the case with methionine sulfone or cysteine sulfinic acid or with the other amino acids tested. Bleached areas may be more readily observed by the transmitted light of an X-ray viewer.

R_F values (*2*) for the phenol-ammonia-gas system, together with a description of the rapidity and sensitivity of the test, are given in Table 1. The column describing the amounts detectable denotes quantities which we have readily discerned. Sensitivity thresholds are probably much lower. On a nonchromatographed sheet methionine could be detected at a 0.4-γ level. Because of spreading during chromatographing, sensitivity is decreased.

Of the other amino acids ordinarily encountered, only threonine and serine affect the reagent at all. After 3 min these give only faint tests—not strong enough to be confused with the sulfur-containing amino acids. Except for cysteic acid, the reagent seems to be specific for the sulfur amino acids and is sensitive to the quantities commonly encountered in paper chromatography. It is rapid and convenient to apply and may be considered a useful adjunct to the many other methods rapidly becoming available for the detection and estimation of chromatographed material.

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A Brain-Wave "Correlator"

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What is believed to be a new instrument for analyzing the electrical activity of the brain consists of two square-wave generators, actuated by the outputs of two channels of an electroencephalograph. Each square-wave crosses the axis at the same instant that its original EEG signal does, but is independent of the latter's wave-form and amplitude. Addition of these square-waves produces a voltage which is a three-valued function of time, being positive when both inputs are positive, zero when they are of unlike sign, and negative when both are negative.

The "voltage" of this composite square-wave, as read on a suitably lagged and calibrated a-c voltmeter, indicates the fraction of the total time during which the two inputs are of the same sign. If the inputs are alike, this fraction is unity and the meter reads +1. If one input is reversed, the fraction is zero and the meter reads −1. Unrelated inputs are of the same sign half of the time, and the reading is zero. Intermediate positions of the meter quantify the correlations reproducibly and more precisely than can be done by inspection.

Monopolar leads (the "indifferent" lead being placed on the ear lobe) have been used in observations on man. To date, only positive correlations have appeared between any two electrodes on the scalp; yet low values of correlation obtainable with certain locations of electrodes indicate that the common activity introduced by the ear is not of much consequence. High correlations sometimes occur when electrodes are symmetrically placed. Disturbing noises or hyperventilation reduce the correlation.

In cats and monkeys with exposed cortex, correlation increases with proximity of the electrodes, but it also depends on functional organization. With the two electrodes in one neuronographic area a high correlation is obtained, but this drops—sometimes abruptly—when one electrode crosses the border into another area.

In one cat Metrazol and one other convulsant each lowered the correlation, whereas CO_2 raised it.

These correlators are now in use in clinical electroencephalography and in experimental neurophysiology, but it is too soon to estimate their full utility.

Spontaneous Increase in Potency of Thromboplastin From Acetone-extracted Brain Tissue[1]

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In the course of studies in our laboratory on the one-stage prothrombin test (Quick), a spontaneous increase was observed in the potency of thromboplastin prepared from an acetone-extracted (*2*) human brain specimen. Our methods for the preparation of reagents and for performing the prothrombin test are given in detail elsewhere (*1*). One feature of this procedure of significance in the present observations is the storage of large quantities of acetone-extracted brain in an evacuated calcium chloride desiccator in a refrigerator. We have found no significant change in the potency of thromboplastin prepared from brain tissue stored in this manner for periods up to two years, *provided the material is gradually used up during the period of storage.* However, we have found that unused *remnants* of brain tissue, stored in this manner, may exhibit striking increases in potency and other unusual characteristics.

The thromboplastin reagents used in these experiments were prepared by incubating 0.3 gm of acetone-extracted brain tissue in 5 cc of 0.9% NaCl solution for 15 min at 48°–50° C. The milky supernatant fluid was used for testing. Solutions of either 0.0075M or 0.025M $CaCl_2$ were used for recalcification. All dilutions of plasma and thromboplastin reagent were made with 0.9% NaCl.

A specimen consisting of several hundred grams of acetone-extracted human brain (H 46) showed no change in thromboplastic potency during a storage period of 210 days. A remnant consisting of 2.5 gm of this material, which was stored in a 50-cc Pyrex beaker, was

[1] Aided by a grant from the Christine Breon Fund.

not used again until the 257th day. However, the desiccator which contained it was opened daily. Additional prothrombin tests on normal plasma, using thromboplastin reagent prepared from this remnant, were made between the 257th and 701st days of storage.

TABLE 1*

Subject	Duration of storage of original specimen (days)	Duration of storage of remnant (days)	"Prothrombin time" (sec)		
			Concentration of plasma		
			100%	50%	12.5%
T. L.	210	0	10.1	12.3	32.6
J. L.	210	47	8.2	11.0	25.1
T. L.	210	65	8.0	10.2	22.2
N. F.	210	69	6.4	6.2	18.6
T. L.	210	491	4.6	2.7	17.7

* Progressive increase in potency of thromboplastin reagent prepared from remnant of acetone-extracted human brain specimen (H 46) stored *in vacuo*.

These tests showed a striking decrease in the "prothrombin time" of all plasma concentrations tested (Table 1). Furthermore, the "prothrombin time" of plasma diluted to 50% of its original concentration was shorter than that of the undiluted plasma. A solution of 0.0075M $CaCl_2$ was used for these tests.

TABLE 2*

Plasma concentration (%)	"Prothrombin time" in seconds	
	With 0.0075 M $CaCl_2$	With 0.025 M $CaCl_2$
100	4.6	6.4
80	3.7	5.5
60	2.6	4.7
50	2.7	...
40	3.1	5.6
20	12.2	16.8
12.5	17.7	...
10	25.8	35.3

* "Prothrombin time" of progressive dilutions of normal plasma tested with thromboplastin reagent prepared from remnant of acetone-extracted human brain (H 46) stored *in vacuo*.

Between the 694th and 701st days of storage, progressive dilutions of normal plasma were tested with thromboplastin reagent prepared from this remnant, using both 0.0075M and 0.025M $CaCl_2$ solutions (Table 2). In Fig. 1, the results obtained are contrasted with those observed using a thromboplastin reagent prepared from a human brain specimen (H 47) which had been stored in the usual manner and had shown no spontaneous increase in thromboplastic potency. The reagent prepared from the remnant showed a progressive shortening of the "prothrombin time" which reached its nadir in plasma concentrations between 50 and 60%. With further dilution of the plasma, the "prothrombin time" became progressively longer. However, the "prothrombin time" of the 40% plasma concentration continued to be shorter

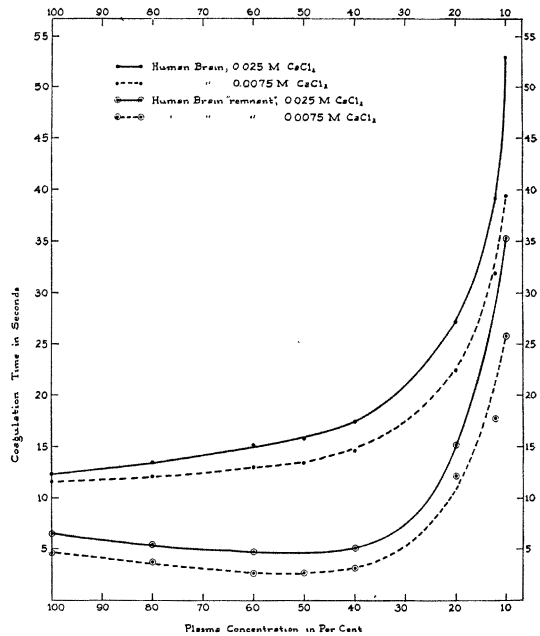

FIG. 1.—"Prothrombin time" of progressive dilutions of normal human plasma obtained with thromboplastin reagents prepared from standard acetone-extracted human brain specimen and its remnant.

than that of the undiluted plasma, and furthermore, at this level there was found the greatest divergence from that prothrombin time obtained with thromboplastin reagent prepared in the usual manner.

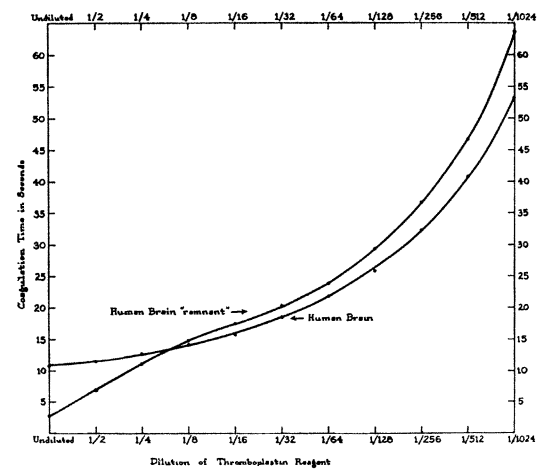

FIG. 2.—"Prothrombin time" of undiluted normal human plasma obtained with progressive half-dilutions of thromboplastin reagents prepared from standard acetone-extracted human brain specimen and its remnant.

On the 701st day of storage, progressive half-dilutions of thromboplastin reagent prepared from the last of the remnant were tested with undiluted normal plasma using 0.025M $CaCl_2$ solution. The results are compared (Fig. 2) with a similar series of observations using a thromboplastin reagent prepared from a human brain in which no spontaneous increase in potency had occurred. From

these data it is apparent that the increased thromboplastic potency disappears in a 1:8 dilution of the reagent, and that on further dilution it becomes even less potent than that of the standard reagent. These results could be explained either by the removal of an anticoagulant from the original brain specimen or by the formation of a coagulation accelerator other than thromboplastin in its remnant.

In order to confirm these observations a second specimen of acetone-extracted human brain (H 47), consisting of 400 gm of dried tissue, and a specimen of rabbit brain (R 47), consisting of 200 gm of dried tissue, were prepared. Aliquots, each consisting of 175 mg of tissue, were stored in Pyrex beakers in the same evacuated desiccator which contained the parent specimens. During the ensuing 120 days thromboplastin reagents prepared from the parent specimens showed no change in potency, whereas those prepared from the aliquots exhibited an increase in potency similar to that observed with the

TABLE 3*

	"Prothrombin time" (sec)					
	Human brain (H 47)			Rabbit brain (R 47)		
	Concentration of plasma			Concentration of plasma		
	100%	50%	12.5%	100%	50%	12.5%
Original specimen stored for 90 days	11.7	15.0	35.7	12.3	16.6	40.9
Aliquot stored additional 120 days	7.7	6.7	22.9	5.7	4.5	17.0
Original specimen stored additional 120 days	11.8	15.4	36.8	12.5	16.7	40.8

* Spontaneous increase in potency of thromboplastin reagent prepared from small aliquots of acetone-extracted human and rabbit brains stored *in vacuo*.

remnant of tissue with which our original observations were made (Table 3). (Solutions of 0.025M $CaCl_2$ were used in these experiments.) However, other aliquots of of human brain specimen (H 47) have so far shown no significant change in potency after 300 days of storage.

That these changes were not due to further desiccation of the brain tissue was shown in the following manner: A portion of rabbit brain specimen (R 47) was found to have a moisture content of 2.4%. This was reduced to 0.01% by intense dehydration *in vacuo*. The dehydrated specimen showed no change in thromboplastic potency.[2]

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[2] We wish to thank Walter E. Ward, of the Cutter Laboratories, Berkeley, California, for desiccating and determining the moisture content of this sample.

Standardized Pain Stimulation as Controlled Stress in Physiological Studies of Psychoneurosis[1]

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It is generally considered that the psychoneurotic is characterized by instability of various physiological systems and that this lability is particularly evident during stress. However, there is need for more specific information about the nature and extent of physiological changes which take place in the psychoneurotic patient under stress. It was considered that the experimental attack upon this problem would be greatly facilitated if a standardized situation of stress were available. This would permit comparisons to be made among individuals and among groups of patients, as well as between psychoneurotics and normals. The main requirements of a useful standard stress situation may be set forth as follows: (1) External stimulation should be uniform and controlled. (2) The stimulation should be relatively mild. Overstimulation may occur to the point where critical individual differences in reaction are obscured. Also, for practical reasons, a procedure to be used with psychiatric patients as subjects must not be frankly traumatic. (3) The stimulation, although relatively mild, should produce definite objective changes known to be associated with stressful experience. (4) Definite differences in test reaction should be detectable when individuals whose reactions differ clinically are exposed to the situation.

As a technique which appeared likely to satisfy these requirements, we selected a pain stimulation series presented by a Hardy-Wolff thermal stimulator (*4*) and carried out the present study to determine whether a series of pain stimuli of fixed order and intensity could be used as a standard stress situation. Since this procedure obviously satisfies the first two criteria, the investigation centered upon the remaining two, for which the technique seemed promising. Pain is generally associated with stress; and clinical observations suggest that the psychoneurotic overreacts to pain. The experimental work of Chapman (*1*), who used the Hardy-Wolff apparatus, has demonstrated differences between psychoneurotic patients and controls. Chapman has shown that the threshold for pain perception was almost exactly the same, but that patients reacted grossly to a degree of pain that was tolerated without reaction (head-withdrawal or wincing) by the controls. The present experiments were designed not for threshold-taking but for the presentation of a fixed series of standard intensities

[1] This research was assisted by a grant-in-aid from the National Research Council of Canada.

[2] The authors wish to acknowledge gratefully the advice and guidance of H. H. Jasper, of the Montreal Neurological Institute.