

est for atmospheric regeneration systems. It has practically no tendency to cause foaming or to stick to the surface of culture vessels, even in old or mismanaged cultures. The higher culture temperature results in more efficient cooling systems, while discouraging fungal and bacterial contamination (5).

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#### References and Notes

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  2. R. D. Gafford and C. E. Craft, *USAF School of Aviation Medicine Rept. No. 58-124* (Jan. 1959). We have modified the original medium slightly to improve growth: the composition in grams per liter is:  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.15;  $\text{KH}_2\text{PO}_4$ , 0.25; tris(hydroxymethyl)aminomethane, 0.50;  $\text{NaNO}_3$ , 0.50;  $\text{KNO}_3$ , 0.50;  $\text{CaCl}_2$ , 0.06;  $\text{H}_3\text{BO}_3$ , 0.034; trisodium ethylenediaminetetraacetate, 0.03;  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , 0.022;  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.004;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.004;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.00066;  $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $1.5 \times 10^{-8}$ ;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $4.7 \times 10^{-6}$ . The tris(hydroxymethyl)aminomethane serves as buffer only and can be replaced by  $\text{K}_2\text{HPO}_4$ . If the medium is to be autoclaved, the phosphate should be kept separate.
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- 4 May 1961

### Localization Effects with Steady Thermal Noise in One Ear and Pulsed Thermal Noise in the Other

**Abstract.** When the duration or repetition rate of pulses in the left ear is increased, while steady, in-phase, thermal noise sounds in the right ear, the pulses are heard to move toward the median plane. At still longer durations (for a given repetition rate) the loudness of noise on the right diminishes, until finally all sound is localized at the median plane.

In these experiments thermal noise was led through a mixing circuit such that part went through an electronic switch and interval timer (Grason-Stadler) to the left ear, and the other part went to the right ear. Thus pulses were presented to the left ear, and steady, in-phase noise to the right ear. Over-all presentation time of stimuli to both ears for a given judgment was set at 10 sec by a Hunter interval timer. Signals in each channel went through an attenuator and transformer before arriving at the earphone (Telephonic TDH-39).

*Perception corresponding to left ear pulses.* The left ear attenuator was set at 40 db above threshold for each subject. (Thresholds were obtained for 200 msec, 1 per sec bursts of noise.) The right ear attenuator was then set to give voltage into the right phone equal to that in the left phone. Repetition rates of pulses used were 1.4, 4.7, 13.9, 58.8, and 105.3 per second. For a given pulse repetition rate, the duration of the pulse could be increased until the subject heard the pulses at the median plane. (The median plane is defined as the plane passing between the cerebral hemispheres.) Thresholds for such centering were obtained by the Method of Limits, using four crossings.

Figure 1, curve A, shows data (medians) for the ten subjects used. The duration required to center the pulses decreases as pulse repetition rate is raised. Individual differences in required duration are more marked at low pulse rates, but all subjects tended to require increasingly smaller durations with increasing repetition rates. (A Friedman rank order test shows significance beyond the .001 level.)

The greatest interaction occurs when the noise in the two ears is in phase (perfectly correlated); for in another experiment with an additional ten subjects, we found that reversing the phase of noise coming to the two ears significantly raised the duration required to center the pulses. It should be noted that Pollack (1) did not obtain significant localization effects by using partially correlated noise.

The present results can be said to show summation effects, since increasing pulse duration increases center localization effects. Tobias and Schubert (2) also found summation effects in overcoming an initial binaural transient disparity. They suggest that the power of the initial transient disparity on localization may be related to neural onset responses. It is possible that in the present experiment the localization "power" of the neural onset response in the left ear must be overcome by increasing pulse duration and thus increasing duration of interaction with the right ear stimulus.

*Right ear perception.* The duration of the gap in the left ear noise was increased until the subject could first detect noise at the right ear position. Thresholds for this detection were determined by the Method of Limits, with four crossings. Half of the subjects obtained these thresholds before those

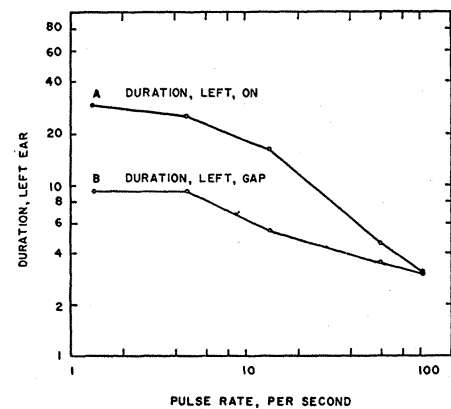


Fig. 1. (Curve A) Median duration (left ear) required by subjects to center the pulses, decreasing as the pulse repetition rate is raised. (Curve B) Median duration of gap (left ear) required by subjects for the detection of noise in the right ear, decreasing as the pulse repetition rate is raised.

described previously. Order of conditions, as in the preceding experiment, was determined for each subject from a set of randomly drawn numbers.

Figure 1, curve B, shows that the median duration of gap required for the detection of noise in the right ear falls for the higher repetition rates used. (A Friedman rank test shows significance beyond the .001 level.)

Our tentative interpretation of these results is as follows: Conduction through the neural channel corresponding to right ear localization perception is suppressed when the stimulation occurs in the left ear. When the left ear stimulus ceases, nerve impulses can pass through this channel; but beyond this point is a summation process, because of which detection depends on repetition rate and duration.

Questioning of the subjects revealed that the right-ear signal was heard to increase in loudness as gap duration was increased. Furthermore, at low repetition rates (1.4 and 4.7), the right-ear signal was heard typically as pulsing; at higher rates the signal heard at the right ear location was heard as continuous. These latter continuity effects (see 3) may occur over such long periods of time because of the facilitating effect of the continuous input to the right ear. A possible mechanism might be an additional neural "facilitation" circuit from a region prior to a localization gating mechanism to a region beyond (4).

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4. This research was supported by a grant from the University Research Committee from funds provided by the Wisconsin Alumni Research Foundation.

6 April 1961

## Polymeric Particles of Protein Insoluble at pH 5 from Rat Liver

**Abstract.** A procedure is given for approximating the volumes of small particles of protein insoluble at pH 5. Among particles smaller than  $2.08 \times 10^7$  cubic angstroms the change in size was linear. The distribution of sizes indicated a polymeric relationship among the particles.

Approximately 25 percent (1) of the soluble protein fraction (2) from rat liver is insoluble at pH 5. When fixed in  $\text{OsO}_4$  and viewed in an electron microscope, this insoluble protein has some of the dimensional characteristics of the endoplasmic reticulum (3). Because of the presumed role of the ergastoplasm (4) in the synthesis of cellular end products and the relations of the microsomal fraction of cells to this organelle, we examined the further possibility that the soluble proteins might serve as a source of  $\alpha$  and  $\gamma$  cytomembranes (5). This study was primarily concerned with the distribution of the sizes of the smallest particles derived from the insoluble protein of the "soluble protein fraction" of liver cells.

Rats were killed by decapitation, and the livers were immediately removed and placed in cold pH 7.12 phosphate-buffered 0.25M sucrose solution (2.5 ml of sucrose solution per

gram of tissue). Homogenization was begun within 10 min post mortem. At 20 min post mortem the homogenate was spun in the centrifuge at 20,000g for 90 min to remove mitochondria, and 2 days after that it was spun at 102,000g for 70 min to remove the microsomal fraction. The pH of the resulting supernatant was adjusted to 5.07 with 0.10N HCl. The precipitate that formed promptly was spun down to give a pellet of protein insoluble at pH 5.

Only a slight amount of precipitate developed from the 20,000g (mitochondrial) supernatant in the 2 days preceding the final spin. It was assumed, therefore, that the  $\text{Mg}^{++}$  concentration was high enough (6) to prevent clumping of the cytomembranes and that a normal microsomal fraction could be removed from this sample by centrifugation. We have found that the precipitation of this insoluble protein from a mitochondrial supernatant also removes the microsomal fraction. However, because this insoluble protein from the soluble protein fraction, prepared as described, had none of the electron-dense characteristics (4) of the microsomal fraction, we considered the precipitate obtained to be essentially free of microsomes.

The pellet of protein was suspended in 10 ml of a 1-percent  $\text{OsO}_4$  solution at pH 5.04. The fixed pellet was triturated by using a glass grinder and resuspended in a test tube. Those particles small enough to remain in suspension after 24 hours in the  $\text{OsO}_4$  were examined in the electron microscope. Small drops of the suspension were dried on collodion-covered RCA stainless steel grids in a desiccator for 1 week. The grids were shadowed with chromium at an angle of 5 : 1 ( $\tan \angle$

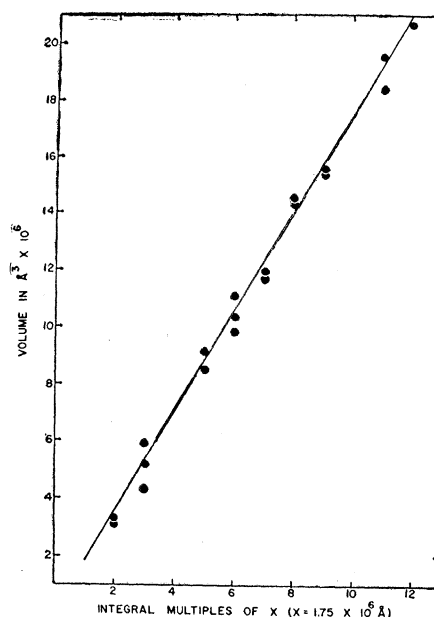


Fig. 2. Plot of the particle sizes of protein insoluble at pH 5, expressing the linearity of the volumes with respect to integral multiples of the smallest volume.

$= 0.2$ ). The particles on the grids were viewed and photographed in a Philips model EMU electron microscope. A magnification of 40,000 was the most useful for measuring the dimensions of the particles.

Our measurements indicated that the particles were not spherical, but ellipsoid. By assuming that they are probably flattened ellipsoids, useful presumptive calculations of the volumes of the particles are possible. A further assumption, obviously inaccurate but reasonable for the purposes of such determinations, was that the beam of chromium was perpendicular to the major axis of the ellipsoids. A fair approximation of the volume of the particles was calculated in the following manner (Fig. 1):

Given that  $\tan \alpha = 0.2$ , and by measuring the length of the shadow,  $X$ , the apparent or measured height of the particle,  $M$ , can be calculated as

$$M = X \tan \alpha$$

By construction,  $\triangle ADE \sim \triangle ABC$ , therefore,  $\angle DEA = \angle ABC = \alpha$ . From this, it is apparent that

$$M = R + (R \cos \alpha)$$

where  $R$  is the radius of the hypothetical sphere and

$$R = M / (1 + \cos \alpha)$$

The formula for the volume of an ellipsoid is  $V = 4/3 \pi AB^2$  where  $A$  is the length of the semimajor axis and  $B$  is

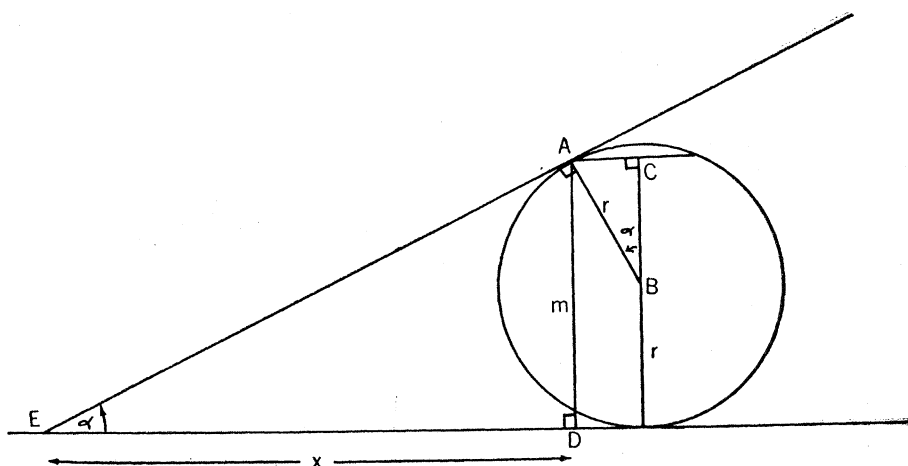


Fig. 1. Diagram of hypothetical particle upon which were based calculations of the volumes of the particles of protein insoluble at pH 5.