Reports and Letters

High-Resolution Microradiography

A method has been developed for coupling the high penetrating power of x-rays with the high resolving power of the electron microscope (*1*). A high-resolution x-ray microscope has long been sought. Recent work by others has given a method for producing good x-ray micrographs with a useful magnification of about 1000 to 1500 diameters but with resolution far below that of the electron microscope. Our technique combines the best features of both x-rays and the electron micrscope and for the first time gives x-ray micrographs at useful magnifications of 10,000 to 25,000 diameters, showing detail of the order of that hitherto seen only with the electron microscope. Biological specimens can now be examined in air or in the growing medium, so that the destructive and desiccating effects of the high vacuum and electron beam in the electron microscope are eliminated.

The basic technique consists of three steps: (i) making a relief image of the specimen in a grainless medium by means of the high penetrating power of x-rays; (ii) obtaining a thin replica of this relief, which is then shadow-cast and made suitable for examination with an electron microscope; and (iii) examining this replica under the high resolving power of the electron microscope.

The first step is the essential one and involves new techniques that make the subsequent steps practicable. Many substances are adequately sensitive to x-rays, particularly some of the finest-grained photographic emulsions, but all of them possess grain sizes that are readily apparent even under a light microscope and are far larger than the details we wish to examine. Even the finest grains of silver in available photographic emulsions are larger than the particles of carbon black, for instance, that we sought to resolve. Therefore, we initiated a search among known photo- and x-ray-sensitive substances for one that would show no structure in the electron microscope range. Bichromated gelatin is such a material, but it is not suitable for preparing replicas by presently known methods. Subsequently, we found that faces of crystals of ammonium dichromate are adequately sensitive to x-rays and at the same time are free from structure in the interfering range.

When we attempted to grow large crystals of dichromate for this use, we found that the slight amount of solution adhering to the crystal as moisture deposits tiny crystals on the otherwise plane surface and that the plane surfaces crack on drying. The cracking and the infinitesimal crystals adhering tenaciously to the crystal faces destroyed the value of the crystals for our purpose. This necessitated development of a method whereby we might prevent both cracking and the formation of fine "after" crystals by diffusing away the thin residual layer of mother liquor.

We finally developed a successful technique that consisted of removing the crystal from the mother liquor and immediately agitating it in a viscous collodion solution. The collodion solution aids in washing off the residual mother liquor, and because it dries to a film on the crystal face, it provides a membrane through which moisture can diffuse slowly. When the collodion film has become completely dry, it can be stripped from the crystal, leaving a completely plane surface without either grain or roughness. Later, we found other materials—particularly certain types of plastic sheetings—that are free from structure in our working range and are also adequately sensitive to x-rays. Such a material is the commercial polyvinyl chloride-acetate film that undergoes change of solubility under x-ray bombardment.

The techniques for using either dichromate crystal faces or polyvinyl films are quite similar. The specimen is mounted tight against the sensitive surface so that there is no space between them and so that no motion of one with respect to the other is possible. This is necessary to cut down the width of the penumbra due to the 1-mm focal spot. Thicker specimens will require a finer focal spot or increased target-to-specimen distance. The combination is then mounted in the path of a carefully limited beam of soft x-rays for a sufficient period to form the image. Exposure for the Thermax particles shown is approximately 23 hours at 9 to 10 kv and 20 ma, using vinyl film as a recorder.

The effect of the x-ray exposure is to change the solubility of the plane surface; the image is developed in relief by dissolving away the more soluble portions of the exposed surface. We have found a mixture of anhydrous alcohols satisfactory for developing the x-ray image on the dichromate crystal face. With polyvinyl films, we use 30-percent acetone in water. This enables us to secure satisfactory relief without softening the film.

A cast thin enough to permit the passage of the electron beam is then made of the relief surface, using silicon monoxide or other suitable material by familiar methods. The replica is mounted on the customary screen. Finally, the relief of the replica is emphasized by the familiar method of volatilizing a thin metal coating on it from an angle. We apply chro-

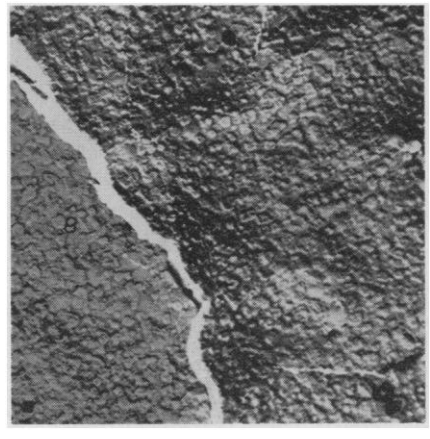

Fig. 1. X-ray micrograph showing thickly populated field of well-dispersed Thermax particles in polyvinyl alcohol; Particles show up as light "hollows." Two particles have been outlined in ink. The two solid black particles are actual Thermax particles (approximately × 3333).

Fig. 2. X-ray micrograph showing "clump" or aggregate of Thermax particles (× 5000).

mium. The shadow-cast replica is then ready for examination in the electron microscope.

By this means, we have been able to obtain excellent electron micrographs of carbon black particles showing variation in dispersion (Figs. 1 and 2). We have made pictures of blood cells at 5 kv, but much control work must still be done before proper interpretation can be made of the results. New techniques will enable us to picture bacteria and other living matter without exposure to the high vacuum of the electron microscope. We have pictured the overlapping of lead particles and structural differences that would not be resolved by the light microscope.

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Notes

1. W. B. Wiegand, H. A. Braendle, and M. T. Karves helped with this work. It was encouraged and financed by Columbian Carbon Company, 380 Madison Ave., New York, to whom applications for patents covering appropriate parts of it are assigned.

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Effects of Pentobarbital Sodium on Adaptive Behavior Patterns in the Rat

We have previously reported a relationship between the rat's behavior in our maze and cholinesterase (ChE) activity in the cerebral cortex (*1*). In each unit of our maze, the rat chooses between a lighted and a dark alley, only one of which is open. The lights and correct alleys are changed after each trial; thus following any one cue can bring only chance success. Nevertheless, the rat usually displays consistent choices ("hypotheses")—for example, consistently choosing lighted alleys or the left alleys. This hypothesis behavior represents a perceptual selectivity that is significant in the organism's normal adjustments (*1*).

The present experiment derived from the following considerations. (i) Illumination is evidently the dominant cue in our maze, since most animals start with a light-going preference. Persistence in this preference results in a light hypothesis. A spatial hypothesis requires *ignoring* the dominant visual cue. (ii) Animals with lower ChE activity display a preponderance of light hypotheses. Animals with higher ChE activity tend to abandon this preference and adopt spatial hypotheses (*1*). (iii) ChE activity is assumed to be an index of the rate of acetylcholine (ACh) metabolism. (iv) Pentobarbital sodium reduces the rate of synthesis of ACh in the cortex (*2*).

These four points can be rephrased in the following hypothesis: Animals with low rates of cortical ACh metabolism are relatively incapable of ignoring the dominant visual cue. Therefore, pentobarbital, by depressing ACh metabolism, should increase light-going behavior.

Figure 1 shows the percentage of light-going choices per trial for three groups of 80-day-old male rats. Group I (control) choices showed the initial light-going tendency but soon fell to about 50 percent and remained there. Their hypotheses during days 1–4 were distributed fairly evenly among light, dark, left, and right (Table 1). Group I was of our C strain, a cross between maze-bright and maze-dull (D strain) rats (*3*). D rats (group IV, $n = 24$) were more persistent in light-going behavior and rarely adopted other hypotheses. In comparison with C rats, D rats have lower cortical ChE activity (*1*).

Group II consisted of C rats run under pentobarbital on days 1–4. Ten milligrams per kilogram of body weight was injected intraperitoneally about 15 minutes prior to testing. This dosage did not reduce eating or increase maze running time. Figure 1 shows that their behavior was preponderately light-going as long as the barbiturate was used, most animals persistently displaying light hypotheses. When pentobarbital was not used (days 5 and 6) light-going choices fell off. Administering the drug on day 7 raised them again. The *t* test (Table 2) indicates highly significant differences between groups I and II. Peculiarities in the behavior of the drugged animals included a rapid gait and lack of exploratory and "vicarious-trial-and error" behavior. The whole picture was one of sterotypy.

To test whether the drug might have made the animals phototropic and unresponsive to the problem nature of the situation, ten animals of group II were later trained with the maze made solvable. On successive days the right, left, and dark alleys were correct. Most animals reduced their light-going behavior and followed these cues, even under the

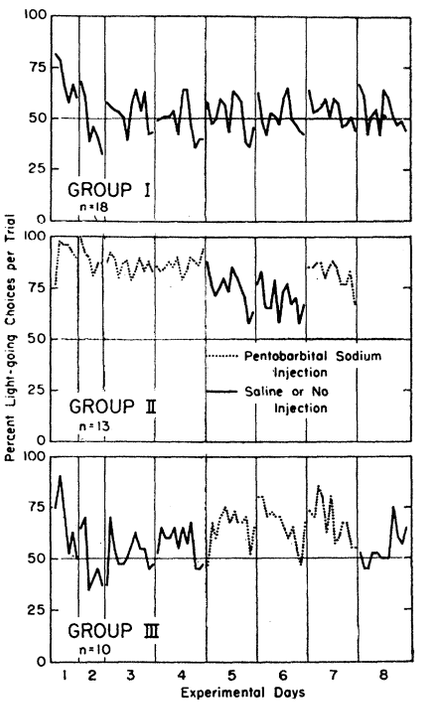

Fig. 1. Percentage of light-going choices per trial for three groups of rats.

Table 1. Frequency of various hypotheses per rat per day. An animal is credited with displaying a hypothesis if its choices during a day deviate from 50 percent, for any cue, by at least 2.5 standard deviations.

Group	Strain	Days 1–4				Days 5–6			
		Light	Dark	Left	Right	Light	Dark	Left	Right
I	C	.19	.11	.19	.15	.20	.06	.30	.08
II	C	.90	.00	.00	.05	.58	.00	.04	.04
III	C	.30	.10	.07	.10	.40	.10	.05	.10
IV	D	.36	.03	.14	.07				

Table 2. *t* Tests of differences between groups in the pentobarbital experiment (light-going scores).

Groups compared	Experimental days					
	1–2	3	4	5	6	7
I and II	7.62*	5.81*	6.15*	3.88*	3.07†	4.08*
II and III	7.61*	4.14*	3.35†	1.06	0.32	1.65
I and III	0.08	0.14	1.10	1.88	2.37‡	1.86

* *t* significant at .001 level of confidence. † *t* significant at .01 level of confidence.
‡ *t* significant at .05 level of confidence.