sory paths except the ones concerned with the object of attention, such an inhibitory mechanism might lead to favoring of the attended object by the selective exclusion of incoming signals. It is conceivable not only that such a selective sensory inhibition might operate simultaneously for various sensory modalities, leaving one or more unaffected but that the selectivity could extend to some discriminable aspects of any single modality—for example, to one tone and not to others. This suggestion finds support in the recent demonstration that sensory "habituation" may occur to a particular tone—that is, a slowly developing inhibitory effect on auditorily evoked potentials observed in the cochlear nucleus on prolonged repetition of a given tone, an influence that does not affect other frequencies that are novel to the animal (*6*). The pathway by which this inhibitory influence acts on incoming auditory impulses remains to be determined, but experiments now in progress have shown that during electric stimulation of the midbrain reticular formation, the auditory potential in the cochlear nucleus is depressed (*7*).

The present observations suggest that the blocking of afferent impulses in the lower portions of a sensory path may be a mechanism whereby sensory stimuli out of the scope of attention can be markedly reduced while they are still in their trajectory toward higher levels of the central nervous system. This central inhibitory mechanism may, therefore, play an important role in selective exclusion of sensory messages along their passage toward mechanisms of perception and consciousness. In a recent symposium on brain mechanisms and consciousness, Adrian pointed out that "the signals from the sense organs must be treated differently when we attend to them and when we do not, and if we could decide where and how the divergence arises we should be nearer to understanding how the level of consciousness is reached" (*8*).

RAÚL HERNÁNDEZ-PEÓN*
HARALD SCHERRER†
MICHEL JOUVET‡
School of Medicine, University of California, Los Angeles, and *Veterans Administration Hospital, Long Beach*

References and Notes

1. J. D. French, M. Verzeano, H. W. Magoun, *Arch. Neurol. Psychiat.* 69, 505 (1953).
2. K.-E. Hagbarth and D. I. B. Kerr, *J. Neurophysiol.* 17, 295 (1954).
3. R. Hernández-Peón and K.-E. Hagbarth, *ibid.* 18, 44 (1955).
4. H. Scherrer and R. Hernández-Peón, *Federation Proc.* 14, 132 (1955).
5. This work was aided by grants from the Commonwealth Fund, the National Institute for Neurological Diseases and Blindness of the U.S. Public Health Service, and Eli Lilly and Company. This report is based on a paper presented before the American Physiological Society on 12 Apr. 1955.
6. R. Hernández-Peón and H. Scherrer, *Federation Proc.* 14, 71 (1955); R. Hernández-Peón, M. Jouvet, H. Scherrer, in preparation.
7. M. Jouvet, E. Berkowitz, R. Hernández-Peón, in preparation.
8. E. D. Adrian, in *Brain Mechanisms and Consciousness*, J. F. Delafresnaye, Ed. (Blackwell, Oxford, 1954), p. 238.
* Present address: University of Mexico, Mexico D.F., Mexico.
† Present address: University of British Columbia, Vancouver, B.C., Canada.
‡ Present address: University of Lyon, Lyon, France.

25 August 1955

Effect of Barbiturates on Acetylation

Several different groups of investigators have recently attempted to demonstrate a biochemical action of barbiturates and other central nervous system depressants. After McLennan and Elliott (*1*) showed that acetylcholine synthesis by brain slices was inhibited by these agents, interest focused on the study of acetylation reactions generally, either in tissue slices or in relatively purified enzyme systems. The results of these studies, however, have been distinctly at variance with one another so that it has not been possible to draw any clear conclusions other than that the experimental methods offered some unseen difficulties (*2–4*). Experiments carried out in this laboratory may shed some light on this problem.

The acetylation system that was studied was that described by Kaplan and Lipmann—namely, the acetylation of arylamines by a pigeon liver enzyme in the presence of adenosine triphosphate (ATP) and coenzyme A (coA) (*5*). Both the crude coA and purified coA were used (Nutritional Biochemicals and Pabst Laboratories, 300 units/mg, 75 percent pure). The analytic procedure for sulfanilamide was that of Bratton and Marshall (*6*), using a photoelectric colorimeter. Similar results were obtained from the use of the pure and the crude coA preparations.

Purified coA was stable when it was kept cold and dry, but aqueous solutions rapidly lost their activity, presumably through oxidation. By dissolving the coA in 1.0*M* cysteine at *p*H 6.8, flushing the vessel with nitrogen, and storing in the freezing chamber of a refrigerator, it was possible to keep the coA solution active for 1 to 2 weeks.

The effect of various barbiturates on this "pure" acetylating system can be seen in Fig. 1. All the barbituric acid derivatives used inhibit acetylation, the amount of inhibition being related to the concentration of the drugs. The concentrations used included the range achieved pharmacologically in the use of these agents as anesthetics. One of the drugs was a convulsant barbiturate, 1,3-dimethylbutyl barbituric acid (*7*), and it too inhibits acetylation. Also tested were MC 1415 (2,2-diethyl-1,3-propanediol) and MC 2973 (2,2-diethyl-1,4-butanediol (*8*). Neither of these substances produces any significant inhibition of acetylation.

With respect to the mechanism of inhibition, addition of extra purified coA will alleviate the inhibition, but addition of extra ATP to the incubating mixture will not (Table 1). Addition of magnesium ions increases the inhibition by barbiturates rather markedly, perhaps by activating some residual ATPase, which may still contaminate the enzyme preparation. When the enzyme system from liver is further fractionated, it has been shown that it is stimulated, rather than inhibited, by magnesium (*9*).

The results presented, in agreement

Table 1. Effect of coA and ATP on inhibition of acetylation by phenobarbital.

coA (units)	ATP (μmole)	Phenobarbital ($10^{-3}M$)	Acetylation (%)	Inhibition (%)
Experiment 1				
0	4	0	0	
1.5	4	0	64.8	
1.5	4	1	50.0	22.8
3.0	4	0	75.1	
3.0	4	1	73.6	2.0
Experiment 2				
0	4	0	0	
1.5	4	0	70.8	
1.5	4	5	41.6	41.2
1.5	8	5	38.0	46.3

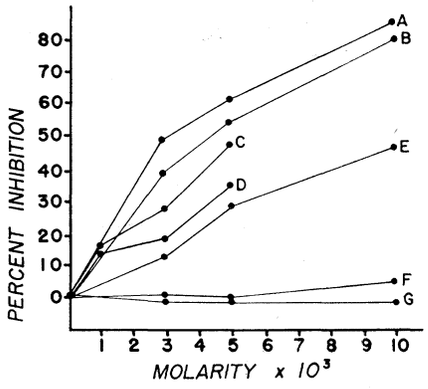

Fig. 1. Each value represents the average of duplicate determinations. Contents of each tube included 4 μmole of ATP, 0.4 μmole of sulfanilamide, 25 μmole of sodium acetate, 20 μmole of sodium citrate, 10 μmole of cysteine, 150 μmole of tris buffer at *p*H 8.3, 0.25 ml of aged enzyme solution, and 1.2 units of coA. Total volume 1.0 ml; incubated for 2 hours at 37°C. *A*, thiopental; *B*, 1,3-dimethylbutyl ethyl barbituric acid; *C*, phenobarbital; *D*, pentobarbital; *E*, amobarbital; *F*, MC1415; *G*, MC2973.

with the views of Govier and Gibbon (*3*), support the idea that barbiturates may produce their effects by interfering with the activation of acetate and consequently the acetylation of choline. The active site in the barbiturate molecule may reside in the "urea" portion, as evidenced by the lack of any effect of the two nitrogen-free substances tested.

The contradiction of the results of Mendelson and Grenell (*4*) remains unexplained. Their procedure was very similar to that used here, the major difference being that the present results were obtained by using a somewhat more than half-activated system.

In summary, a series of barbituric acid derivatives has been shown to produce inhibition of acetylation in a relatively "pure" acetylating system. A convulsant barbiturate also produces marked inhibition, but compounds that might be considered as "urea-free" depressants and convulsants (*10*), respectively, do not produce significant inhibition. Inhibition produced by phenobarbital could be relieved by addition of extra coA, but was not found to be relieved by addition of ATP.

BERNARD H. MARKS*
Department of Physiological Chemistry and Pharmacology, Ohio State University, Columbus

References and Notes

1. H. McLennan and K. A. C. Elliott, *J. Pharmacol. Exptl. Therap.* 103, 35 (1951).
2. W. J. Johnson and J. H. Quastel, *Nature* 171, 602 (1953).
3. W. M. Govier and A. S. Gibbons, *Science* 119, 185 (1954).
4. J. Mendelson and R. G. Grenell, *ibid.* 120, 802 (1954).
5. N. O. Kaplan and F. Lipmann, *J. Biol. Chem.* 174, 37 (1948).
6. A. C. Bratton and E. K. Marshall, *ibid.* 128, 537 (1939).
7. Kindly supplied through the courtesy of K. K. Chen of the Lilly Research Laboratories.
8. Kindly supplied through the courtesy of B. Stearns of the Squibb Institute for Medical Research.
9. T. C. Chou and F. Lipmann, *J. Biol. Chem.* 196, 89 (1952).
10. I. H. Slater, D. E. Leary, P. E. Dresel, *J. Pharmacol. Exptl. Therap.* 111, 182 (1954).

* With the technical assistance of Robert McCoy.

6 September 1955

Color Autoradiography

Although the effect of radioisotopes on ordinary photographic emulsion is well known, there are no reports on results obtained with color film. It occurred to us that multilayer color film is, in effect, a stack of absorbers that should yield, after photographic development, a hue dependent on the ratio of absorption in the layers. This ratio should be a function of the energy spectrum of emitted radiation, and hence each radioisotope should produce a specific hue. Furthermore, the amount of radiation from a given isotope absorbed by the film should affect only the brightness but not the hue of the final product. The present study bears out these considerations and indicates that color-film autoradiography is practicable (*1*).

Table 1. Effect of radioisotopes on color film.

Isotope	$E_{max.}$* (Mev)	Trials (No.)	Munsell color No. (mean ± S.D.†)	Actual color range ± 1 S.D.†
Carbon-14	0.16	12	68.5 ± 4.7	Bluish, blue-purple to blue
Sulfur-35	0.17	16	64.4 ± 3.1	Purplish, blue to greenish blue
Calcium-45	0.25	8	51.9 ± 5.5	Bluish, blue-green to bluish green
Iodine-131	0.60‡	23	46.3 ± 10.6	Bluish, blue-green to greenish green-yellow
Phosphorus-32	1.7	8	35.9 ± 1.2	Greenish, green-yellow to green-yellow

* Values for $E_{max.}$ were obtained from *Isotopes* (Oak Ridge National Laboratory, Oak Ridge, Tenn., 1954). † Standard deviation. ‡ Most abundant β.

One-inch diameter filter paper disks (Whatman No. 1) were dipped into solutions of P^{32}, I^{131}, Ca^{45}, S^{35}, and C^{14} ($25–25 \times 10^{-5}$ μc/ml) and air-dried. Approximately 0.08 ml was absorbed per paper in this manner. The disks were pressed against the emulsion side of Ektachrome film (Eastman Kodak Company, daylight type), and the film was exposed from 1 to 40 days. Photographic development followed the manufacturer's recommendations (*2*). Classification of the resultant hues was made by visual comparison with Munsell color standards (*3*).

Table 1 lists the characteristic hue produced by each isotope. The range of color encompassed the blues for the least energetic particles to the green-yellows for the most energetic. The usable range appeared to be 10^7 to 10^{10} total disintegrations per square centimeter. Within this range, the characteristic hue for each isotope remained constant, regardless of the density of the image. It is interesting that thin absorbers (cellulose tape, 9 mg/cm²), when they were placed between the source and the film, produced an increased density with no appreciable change in hue. In the case of I^{131}, which emits both β and γ rays, the hue indicates that the former radiation was primarily responsible for exposing the film.

From these experiments, it is evident that color autoradiography will permit differentiation of isotopes, provided that sufficient difference exists between energy spectra. For example, a mixture of inorganic I^{131} and C^{14}-tyrosine was separated chromatographically on paper and identified by this technique.

Other possible applications for color autoradiography may be (i) demonstration of isotope emitter characteristics, (ii) determination of proportions of two or more isotopes in a mixture, and (iii) improvement of image detail in autoradiography of tissues. Of course, the value of such results must depend on the type of film that is available. The film used in these studies permitted only a limited approach to these applications. Furthermore, the complex method of photographic development is a decided deterrent to the use of color film as compared with standard film. Perhaps the introduction of special types of multilayered emulsions might make color autoradiography a valuable and convenient tool for the laboratory.

G. W. BUCKALOO
DAVID V. COHN
Radioisotope Unit, Veterans Administration Hospital, Kansas City, Missouri, and *Department of Pharmacology, University of Kansas Medical Center, Kansas City*

References and Notes

1. An excellent discussion of the color film used in this study appears in *Kodak Color Handbook* (Eastman Kodak Co., Rochester, N.Y., 1953).
2. We are indebted to Kenneth S. Carnes and Earl E. Powers of the Medical Illustration Laboratory of this hospital for processing the film.
3. In the Munsell system, the complete visual color wheel is divided into 100 parts consecutively numbered 1 to 100 with each hue assigned a specific number within this range. See *Munsell Book of Colors* (Munsell Color Co., Baltimore, Md., 1942).

22 August 1955

I feel more vexed at impropriety in a scientific laboratory than in a church. The study of nature is intercourse with the Highest Mind.—LOUIS AGASSIZ.