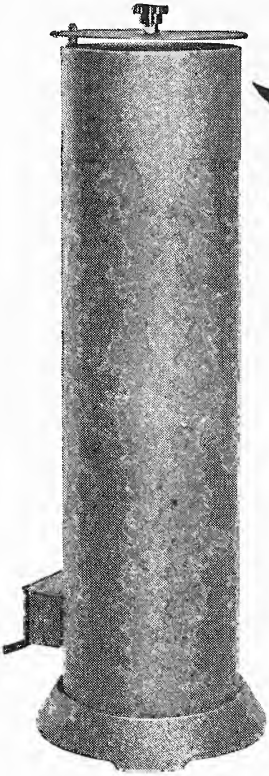
ENGINEERING SECTION

Stricken by heart attacks during the Fall, both Professor Fred Bull of the chemical engineering department at Virginia Polytechnic Institute and Mr. Tilton E. Shelburne of the Virginia Council for Highway Investigation and Research are reported well on the road to recovery and expect to resume their duties early in 1961.

Dean Lawrence R. Quarles of the School of Engineering, University of Virginia, attended a conference on University Reactors at Gatlinburg, Tennessee, sponsored by the Oak Ridge Institute of Nuclear Studies and presented a paper "Construction of the University Reactor." In October Dean Quarles attended a meeting of the ANS-ASEE Committee on Objective Criteria for Nuclear Engineering Education in Chicago and also a meeting of the Region IV Executive Committee of the AIEE in Charlotte, North Carolina. Professor Orville R. Harris of the electrical engineering department of the University of Virginia attended a symposium in New York in November on Magnetism and Magnetic Materials. In December Dr. Harris attended the annual meeting of the American Rocket Society in Washington.

Professor R. E. L. Gildea spent a portion of the summer of 1960 on active duty with the U. S. Public Health Service during which time he helped organize a regional meeting on Radiological Health to be sponsored during the spring by the University of Virginia, the Virginia Department of Health, and the U. S. Public Health Service. Major D. M. Crim of the civil engineering department at Virginia Military Institute is on leave of absence during the first semester of the 1960-'61 academic year and is studying for a graduate degree in civil engineering at the University of Virginia.

Professors Otis L. Updike and Robert M. Hubbard of the chemical engineering department, University of Virginia, attended the annual meeting of the A. I. Ch. E. in Washington in December. Professor Updike was co-chairman for two sessions of the symposium on "The Unsteady State as an Analysis Tool in Chemical Engineering." Professor Hubbard is chairman of two subcommittees of the A.I.Ch.E. Chemical



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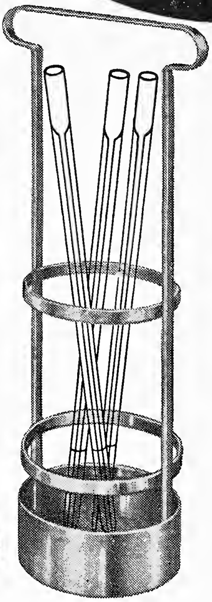

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Engineering Education Projects Committee. A paper by Professor Hubbard "Teaching with a Pneumatic Analog" was published in the August and September issues of the ISA Journal.

Professor Bull has announced that Mr. R. H. Pusey has assumed a teaching position on the staff of his department at VPI taking the place of Mr. G. F. Meehan who recently resigned.

Professor Nelson F. Murphy of the chemical engineering department at VPI is a coauthor with Professor F. W. Bull of a bulletin from the Virginia Engineering Experiment Station entitled "Decolorization of Waste from Caustic Wash of Chlorine-Bleached Kraft Pulp." Professor Murphy attended a meeting of the Committee on Education of the American Electroplaters' Society in Hartford, Connecticut in February. In November he spoke to the Blue Ridge Branch of the American Electroplaters' Society in Roanoke on the subject "How Acid Inhibitors Work."

Professor Robert Truitt of Virginia Polytechnic Institute has announced that the name of his Department of Aeronautical Engineering has been changed to the Department of Aerospace Engineering.

Mr. Tilton E. Shelburne of the Virginia Council for Highway Investigation and Research attended a meeting in Chicago in February of the Data Analysis Panel on the AASHO Road Test.

Dean Lawrence R. Quarles of the Engineering School of the University of Virginia attended a meeting of the American Society for Engineering Education-American Nuclear Society Committee on Objective Criteria for Nuclear Education in Dallas, Texas in January. Also during that month Dean Quarles attended a national meeting of the American Institute of Electrical Engineers in New York. In February he attended a Conference on Higher Adult Education sponsored by the Southern Regional Education Board. He also met with the Regional Advisory Council on Nuclear Energy in Atlanta, Georgia in February.

Professors Otis L. Updike and Robert M. Hubbard of the chemical engineering department of the University of Virginia attended the annual meeting of the American Institute of Chemical Engineers in Washington in December. Dr. Updike was co-chairman of two sessions of a symposium on the subject "The Unsteady State as a Tool for Analysis in Chemical Engineering." Professor Updike has recently received a subcontract from the Oak Ridge National Laboratory for research on the dynamics of solvent extraction cascades.

AGRICULTURAL SCIENCE

Drs. D. G. Cochran and J. M. Grayson, V. P. I. Entomologists, have received a grant of $3,000 for studies in cross resistance and control of German cockroaches resistant to organic phosphate insecticides. The research will be in cooperation with the National Pest Control Association. Both investigators were speakers at recent Regional Pest Control Operator's Conferences. Dr. Cochran spoke at Purdue University and Dr. Grayson at the University of Massachusetts.

Dr. Grover C. Smart, Jr., has joined the staff of the Holland substation as Nematologist. He received his B. A. and M. A. from the University of Virginia and the Ph. D. degree from the University of Wisconsin.

Several staff members in the Agronomy Department at Virginia Polytechnic Institute have recently received grant-in-aids. Dr. G. W. Thomas will study "Electrolyte Inhibition by Soil" on a $19,000 NSF grant, and Drs. R. E. Blaser and R. E. Schmidt have received $2,000 from the American Potash Institute to conduct a project on the "Effect of Potash with Rates of Nitrogen and Phosphorus on Tifgreen Bermuda Grass Turf and Winter Growing Ryegrass." Dr. Blaser has also received a $500 grant from the Golf Course Superintendents Association of American for an undergraduate scholarship in turf.

New members of the Agronomy Department, Virginia Polytechnic Institute are Mr. H. H. Thomas, Mr. S. J. Ross, Jr., and Dr. J. Crews. Mr. Thomas and Mr. Ross will be stationed with the soil survey group in Madison and Dr. Crews will head up the department's program in flue cured tobacco breeding.

The Virginia Tech Chapter of A.D.S.A. (student Dairy Club) was honored as the outstanding dairy club in the United States at the recent A.D.S.A. meeting in Logan, Utah.

The Annual Subscription Rate is $3.00, and the cost of a single number, $1.00. Reprints are available only if ordered when galley proof is returned. All orders except those involving exchanges should be addressed to Robert Kral, Virginia Polytechnic Institute, Blacksburg, Virginia. The University of Virginia Library has exclusive exchange arrangements, and communications relative to exchange should be addressed to The Librarian, Alderman Library, University of Virginia, Charlottesville, Virginia.

Notice To Contributors

Contributions to the Journal should be addressed to Robert D. Ross, Virginia Polytechnic Institute, Blacksburg, Virginia. If any preliminary notes have been published on the subject which is submitted to the editors, a statement to that effect must accompany the manuscript.

Manuscripts must be submitted in triplicate, typewritten in double spacing on standard 8½" x 11" paper, with at least a one inch margin on all sides. Manuscripts are limited to seven pages, with the proviso that if additional pages are desired, the author may obtain them at cost. The author may estimate the length of his paper by counting the total number of characters, including blank spaces, and dividing this by 3300. The result is the approximate number of printed pages in the Journal.

Division of the manuscripts into subheadings must follow a consistent plan, and be held to a minimum. It is desirable that a brief summary be included in all manuscripts.

Footnotes should be included in the body of the manuscript immediately following the reference, and set off by a dashed-line above and below the footnote content. Footnotes should be numbered consecutively from the beginning to the end of the manuscript.

Bibliographies (Literature Cited, References, etc.) should be arranged alphabetically according to author. Each reference should include the date, full title of the article, the name of the Journal, the volume, number (optional), pages, tables and figures (if any). For example: "Sniffen, Ernest W. 1940. Cobbles from the Pleistocene Terraces of the Lower York-James Peninsula Va. Journ. Sci., 1 (8): 235-288 1 fig., 1 tab. Reference to the bibliographic citations should not be made by numbers. Instead, using the above citations, where a reference is desired: either "Sniffen (1940)", (Sniffen, 1940: 186)", or "Sniffen (1940) states that . . ."

Explanation of figures, graphs, etc., should be typed on separate pages. All figures should be numbered consecutively beginning with the first text figure and continuing through the plates. If figures are to be inserted in the text this should be clearly indicated by writing "Figure —" at the appropriate place in the margin.

Illustrations including lettering, should be arranged so that on reduction they will not exceed the dimensions of the maximum size of a printed page. 4-1/2" x 6-1/2", and so that they are well balanced on the page. Large plates must be accompanied by 8-1/2" x 11" photographic copies which can be sent to the reviewers. The Journal will furnish the author with one plate (halftone or line reproduction) or its equivalent; additional figures, colored illustrations or lithographs may be used only if the author makes a grant covering the cost of production. Original drawings (which must be done in black drawing ink) not photographs of drawings, should accompany the manuscript. Photographs should not be used if a line and dot (stippled) drawing will suffice. If photographic prints are to be used they should be glossy, sharp and show good contrast. Drawings not neatly executed and labeled (do not use a typewriter), or which are submitted on yellow or yellowish-white paper will not be accepted.

Galley Proofs and engraver's proofs of figures are sent to the author for correction. Costs of excessive changes from the original manuscript must be defrayed by the author.

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