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]

References

1. AGGELER, P. M., HOWARD, J., LUCIA, S. P., CLARK, W., and ASTAFF, A. *Blood*, 1946, **1**, 220.
2. QUICK, A. J. *Science*, 1940, **92**, 113.

[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]

R. B. MALMO, C. SHAGASS, J. F. DAVIS, R. A. CLEGHORN, B. F. GRAHAM, and A. JOAN GOODMAN[2]

Allan Memorial Institute of Psychiatry, McGill University, Montreal

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.

to every subject, with measurement of physiological responses to stimulation as indices of reaction.

An objective indication of stressfulness is provided by the lymphocyte count, which has been shown to drop following certain forms of stress (*3, 5, 7, 9*). This involves activation of the anterior pituitary by the central nervous system, resulting in liberation of adrenocorticotropic hormone. The consequent stimulation of the adrenal cortex leads to secretion of 11–17-oxysteroids, which promotes dissolution of the lymphocytes. Stress employed by former workers, viz., exposure to heat and pursuitmeter tests (*6, 10*), would seem to have been more strenuous and prolonged than the procedure employed in the present study. The lymphocyte study was made on a group of 10 patients, 8 psychoneurotics with anxiety, and 2 depressives.

As representative physiological measures serving as criteria of differentiation, records of finger movement and skin resistance, and electroencephalograms, were taken. These measurements were obtained during the pain test from a second group of 10 patients, selected so that most of the following characteristics were present: (1) conscious fears and anxiety—continuous, rather than intermittent, with depressed mood; (2) "poor control"—anxious manner, restlessness, some impairment to speech (stuttering, subdued tone of voice, unsteadiness of voice, tightening of throat); (3) increased "tension"—fatigue, increased tendon reflexes, and irritability; (4) tremor; (5) flushing; (6) palmer sweating; (7) anorexia; (8) insomnia. Absence of the following characteristics was required for selection: (1) anxiety which was specific to certain limited situations only; (2) obsessive-compulsive symptoms; (3) hysterical symptoms; (4) psychosis. These selective criteria served to bring together a group of patients whose anxiety level was high. Ten controls, who matched the patients in age and sex, were employed for purposes of comparison. These were taken from the medical and nursing staffs of the hospital. Anxiety level for this group, as a whole, would be expected to be considerably lower than that for the patient group.

The total period of examination, which lasted about 1 hr, was divided into three parts. During the first part the subject was reassured, given instructions, and prepared for the test (*i.e.* his forehead was blackened and electrodes were attached). A definite effort was made to reassure every subject about the relatively nontraumatic nature of the test. Details concerning the stimulation series were withheld, but the patient was assured that no electric shock would be administered.

Following this there was a 7-min rest period during which the subject was asked to relax. The test proper was then begun. During stimulation, light from a 500-watt lamp was focused on the forehead of the subject by means of a condensing lens. Stimuli were presented by means of an electronically controlled shutter which, when open, permitted light to pass to the subject's forehead.

Twelve stimuli were spaced at intervals of exactly 1½ min. All stimuli were 3 sec in duration, except the last stimulus, which lasted for only 1 sec. Intensities were varied by means of a Variac transformer which controlled the voltage through the lamp. The intensity series, expressed in watts, was 500, 270, 340, 400, 270, 340, 400, 270, 340, 400, 500, 500. The range of intensities for the 3-sec stimuli was from approximately 0.23 gm cal/sec/cm² to approximately 0.44 gm cal/sec/cm². The most intense stimulus felt definitely painful to everyone.

During the test the subject sat leaning forward slightly with his chin in a rest. His left hand was strapped down (palm up) for skin-resistance recording, and his right forefinger rested on a button which he was instructed to press during stimulation when he thought that the heat on his forehead was about to become painful. The subject's eyes remained closed throughout the test.

During instructions the subject was informed that the forehead would be the place stimulated. He was told to expect a sensation of warmth, mounting into heat which might suddenly swell into a stab of pain. The subject was requested not to talk during the test, except when the examiner asked him a question. Exactly 30 sec following each stimulus the examiner asked the subject two questions: "How did that feel to you?" and "Did you press the button?"

The group of 10 patients whose lymphocyte counts were studied were fasted on the morning of the test, which was conducted in each case from 1:00 P.M. onward. Blood samples for white-cell count and differential were taken at 1:00, 3:00, and 5:00 P.M. Control lymphocyte counts also were made at the same times on the same subjects the day following this stress situation, when no testing was being done.

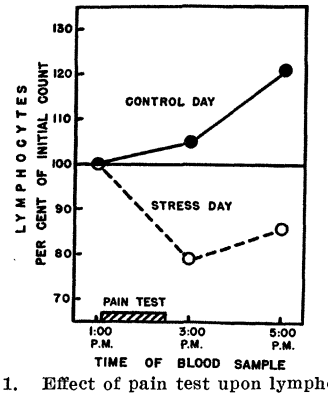

FIG. 1. Effect of pain test upon lymphocyte count.

The mean lymphocyte counts for the group on stress and control days are plotted in Fig. 1. The difference between these mean counts was reliable at the 3% level of confidence, both when the 3:00 P. M. and 5:00 P.M. counts were compared with the 1:00 P.M. count.

For the purpose of recording finger movements, a rugged Rochelle salt crystal was converted into a push-button pick-up, and the output of the crystal was fed into an amplifier of an Offner electroencephalograph. For purposes of analysis, we divided the record into four time segments: (1) during the 3-sec stimulation, (2) 27 sec immediately following stimulation, (3) 20 sec following the question, and (4) 40 sec preceding the next stimulus.

If during any of these intervals finger movement greatly exceeded the base-line level of oscillation, a plus rating was scored for that particular time segment. A plus rating was also made if the subject pressed the button voluntarily. Thus, it was possible to score 5 plusses for each of 12 stimuli and to grade all subjects on a scale from 0 to 60. Ratings were made by a technician from whom identity of the records was withheld in order to eliminate subjective bias.

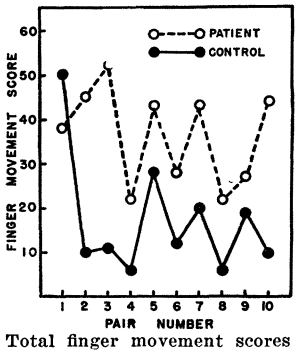

FIG. 2. Total finger movement scores for matched pairs; comparison of each patient with his matched control (maximum score = 60).

The finger movement data from the matched groups are presented in Fig. 2. The only reversal was that of a control with a score of 50. This finding is of particular interest because, actually, it turned out not to be a reversal at all. Each subject was questioned at the conclusion of the test. The relevant part of this control's protocol reads as follows: (Question) "What was the most unpleasant experience which you have ever had?" (Answer) "I have experienced considerable anxiety. I had an acute anxiety state for about 3 years (about 6 years ago). I got over it with psychotherapy. The unpleasant feelings were feelings of panic and tension. I was so tense that noises (such as talking or the sound of a door closing) actually hurt me; they were physically painful." In spite of this reversal, which was treated statistically as though it were a true reversal, the difference between patients and controls is reliable at the 1% level of confidence. Differences between groups were significant for each of the four separate time segments. The difference for the number of voluntary pressures on the button was not significant.

Analysis of skin-resistance records showed a higher frequency of anticipatory responses in the patient group. There was only one reversal (again the case of the control with the history of anxiety state); all of the other pairings showed a higher frequency of anticipatory responses for the patient member (see Fig. 3).

One EEG lead (midline parieto-occipital) was employed. EEGs were analyzed by a modification of the Davis (*2*) technique for the variables per cent alpha, alpha frequency, fast frequency content, and for the presence of abnormalities (any waves of 6/sec or less). No significant differences between matched groups were found with respect to any of these variables.

The results of the investigation show that the standard conditions of stimulation met the two basic requirements of a good experimental stress situation. The measures of finger movement and skin resistance demonstrated in clear-cut fashion the differences between normals and anxiety cases. The drop in lymphocyte count also indicates objectively that the stress of the situation produced far-reaching physiological effects of pituitary-adrenal cortex stimulation. The pain series is therefore a simple, easily controlled stressful situation by which differential physiological response may be studied.

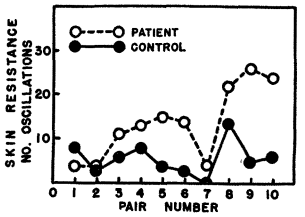

FIG. 3. Number of oscillations in galvanic skin response during anticipatory period; comparison of each patient with his matched control.

The finger movement recordings appear to be a particularly important indicator of motor disturbance associated with anxiety. Our findings in this regard are in accord with those of Luria (*8*), who conducted similar studies, although Luria's testing technique was designed more for analysis of disorganization of voluntary response. Our technique was such as to bring into focus the intrusion of "needless" muscle activity at times when no movement of the finger was required—that is, when the subject's finger was supposed to be at rest.

Luria relied almost exclusively upon measurements of finger movement in his studies of emotion. Our findings support his argument for the importance of motor disturbances in revealing general emotional disturbances. But it would seem desirable to conduct further research with more measures, tapping other physiological systems, such as the cardiovascular system, to obtain a broader picture of disturbances under stress and to provide the means for an objective comparison of reacting systems.

It appears likely that the pain series which was found to be useful in the present investigation may profitably be employed in more extensive analyses of disturbances in mental patients undergoing stress.

References

1. CHAPMAN, W. P. *Psychosom. Med.*, 1944, **6**, 252–257.
2. DAVIS, P. A. *J. Neurophysiol.*, 1941, **4**, 92–114.
3. ELMADJIAN, F., and PINCUS, G. *Endocrinology*, 1945, **37**, 47–49.
4. HARDY, J. D., WOLFF, H. G., and GOODELL, H. *Res. Publ. Ass. nerv. ment. Dis.*, 1943, **23**, 1–15.
5. HARLOW, C. M., and SELYE, H. *Proc. Soc. exp. Biol. Med.*, 1937, **36**, 141–144.
6. HOAGLAND, H. *J. aviat. Med.*, 1947, **18**, 450–464.
7. HOAGLAND, H., ELMADJIAN, F., and PINCUS, G. *J. clin. Endocrinol.*, 1946, **6**, 301–311.
8. LURIA, A. R. *The nature of human conflicts.* New York: Liveright, 1932.
9. PINCUS, G. *Recent progress in hormone research.* (Vol. 1.) New York: Academic Press, 1947. Pp. 123–145.
10. PINCUS, G., and ELMADJIAN, F. *J. clin. Endocrinol.*, 1946, **6**, 295–300.