the inferior colliculus which, recorded with a single active micro-electrode, show a slow positive deflection followed by a longer negative one. Spikes, indicating the activity of single cells near the tip of the micro-electrode appear as the potential goes from positive to negative. The overall length of the potential is about 20 milliseconds.

The inferior colliculus will also respond to stimulation of contralateral cutaneous nerves. Those tested were the great auricular, median, ulnar, saphenous, tibial. The nerve to the semimembranosus failed to evoke a response in this region. Apparently the whole nucleus of the inferior colliculus was involved in these responses, but the records were easier to secure and showed more extensive firing of cells in the lateral portion of the nucleus. Instead of showing the characteristic positive-negative succession of deflections, the potentials evoked by stimulation of spinal nerves usually were characterized by a spikiness of the line, all discrete spikes being (as in the auditory potentials) negative. (Fig. 1, b). The earliest activity recorded following stimulation of the forelimb nerves in the cat was observed at 4 milliseconds, and following hindlimb stimulation 8 milliseconds.

When the spinal nerve stimulation was used to condition the auditory potential by placing it about 12 milliseconds ahead of the latter, 2 effects were noted. The first was a decrease in the duration of the spiking component of the auditory potential. This is due to the shortening of the train of spikes in the individual units (Fig. 1, c). The second effect of the conditioning is the decrease in the number of units involved in the auditory potential. Detailed analyses of the potentials of the inferior colliculus will be published elsewhere.

Summary. Stimulation of cutaneous nerves and of the cochlea evokes characteristic potentials in the inferior colliculus. With micro-electrode recording, the activity of individual cells may be observed. Placing the cutaneous stimulation before the auditory stimulation demonstrates a conditioning of the auditory potential which decreases the number of cells fired and shortens the train of impulses from those cells left active.

14548

Ultraviolet Absorption Spectrum of Human Seminal Plasma.*

VICTOR ROSS AND LUCILLE ROSS.

From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University.

Although ultraviolet absorption spectra of several body fluids have been published, we have found none of seminal plasma. Accordingly, during the course of electrophoretic and chemical examination of human seminal plasma, the following measurements of such absorption spectra were made and some factors affecting these spectra were studied.

Methods and Observations. The spectra were measured with a Hilger quartz spectroscope No. E-316 equipped with a Spekker photometer and with a hydrogen discharge tube designed by Darby.[1] Details of curve structure were not sought, principal interest being in the positions of maxima and minima and in shifts resulting from changes in pH values of the solutions.

Semen was centrifuged and the plasma dialyzed at 3°-5° in viscose casing against 0.02 M phosphate buffer containing 0.055 M NaCl (pH 7.85) and diluted for spectroscopic

* This work was made possible by a grant from the Committee on Maternal Health, Inc., whose aid is gratefully acknowledged.

[1] Darby, Hugh H., *J. Am. Chem. Soc.*, 1940, **62**, 1874.

[2] Ross, V., Moore, D. H., and Miller, E. G., Jr., *J. Biol. Chem.*, 1942, **144**, 667.

examination. Electrophoretic patterns,[2,3] following such treatment, show a slow moving component P1 (μ = —0.6)† representing proteose, a considerable portion of which is lost in dialysis. There are 3 globulins,‡ P2 (μ = —2.9), P2a (μ = *ca.* —3.8), and P3 (μ = —4.6). In addition, 2 faster moving peaks are seen, P4 (μ = —5.7) and P5 (μ = —6.3), though not frequently together in the same pattern. These represent the glycoproteins. Serological tests indicate that a little serum albumin is present also, but that P2, P2a and P3 are not identical with serum globulins.

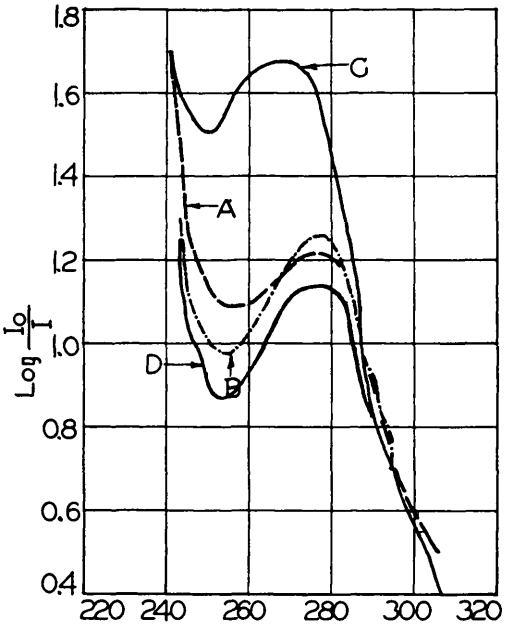

FIG. 1.

Curves A and B, normal seminal plasma. Curve C, normal seminal plasma not dialyzed. Curve D, mixture of globulins P2 and P3. Nitrogen content of solutions: A = 0.0109%; B = 0.0208%; C = 0.0102%; D = 0.0155%. All were done at pH 7.85 in a 1 cm cell. Electrophoretic analyses of plasmas corresponding to Curves A and B appear as Patterns 1 and 3 in Reference 3.

Curves A and B in Fig. 1 represent the spectra for 2 normal plasmas dialyzed as described. The maxima are at 276 and 277 mμ, the minima at 256 and 254 mμ respectively. Curve C in Fig. 1 shows absorption by an *undialyzed*, normal specimen which was simply diluted with phosphate buffer for examination. The maximum is at 268 mμ and the minimum at 250 mμ. The shift toward the more characteristic positions for proteins, following dialysis, may be due to the removal of dialyzable constituents.§

An absorption curve for a mixture of the globulins P2 and P3, in phosphate, is shown in Curve D, Fig. 1. The maximum and minimum are at 278 mμ and 253 mμ respectively. The globulins, therefore, determine the general shape of the curve for the whole plasma just as the proteins of blood serum determine the shape of the absorption curve of that fluid.[4]

Resembling the absorption by normal plasmas was that (not illustrated) by dialyzed seminal plasma from 3 "abnormal" semens: (a) in which the sperm were non-motile and included many morphologically abnormal forms; (b) in which repeated specimens from the same individual had cells of poor motility; and (c) in which both numbers and motility were poor. The absorption densities, log (I_0/I) of normal and abnormal specimens, were not proportional to their nitrogen content, perhaps owing to the presence of different amounts of glycoprotein.

The influence of marked changes in hydrogen ion concentration on absorption by a normal, dialyzed seminal plasma is shown in curves A (pH 7.85) and B (pH 12), Fig. 2. At pH 12 the peak has shifted to 283 mμ and the valley to 272 mμ, while the ratio of

[3] Ross, V., Miller, E. G., Jr., Moore, D. H., and Sikorski, H., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 179.

† The symbols P1, P2, P2a, P3, P4, and P5 have been used in an earlier publication[3] for the protein components of human seminal plasma in the order of increasing mobility. Electrophoretic mobilities, as given in the text, are in cm^2 $volt^{-1}$ $sec.^{-1}$ $\times$ 10^{-5} and are averages of a number of determinations.

‡ We refer to components P2, P2a, and P3 as globulins because there are water-soluble and water-insoluble fractions of each in *fresh* seminal plasma.

§ Some of the additional substances present in seminal plasma, and presumably removed by dialysis, are urea, glucose, lactic acid, ascorbic acid, spermine, bicarbonate, phosphate, chloride, and citrate.

[4] Lewis, S. Judd, *Proc. Roy. Soc. Lond.*, 1915-17, **89**, 327; Stenstrom, W., and Reinhard, M., *J. Biol. Chem.*, 1925, **66**, 819.

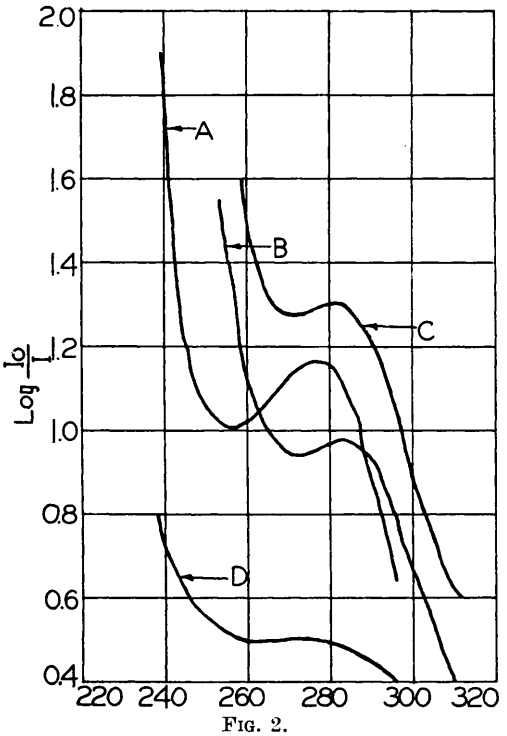

FIG. 2.

Normal seminal plasma at (A) pH 7.85 and (B) pH 12. Electrophoretic analysis of this plasma appears as Pattern 2 in Reference 3. Mixture of globulins P2, P2a and P3 at (C) pH 12 and (D) pH 2. Nitrogen content in (A) and (B) = 0.0101%; in (C) = 0.0028%; in (D) = 0.0021%. 1 cm cell used in (A) and (B), 4 cm in (C) and 2 cm in (D).

the density at the peak to that at the valley has decreased. At pH 2 the peak and valley disappeared, the curve became almost horizontal between 250 mμ and 270 mμ and the densities increased. Because the solution was somewhat cloudy and consequent scattering of light introduced errors, the curve is not reproduced. The effect of pH on the absorption by a mixture of the globulins P2, P2a, and P3 appears in curves C and D, Fig. 2. Here the shift, at pH 12 (C), was to 283 mμ for the maximum and to 272 mμ for the minimum, values identical with those for the plasma after adjustment to this pH. A similar, but more marked shift occurs with solutions of tyrosine,[5] and to this is ascribed the behavior, under these conditions, of proteins containing this amino acid. At pH 2 (D) the peak and valley disappeared but no significant change in densities occurred. At pH 7.85 the curve was almost the same as A.

Curves A and B, Fig. 3, show absorption by two preparations of P5, while curve C represents absorption by P4; there is little or no selective absorption by these glycoproteins. All 3 preparations were electrophoretically homogeneous at pH 7.85. The similarity in the curves for P5 and P4 is paralleled by the similarity in their nitrogen content and in their content of reducing substance after hydrolysis. At pH 12 (Curve D), the intensity of absorption by P4 is somewhat depressed and there is, perhaps, a slight shift toward the longer wave lengths of the small inflection in the region of 285 mμ. At pH 2 the solution became cloudy and the curve, which showed no significant change in shape but a 5-fold increase in density values, is therefore not reproduced. There is no spectrographic evidence for the presence of nucleoprotein in either of the two acetic acid precipitable components.

Summary. At pH 7.85 the peak of ultraviolet absorption by human seminal plasma and of a mixture of globulins derived from it lies in the region of 276 to 279 mμ and the valley lies between 254 and 256 mμ. No significant differences were observed between the curves for plasmas from normal semen and the curves for plasmas from 3 semens which contained sperm considered to be defective in motility or morphology. Absorption by 2 acetic acid precipitable components (glycoprotein) shows little selectivity; there is only a slight inflection in the curve in the region of 285 mμ. The absorption increases sharply in the lower wave lengths. The effect of increasing the pH of the solution to 12 is to shift absorption, by both seminal plasma and by the globulins, toward somewhat longer wave lengths. At

[5] Holiday, E. R., *Biochem. J.*, 1936, **30**, 1795; Dhéré, C., *Recherches Spectrographiques sur l'absorption des Rayons ultraviolets par les albuminoïdes, les protéides et leures dérivés*, Fribourg, 1909; Kober, P. A., *J. Biol. Chem.*, 1915, **22**, 433; Gróh, J., and Hanák, M., *Z. Physiol. Chem.*, 1930, **190**, 169; DeGouveia, A-J. A., Coelho, F. P., and Schön, K., *Fevista da Faculdade de Ciencias da Universidada de Coimbra*, 1938, **6**, No. 4.

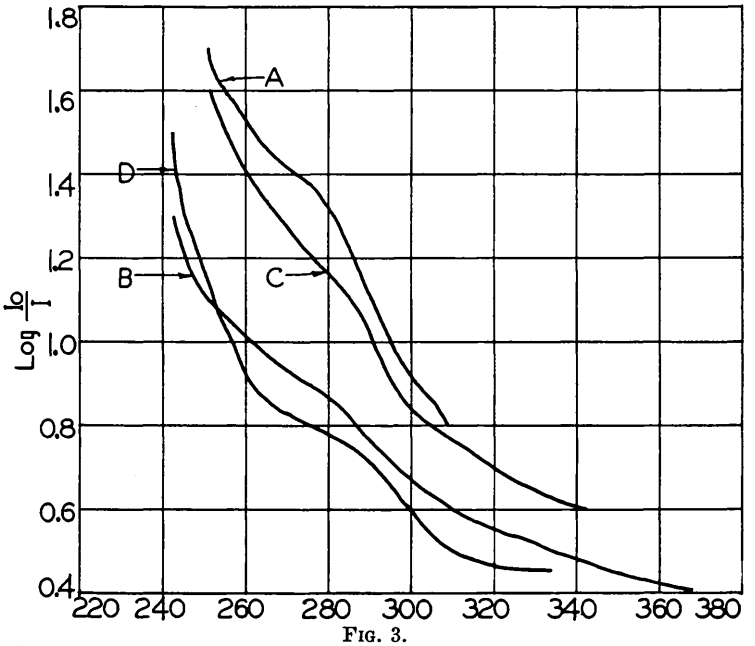

FIG. 3.

Curves A and B, two preparations of P5 (glycoprotein) examined at pH 7.85. Nitrogen content in A = 0.0022%; cell length 1 cm. Curves C and D, preparation of P4 (glycoprotein) examined at pH 7.85 and 12 respectively. Nitrogen content = 0.00136% in both; cell length 2 cm.

pH 12 there is a shift toward the longer wave lengths of the slight inflection at 285 mμ in the curve for glycoprotein.

We wish to record our appreciation to Dr. Hugh H. Darby for a discussion of the contents of this report.

14549 P

Studies on Synergism of Leukemogenic Agents in Mice.*

HENRY S. KAPLAN† AND ARTHUR KIRSCHBAUM.

From the Departments of Radiology and Anatomy, University of Minnesota Medical School, Minneapolis, Minn.

Various agents have been shown to act synergistically with carcinogens when applied simultaneously to susceptible mice.[1,2] Furth and Boon[3] recently reported a synergistic leukemogenic effect of combined application of methylcholanthrene and irradiation in Rf/Ak hybrids. The incidence of leukemia was 6% in irradiated mice receiving a single dose of 175 r, 16% in others treated only with the carcinogen, and 64% in irradiated and carcinogen-treated mice. The onset of leukemia was also accelerated in animals receiving combined treatment. Preliminary work revealed a 59% incidence of leukemia in animals

* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and the Cancer Fund of the Graduate School of the University of Minnesota.

† National Cancer Institute Trainee in Radiology.

1 Berenblum, I., *Cancer Research*, 1941, **1**, 807.

2 Shear, M. J., *Am. J. Cancer*, 1938, **33**, 499.

3 Furth, J., and Boon, M. C., *Science*, 1943, **98**, 138.