mens. However, this material can be removed by eluting it from paper and passing it through a Dowex 50 – × 8 200-400 mesh column. Compound A is not affected by this procedure.

A visual comparison with known standards of the size of the spots and the intensity of the Ninhydrin color developed by glycine and glutamic acid derived from the BSP conjugate suggests that these amino acids are present in equimolar concentration and that for each mole of BSP there is a mole of glycine and a mole of glutamic acid. Chemical analyses to confirm this impression are now in progress.

Tests on band A for free or combined sulfhydryl groups were negative. It should be mentioned that no evidence of a BSP-glucuronic acid conjugate was found. Thus, the quantity of hexuronic acid (*8*) in compound A did not differ significantly from that contained in control bile migrating with the same R_f as A, and after incubation of A with β-glucuronidase (Worthington) at 37°C, *p*H 4.9, for 1 hour, no free BSP was detected by paper chromatography. Under similar conditions, the activity of the enzyme was demonstrated by its capacity to liberate phenolphthalein from phenolphthalein glucuronide.

The results of this study indicate that BSP is excreted in the bile of the rat as at least three and perhaps four compounds. This finding introduces a new complexity in the interpretation of the values for BSP T_m obtained in previous investigations (*4, 9*). Any analysis of biliary secretory T_m of BSP must henceforth take into account the probability that more than one transport process is operative in the movement of BSP from blood to bile.

The primary purpose of this communication is to describe the nature of the major excretory product of BSP in bile. This compound and its hydrolytic products were subjected to both chromatographic and chemical analysis. The results of these studies indicate that BSP is excreted by the liver of the rat primarily as a conjugate of glycine and glutamic acid, over 75 percent of the excreted BSP being found in this compound.

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5. This investigation was supported by research grants from the Dallas Heart Association and the U.S. Public Health Service (H-3439). I am indebted to Dr. Marvin D. Siperstein for many helpful suggestions and discussions and to Mrs. Mary Hamilton for valuable technical assistance.
6. Standard BSP, when subjected to acid hydrolysis and then chromatographed in the acetic acid:water:*n*-propyl alcohol solvent system, yields two BSP bands, one migrating with the same R_f as BSP, the other moving faster. These two bands are also identified after A is hydrolyzed.
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An Artificial Reagent for the Diagnosis of Classical Hemophilia

Present methods for the laboratory diagnosis of classical hemophilia [hemophilia A, or antihemophilic globulin (AHG) deficiency] are modifications of the thromboplastin generation test (*1*) or are dependent upon the use of plasma from a patient with classical hemophilia as reagent (*2*). The first of these methods, although of confirmed value, is time-consuming; a disadvantage of the second method is that adequate amounts of hemophilic blood are not always easily available. For these reasons an alternative rapid method in which readily available materials are used would be of value.

The principle of an alternative method was suggested by the observations (*3*) of the effects of disodium ethylenediaminetetracetic acid (Na_2-EDTA) on certain clotting factors; rapid and pronounced losses of proaccelerin (factor V) and AHG have been the most marked findings (*4*).

Normal human blood was collected into Na_2-EDTA (*5*) (150 mg/100 ml of blood). After centrifugation the plasma was decanted and stored at +4°C for 30 days. Plasma so treated retained normal activities of plasma thromboplastin component (PTC), plasma thromboplastin antecedent (PTA), Hageman factor, prothrombin, proconvertin [factor VII, or serum prothrombin conversion accelerator (SPCA)], Stuart factor, and fibrinogen; however, AHG and proaccelerin fell to undetectable levels (< 0.1 percent). Such plasma was stored at −20°C in small aliquots until needed. Optimal quantities of Asolectin (*6*) and barium sulfate adsorbed oxalated bovine serum were added to such plasma immediately before the test, as sources of platelet prothromboplastic-factor–like activity and of accelerin, respectively. The buffer for all dilutions was Veronal acetate, *p*H 7.3 (*7*).

In a series of 12- by 75-mm tubes were placed 0.1 ml of this aged Na_2-EDTA plasma; 0.1 ml of Asolectin (0.005 percent); 0.05 ml of barium sulfate adsorbed bovine serum, diluted 1 to 80; and 0.05 ml of either normal or unknown plasmas in serial dilutions. The coagulation times for these mixtures after recalcification with 0.1 ml of 1/70*M* $CaCl_2$, were recorded, and the values for tubes containing the unknown plasma were compared with those for tubes containing serial dilutions of normal plasma (see Table 1). The average recalcification time for such a system, when buffer is substituted for the unknown plasma, is 14 minutes; the time is shortened to 5 minutes or less (normal, 4¼ minutes) when any one of the following is added instead: platelet-poor normal plasma; plasma from a patient receiving Dicumarol; Seitz-filtered normal plasma; barium sulfate adsorbed normal plasma; and plasmas from patients congenitally deficient in PTA (one patient), Hageman factor (one patient), PTC (three patients), proconvertin (one patient), and proaccelerin (one patient). However, in none of six patients diagnosed by the method of Soulier and Larrieu (*2*)

Table 1. Coagulation time with artificial reagent to which the various plasmas listed were added prior to recalcification.

Plasma	Av. time (min)
Normal, platelet-poor	4¼
Normal, Dicumarol	3
Normal, Seitz-filtered	3¾
Normal, $BaSO_4$-adsorbed	2½
From PTA-deficient patient	4¾
From individual with Hageman trait	3¾
From PTC-deficient patient No. 1	4¼
From PTC-deficient patient No. 2	4¼
From PTC-deficient patient No. 3	4½
From proconvertin-deficient patient	2½
From proaccelerin-deficient patient	5
From AHG-deficient patient No. 1	6¼
From AHG-deficient patient No. 2	7
From AHG-deficient patient No. 3	8
From AHG-deficient patient No. 4	8
From AHG-deficient patient No. 5	10¼
From AHG-deficient patient No. 6	15
Veronal-acetate buffer (*p*H 7.3)	14

as cases of classical hemophilia was the prolonged recalcification time corrected to normal in the above system.

It would thus appear that the diagnosis of classical hemophilia can be made with this artificial reagent; diagnosis by this means would have the advantage of simplicity and availability of material (*8*).

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4. I wish to thank Dr. J. P. Soulier and members of his staff for making available the facilities of his laboratory and the plasmas of the various patients studied.
5. The commercial product used was Komplexon III, obtained from A. G. Siegfried, Zofingen, Switzerland.
6. Asolectin is a preparation of mixed soybean phosphatides, obtained from Associated Concentrates, Woodside, Long Island, N.Y.
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Fusion Contour for Intermittent Photic Stimuli of Alternating Duration

Abstract. It is generally thought that fusion of intermittent photic stimuli occurs when the duration between successive pulses of light is reduced to a certain value, this value being a function of the illumination and viewing conditions. The findings described in this report show that fusion is determined not only by the duration between successive stimuli but also by the temporal pattern of successive stimuli.

Most studies of visual flicker are made with intermittent photic stimuli of equal duration repeated serially (Fig. 1, *a* and *b*). Although such stimuli are usually described in terms of their frequency, the reciprocal of frequency, or period, may also be used for this purpose. As the frequency of an intermittent light is increased (that is, as its period is decreased) the apparent rate of flicker increases until a point is reached at which the light ceases to flicker and appears steady. The frequency at which this fusion occurs is called the critical flicker frequency.

The critical flicker frequency is usually taken to be a measure of the temporal resolving power of the eye. One explanation of fusion (*1*) is that at the critical flicker frequency the excitatory effects of one stimulus persist until the arrival of the next stimulus, providing a constant excitation process. A second explanation of fusion (*2*) is that there is a refractory period after each stimulation. Any stimulus arriving during this refractory period will fail to result in excitation. A third explanation (*3*) involves the threshold of brightness discrimination. Stimulation by a flash of light raises the excitation level to a certain value. When the light is turned off, the level of excitation starts to decline. The onset of the next flash of light raises the excitation level to its previous value. At the critical flicker frequency, successive pulses of light occur so rapidly that the excitation level fluctuates between limits which are not detectably different in brightness. All three explanations assume that for given viewing conditions fusion will occur whenever the duration between successive stimuli is less than a certain value.

Recently we have been studying visual flicker with intermittent photic stimuli of unequal periods (Fig. 1, *c*). Note that the train of pulses shown in *c* does not represent the mixing of two independent frequencies. With such stimuli flicker may be obtained even though the duration of each of the alternating periods is less than the duration of that period which, when repeated serially, is seen as fused. For example, if both *a* and *b* in Fig. 1 are above the critical flicker frequency, when alternated serially, as in *c* of Fig. 1, they may appear to flicker. The purpose of this report is to present data on the fusion contour for intermittent photic stimuli of alternating duration (*4*).

"On" and "off" triggers generated by four variable-frequency oscillators produced electric square waves which were the input to a Sylvania R1131C glow-modulator tube. The rise and decay time of the tube is approximately 20 μsec. The four oscillators were set so that two of them determined the "on" time and the "off" time, respectively, of one period; the remaining two determined the "on" and "off" times of the second period. They were then connected in series to produce intermittent electric square waves composed of two alternating periods (as in *c* of Fig. 1). Either of the two periods could be varied independently of the other. Within each period, "on" time always equaled "off" time. The glow-modulator tube illuminated a spot which subtended ½° of visual angle. The luminance of the test spot was approximately 1800 millilamberts (mlam); the luminance of the surround was approximately 22 mlam. All observations were made monocularly.

Measurements were obtained in the following manner: One period (*A*) was set at a fixed value while the other period (*B*) was varied by the observer until a fusion point was obtained. Measurements were first made with *A* set to a value of 2 msec. In successive measurements, the period of *A* was increased in 2-msec steps until it reached a value for which no further fusion points could be found.

Figure 2 shows the data for two subjects. The coordinates of Fig. 2 have been labeled in terms of both period and frequency. The frequency label has reference only to the frequency which either period would have if it were pre-

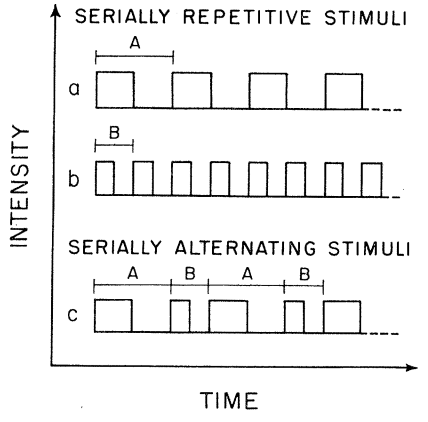

Fig. 1. Schematic representation of intermittent photic stimuli used in studies of visual flicker.

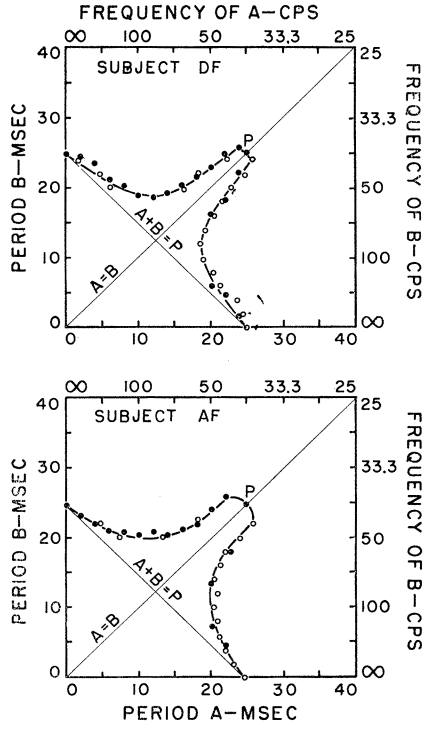

Fig. 2. Fusion as a function of the duration of alternate periods of intermittent photic stimulation. The solid circles are fusion points obtained by varying the period of *B*, with *A* constant. These points are based on ten observations per observer. The open circles are their mirror images.