any species of cell. In the first case, taking into account the percentage error of the method of counting, most probably no less than 91 percent of the population was found to divide synchronously. This figure is based upon a maximal eightfold predicted increase in cell number which was then verified by cytological examination. In the second case, the actual duration occupied by the production of new cells was only 1 hour out of 24 hours.

In similiar attempts to produce synchronously dividing cultures of *Chlorella pyrenoidosa* (*6*) and *C. eugametos* (*7*) by light-dark cycles, fractional centrifugation was found to be necessary to separate the newly released daughter cells from those mother cells which had not yet completed division. On the cycles used, all of the cells could not be kept in phase by varying the light-dark periods alone. Either these two species are more refractive to synchronization than *Chlamydomonas moewusii,* or the period of light to which the cells were exposed was too long. It appears to be important to provide no more light than is necessary for the cells to initiate mitosis, the latter being a light-dependent reaction in these species. Cytokinesis and release of the daughter cells are light-independent reactions. Provision of light beyond the minimum amount of time required for mitosis tends to initiate a second life cycle for a portion of the population, and thus encourages asynchrony.

Recently, Scherbaum reported a new parameter, the synchronization index (SI) for comparing the degree of synchrony in different systems (*8*). The originally derived equation was formulated for cells which divide once to form two cells. The system reported here for *Chlamydomonas* divides three times to produce eight cells. The equation has been modified, so that in

$$SI = n' X (1 - t/gt)$$

n' represents the percentage of cells undergoing synchronous division, t represents the time during which the new cells are being produced, and gt represents the calculated time required for a single cell to produce eight daughter cells during asynchronous exponential growth. In order to determine gt, the "true" generation time was measured first. The latter is an expression of the time taken for a population to double during the exponentional growth phase. It was determined by growing cultures in constant light under asynchronous conditions (*8*). The value was found to be 4 hours. Since a single cell actually gives rise to eight cells during exponential growth and not two, the "true" generation time is not an indication of what is occurring and thus cannot be used in the equation. Theoretically, three times the "true" generation time, or 12 hours, would be necessary for the production of eight cells in constant light. This value of 12 hours was corroborated experimentally. It presents the same type of information as that provided by the term *generation time*; that is, it reveals the amount of time necessary for an *average* cell to produce progeny under "constant" conditions. The synchronization index was 0.83. The range of synchronization indices as computed by Scherbaum (*9*) for all of those species for which data were available was between 0.26 and 0.69.

In conclusion, it has been shown that a single light-dark shift is sufficient to produce a highly synchronized culture of *C. moewusii,* perhaps the highest of any species to date. It would appear that this species, in spite of its "atypical" division pattern, would be an ideal one to study the mechanisms underlying artificially induced growth and division.

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References and Notes

1. This investigation was supported by the University of Connecticut Research Foundation (117-5-9) and the National Science Foundation (G 7643). This report is contribution No. 25 from the Institute of Cellular Biology.
2. A. Campbell, *Bacteriol. Rev.* **21**, 263 (1957); E. Zeuthen, *Advances in Biol. and Med. Phys.* **6**, 37 (1958)
3. The results of work along these lines are being perpared for publication and have already been reported in part before the Society of Protozoologists [**6**, suppl. (1959)].
4. E. Bernstein and T. Jahn, *J. Protozool.* **2**, 81 (1955).
5. E. Bernstein, thesis, Univ. of California, Los Angeles (1956).
6. R. Schmidt, thesis, Univ. of Maryland (1957) and personal communication (1959).
7. D. Wetherell, *Physiol. Plantarum* **11**, 260 (1958).
8. O. Scherbaum, *J. Protozool.* **6**, suppl. (1959).
9. ———, personal communication (1959).

21 January 1960

Subfractionation of Human Serum Enzymes

Abstract. Evidence is given for the subfractionation by starch gel electrophoresis of human serum cholinesterase, aromatic esterase, and acid phosphatase. These subfractions and unfractionated leucine aminopeptidase are localized with respect to the protein zones of the Smithies starch gel pattern.

The remarkable heterogeneity of serum proteins, including enzymes, is being increasingly evidenced today. Proteins, including many that have hitherto been considered homogeneous, are being separated into distinct subfractions by newer and more powerful methods, of which starch gel electrophoresis, introduced by Smithies in 1955, is a leading example. The present work has employed this method to contribute evidence for the subfractionation of several serum enzymes that have been long studied for their biochemical and clinical importance.

The procedure is essentially that given in Smithies' first detailed paper (*1*). Soluble starch (15 gm) (*2*), suspended in 0.03*M*, *p*H 9.00 ± 0.03, borate buffer (100 ml), is heated with swirling to sudden formation of a homogeneous, viscous solution, which is quickly evacuated to remove air bubbles, and poured into plastic trays (2.0 by 25 by 0.65 cm) to cool overnight (final gel, *p*H 8.5 ± 0.05). A small paper rectangle, saturated with the serum sample, is inserted into a vertical slit in the gel and sealed over with a glass cover slip. A coat of waterproofing plastic is applied over the entire exposed gel surface from a spray can. A paper wick (six thicknesses of blotting paper) connects each end of the gel with its corresponding electrode chamber, filled with the same buffer. Electrophoretic conditions are room temperature, constant current 2 ma/cm of gel width, initial gradient 6 volt/cm for a 6-hour run, correspondingly less voltage and current for an overnight 18-hour run. Each gel is cut horizontally into two slices approximately 3 mm thick, each of which may be tested differently. Subsequent cross comparisons are facilitated by a toothed pattern cut through one side of the gel before slicing [after Grouchy (*3*) and Moretti *et al.* (*4*)].

Histochemical methods employed are: (i) protein (blue-black zones), aniline blue black (*1*); (ii) cholinesterase (purple zones), beta-carbonaphthoxycholine iodide (or its 2-bromo-6-naphthyl derivative), *p*H 7.4 Veronal buffer, calcium ions, naphthanil diazo blue B (tetrazotized *o*-dianisidine) as coupling agent (*5*); (iii) aromatic esterase, alpha-naphthyl acetate (purple-gray zones) or beta-naphthyl acetate (red-purple zones), *p*H 7.0 phosphate buffer, diazo blue B as coupling agent (*6*); (iv) acid phosphatase (purple-red zones), naphthyl AS phosphate, *p*H 5.4 acetate buffer, manganese ions, diazotized *o*-aminoazotoluene as coupling agent (*7*); (v) leucine aminopeptidase (orange-red zone), *l*-leucyl-beta-naphthylamide • HCl, *p*H 7.1 tris(hydroxymethyl)aminomethane buffer, diazotized *o*-aminoazotoluene as coupling agent (*8*). Where applicable, the substrate solution is made $10^{-4}M$ in eserine (cholinesterase inhibitor). In most cases incubation for 20 minutes at room temperature is adequate to develop zones. (Control incubations ruled out the possibility of interference by native serum phenols.) After the excess

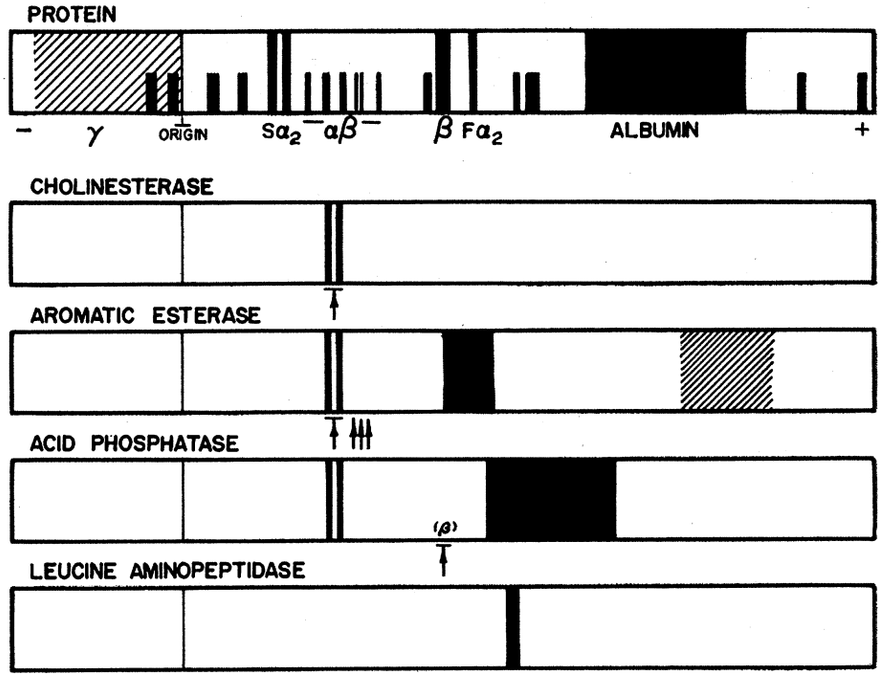

Fig. 1. Starch gel electrophoresis patterns of human serum (schematic). Short bars represent protein zones that are usually less reliable for reference purposes. Shaded areas represent pale diffuse zones. The small arrows indicate locations of corresponding enzyme activities reported by Grouchy (*3*).

reagents are rinsed out, the gels are saturated in glycerol, which increases their transparency, often discloses additional protein zones, and apparently preserves the gels indefinitely.

Results are summarized schematically in Fig. 1. Our typical protein pattern for a normal type 2 haptoglobin serum reveals at least 17 zones, and is distinguished from other published patterns by the doublet or pair of intense zones commonly occurring at the $S\alpha_2$ position.

Cholinesterase activity, which is completely inhibited by $10^{-4}M$ eserine, separates into an intense doublet in the $\alpha\beta$ region. Neither zone can represent specific red cell cholinesterase, since that enzyme does not act on carbonaphthoxy choline (*9*). Among cancer sera, subfractionation into the doublet may occur less readily.

Using conventional electrophoresis, several earlier workers located cholinesterase activity over the α_2-β globulin region. Using starch gel, Grouchy (*3*) found a single zone of cholinesterase activity (acetylthiocholine substrate) at the same position as our doublet. Hunter and Markert (*10*), who were first to demonstrate the value of starch gel electrophoresis in enzyme work, found at least ten different esterases (alpha-naphthyl butyrate substrate) in a wide variety of mouse tissues by starch gel, including two eserine-sensitive zones in liver, but did not report on subfractionation of serum cholinesterase. Kraupp and Werner (*11*) gave evidence for the ammonium sulfate subfractionation of horse serum cholinesterase. However, evidence for subfractionation of human serum cholinesterase has apparently not been given previously.

Aromatic esterase activity separates into four zones. An eserine-sensitive doublet coincides with the previous doublet and probably represents the same enzymes (nonspecific cholinesterases). A third broad zone, resistant to eserine, migrates between β and $F\alpha_2$, while a fourth zone, pale and diffuse, migrates with the leading half of the albumin zone. [The latter two zones may be components of the A-esterase that Augustinsson (*12*) found migrating with albumin in conventional electrophoresis.] Marked substrate specificity is apparent in that, with beta-naphthyl acetate, the third zone is more intense than the doublet, whereas the reverse is true with alpha-naphthyl acetate (and the third zone shows evidence of further subfractionation). Grouchy, using alpha-naphthyl acetate substrate, found a single zone corresponding to his cholinesterase zone and occasionally observed two or three additional zones, but unlike ours, narrow and located in the $\alpha\beta$ region.

Acid phosphatase activity separates into three zones. Two again occur as a doublet which, as nearly as can be determined, also coincides with the cholinesterase doublet. A broader zone migrates between $F\alpha_2$ and albumin. In contrast, Grouchy, using beta-naphthyl phosphate substrate, *p*H 5 to 6, found all activity solely at the β zone.

This subfractionation of unrelated activities into coinciding doublets invites comment, especially since the intense $S\alpha_2$ protein doublet also is close. Offhand, one may suspect that the enzyme doublets arise simply from nonspecific staining of the $S\alpha_2$ protein doublet by dyes that form in the different substrate solutions. However, careful observations by several methods demonstrate that the enzyme doublets do not coincide with the protein doublet, but occur in the $\alpha\beta$ region, and quite independently of the presence or absence of protein zones in that region. Moreover, the stained enzyme doublets develop in substrate solutions that remain pale or have a different color.

Work proceeds to determine how many of the apparent differences between Grouchy's findings and ours may be due to different electrophoretic conditions or substrate specificity, with the added possibility that other substrates may uncover additional esterase and phosphatase subfractions. Also, the authenticity of the reported subfractions is being further examined by appropriate re-electrophoresis and study of the enzymatic properties of each separated subfraction. This work is stimulated by the probability that the blood level of each new subfraction found, by reflecting the healthy or diseased metabolism of its particular source tissue in the body, can offer a more penetrating and selective means of diagnosis, as has proved practical in other cases, notably prostatic acid phosphatase in prostate cancer (*13*).

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References and Notes

1. O. Smithies, *Biochem. J.* **61**, 629 (1955).
2. Several brands of starch have been employed satisfactorily: potato starch, powder, Amend Drug and Chemical, or Mallinckrodt (300 gm, solubilized by heating in 600 ml of acetone and 6 ml of concentrated HCl at 39°C for 45 minutes); or the specially solubilized product of Connaught Medical Laboratories, Toronto (used directly at a recommended lower concentration of 13 percent).
3. J. de Grouchy, *Rev. franç. études clin. biol.* **3**, 881 (1958).
4. J. Moretti, G. Bouissier, M. Jayle, *Bull. soc. chim. biol.* **40**, 59 (1958).
5. A. M. Seligman and M. M. Nachlas, *J. Clin. Invest.* **29**, 31 (1950).
6. G. Gomori, *Microscopic Histochemistry. Principles and Practice* (Univ. of Chicago Press, Chicago, Ill., 1952), p. 207.

7. M. S. Burstone, *J. Natl. Cancer Inst.* **21**, 523 (1958).
8. ———, *J. Histochem. and Cytochem.* **4**, 217 (1956).
9. H. A. Ravin, K.-C. Tsou, A. M. Seligman, *J. Biol. Chem.* **191**, 843 (1951).
10. R. L. Hunter and C. L. Markert, *Science* **125**, 1294 (1957); C. L. Markert and R. L. Hunter, *J. Histochem. and Cytochem.* **7**, 42 (1959).
11. O. Kraupp and G. Werner, *Arch. intern. pharmacodynamie* **76**, 13 (1948).
12. K.-B. Augustinsson, *Nature* **181**, 1786 (1958).
13. This work is aided by grant No. T-77 from the American Cancer Society, and by a gift from Mollie E. Swinney. We wish to acknowledge assistance in this work by J. M. Smith and H. C. Coffey.

30 December 1959

Distortion of the Pyramid of Numbers in a Grassland Insect Community

Abstract. An intermediate size class of the insect component of a natural community contained more individuals than did a small size class, altering the expected ecological pyramid of numbers. The anomaly resulted from the presence of an abundant immigrant species, and it is suggested that departures from the normal pyramid might, in general, imply external disturbance.

Of the various possible ecological pyramids, the one based on the numbers of individuals per size class is the most easily obtained. However, in it the ecological implications are not so clear as in pyramids based on biomass or energy, or pyramids in which the species are grouped according to trophic level, and it is accordingly comparatively little used. Nevertheless, the size-frequency distribution is probably a basic datum which will eventually be incorporated in the main theory of community structure and organization.

As part of an ecological investigation of a grassland insect community, the size-frequency distribution of the components was ascertained for a series of samples collected 14 July 1958 and for a second series taken 21 July 1958 (Table 1). In each case, eight independent samples of 25 sweeps each were secured in the upland herbaceous vegetation of an abandoned field (*1*) by sweeping with an 18-in. canvas net; each set includes all samples obtained on that day. The samples were taken at widely separated localities on the field, and were calibrated for area by comparing the numbers of adult individuals of a key species taken in the net with the number determined by direct counts within frames of known dimensions. The total area covered by each series of eight samples was estimated at approximately 90 m^2.

The sweepings were carefully sorted in the laboratory with the aid of a microscope (*2*), so the smaller size classes are satisfactorily represented in the samples. Although this sweep sampling technique is not adequate for large, active insects, these are insignificant in number as compared to the small and sedentary species, and their under-representation in our samples does not materially affect the conclusions we have drawn.

The striking fact about the size-frequency distribution for both sets of samples is the large numbers of individuals in the 4.6- to 6.5-mm class (Table 1). In every sample, this number exceeds that in the next smaller (2.6- to 4.5-mm) class. This results in a top-heavy pyramid of numbers (Fig. 1*a*). Since insects from 0.6 to 10.5 mm in size made up the overwhelming bulk of all the animals that were taken in the sweep samples, this anomalous size distribution applies as well to the entire fauna of the herbaceous stratum of the field.

Examination of the species composition of the 4.6- to 6.5-mm class shows that it is largely made up of adult individuals of the meadow spittle bug, *Philaenus leucophthalmus* (L.) (Table 1). In fact, exclusion of the *Philaenus* material from consideration in the pyramid of numbers restores that size class to normal proportions (Fig. 1*b*).

It is therefore of interest to note that the vast majority of adult *Philaenus leucophthalmus* on the field are of exogenous origin. As the cultivated crops of clover and alfalfa in the surrounding countryside are cut, these insects move into the grassland in such numbers as to dominate the local fauna during most of the growing season. In both 1958 and 1959, essentially all adult *Philaenus* present in mid-July had come into the field from the outside, the small "native" populations having fallen nearly to zero by the end of June. So far as we know, no other important species taken in the sweep samples behaves in this way.

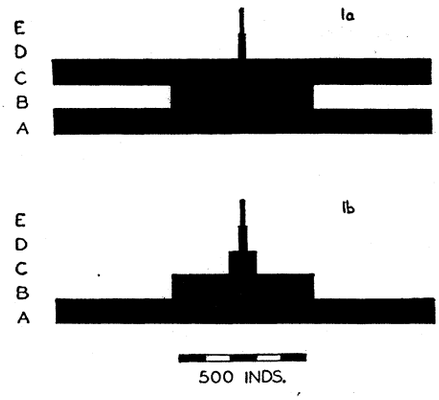

Fig. 1. Ecological pyramids of numbers for the insects of the herbaceous stratum in an old-field upland grassland community, Edwin S. George Reserve, Livingston County, Mich., based on a series of sweep samples collected 21 July 1958. (1*a*) Pyramid for total sample; 1*b*, pyramid after removal of all specimens of the spittlebug, *Philaenus leucophthalmus*. Size classes (in mm): *A*, 0.6–2.5; *B*, 2.6–4.5; *C*, 4.6–6.5; *D*, 6.6–8.5; *E*, 8.6–10.5.

In 1957 (when samples of the sort described here were not taken), *Philaenus* was estimated to be approximately 10 times as abundant on the field as in 1958, and the pyramid of numbers would then presumably have been even more distorted than that shown in Fig. 1*b*.

Table 1. Size-frequency distribution of insects taken in two series of sweep samples from an old-field upland grassland community, Edwin S. George Reserve, Livingston County, Mich., in July 1958. The numbers in parentheses refer to *Philaenus leucophthalmus* (L.); the other numbers refer to all the insects in the class.

Sample No.	Size class category (mm)				
	0.6–2.5	2.6–4.5	4.6–6.5	6.6–8.5	8.6–10.5
			July 14		
1	25	7	16 (15)	0	0
2	100	12	40 (31)	2	1
3	56	28	134 (127)	3	0
4	75	16	43 (36)	2	3
5	70	24	72 (63)	1	1
6	219	17	102 (95)	4	2
7	52	12	36 (31)	3	0
8	81	42	71 (64)	1	0
Total	678	158	514 (462)	16	7
			July 21		
1	230	104	185 (165)	3	4
2	102	31	159 (150)	6	2
3	169	100	147 (139)	5	2
4	209	89	215 (195)	2	2
5	242	54	186 (167)	6	1
6	306	68	186 (173)	2	3
7	86	66	169 (156)	2	1
8	131	42	233 (222)	4	0
Total	1475	554	1480 (1367)	30	15