TABLE VI. Molecules of Antigen Used for Sensitization.

Antigen	γ/ml	Molecular wt	Molecules per ml
Ovalbumin	5×10^3	4×10^4	7.5×10^{16}
Human γ-globulin	100	1.6×10^5	3.8×10^{14}
Polio I	13	6.7×10^6	1.2×10^{12}
II	5	*Idem*	4.5×10^{11}
III	5	"	*Idem*

Type I, 64 CF units/ml; Type II, 25 CF units/ml; Type III, 25 CF units/ml.

titers were 1:40. Nonspecific titers were <1:10. These results suggest that cross reactions were not lessened when purified virus was used to sensitize the cells, but nonspecific titers were lessened.

Data showing the number of molecules used to sensitize the cells are given in Table VI. These calculations were based on the relationship of 5 CF unit/γ of viral antigen (J. Charney, personal communication) while the amounts for the ovalbumin and gamma globulin were taken from Boyden(2). It becomes evident that the higher the molecular weight of the antigen, the fewer number of molecules are required for sensitization.

Statistically, the overall correlation between HA and neutralizing titers was fairly good ($r = 0.75$). However, a statistical analysis is an inadequate criterion when sera do not show any HA titers despite appreciable neutralizing antibody. It should be emphasized that if a serum has no HA titer, this does not mean that the serum contains no neutralizing antibodies (Table V, sera 1, 2 and 3).

Summary. An hemagglutination technic for the titration of antibodies to polioviruses has been described. The HA titers showed approximately a 75% correlation with neutralizing antibody. The virus-sensitized cells did not remain agglutinable for more than 2 days at 5°C, but have proven stable for at least 6 weeks when kept frozen. An antigenic cross among viral types of 12% was observed. Purified virus lessened the nonspecific HA, but not the percentage of cross reactions.

1. Bautista, G., Jr., Jungeblut, C. W., Kodza, H., *J. Immunol.*, 1956, v77, 242.

2. Boyden, S. V., *J. Exp. Med.*, 1951, v93, 107.

3. McKenna, J. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 591.

4. Schwerdt, C. E., Schaffer, F. L., *Ann. N. Y. Acad. Sci.*, 1955, v61, 740.

5. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1955, v85, 527.

6. Salk, J. E., Youngner, J. S., Ward, E. N., *Am. J. Hyg.*, 1954, v60, 214.

7. Charney, J., Fisher, W. P., Machlowitz, R. A., *Fed. Proc.*, 1957, v16, 409.

Received October 29, 1957. P.S.E.B.M., 1958, v97.

Relationship of Mental and Emotional Stress to Serum Cholesterol Levels.* (23676)

P. T. WERTLAKE, A. A. WILCOX, M. I. HALEY, AND J. E. PETERSON
(Introduced by U. D. Register)
Departments of Biochemistry and Internal Medicine, School of Medicine, College of Medical Evangelists, Loma Linda, Los Angeles, Calif.

With the increased incidence of coronary heart disease there has been much speculation concerning a possible cause-and-effect relationship between stress, cholesterol-lipid-lipoprotein metabolism and coronary atherosclerosis(1,2,8). In considering the pathogenesis of a disease such as atherosclerosis or myocardial infarction one must recognize the possibility of direct as well as indirect effects of such factors as stress, diet, sex, age, exercise, etc. This is particularly evident in the case of stress because of its varied nature and the wide spectrum of bodily responses attributed to it(3). That certain forms of physiological stress can elevate serum cholesterol has been demonstrated. In a study of 55 male subjects Kuhl, *et al.*(4) found that brief immer-

* Aided by a grant from Nat. Heart Inst., N.I.H.

TABLE I. Distribution of Subjects According to Serum Cholesterol Changes under Stressful Conditions.

Groups*	Diff. between total cholesterol levels for stress and control periods, mg %	Avg total cholesterol, mg %†		No. of subjects	% of total No. subjects	Significance of diff. from total group (P values)
		Control period	Stress period			
I	<9	218 ± 7.1	216 ± 9.0	14	32	>.1
II	+ 10– 15	210 ± 7.3	223 ± 7.3	10	23	<.1
III	+ 16–137	213 ± 7.3	261 ± 12.2	20	45	<.01
Total	+ 24	214 ± 3.5	238 ± 4.8	44	100	<.001

* The groups are arbitrarily divided with respect to the stand. error of the difference of the means which equals 5.90 mg %.

† Values shown are mean ± stand. error of mean.

sion in cold water was followed by an immediate and significant increase in serum cholesterol. Selye(5) has described a biphasic response of serum cholesterol and lipids in his adaptation syndrome. However, in an attempt to evaluate the influence of "mental stress" Beischer(6) failed to find any significant changes in concentrations of cholesterol, phospholipids, or lipoproteins in the serum following the subjects' first experience of a human centrifuge ride or the experience of simulated failure of a low pressure chamber. Further investigation of this aspect of the problem has therefore seemed advisable.

In the present study 44 male students were studied during periods in which they were free of examinations as well as during an examination week. Thus the purpose of this study was to determine what possible effect mental and emotional stress might have on the level of serum cholesterol.

Method. All those participating in this study were apparently healthy, male, medical students whose ages ranged from 21 to 37, the mean age being 25. The subjects continued their usual program with no major changes in their diet or exercise for the period of observation. All blood samples were collected in the post-absorptive state. Three control days were chosen to come within a period of time in which the students were free of examinations for at least a week before and after the samples were taken. Samples were drawn on the mornings of 4 examination days to determine the effect this form of mental and emotional stress might have on serum cholesterol. Total serum cholesterol was determined in duplicate on each sample by the method of Pearson, Stern, and McGavack(7).

Results. The mean value for serum cholesterol in 44 subjects on the 3 control days was 214 mg %. The mean level of serum cholesterol during the examination days was 238 mg %, which represents an average increase of 11% over the control period. This increase in mean value of serum cholesterol under these conditions of mental and emotional stress is statistically significant ($P < 0.001$).

The 44 subjects were arbitrarily divided into 3 groups with respect to the standard error of the difference of the mean levels of serum cholesterol during the control days and the examination days. Those subjects showing a change less than 9 mg % from their control levels were placed in Group I. Group II includes those subjects in whom serum cholesterol increased 10-15 mg % above the subject's control level. Group III includes those individuals showing an increase of 16 mg % or more above their control values. By reference to the statistical findings for the entire group of 44, Group I can be considered to have a probability ($P > 0.1$) of being due to chance; Group II ($0.1 > P > 0.01$); Group III ($P < 0.01$). Table I shows the distribution of subjects according to their serum cholesterol changes.

There were some individuals in Group III who experienced a remarkable increase in their serum cholesterol. One particularly notable example averaged 259 mg % for the control samples, but this average increased to 395 mg % during the 4 examination days. On 2 of these days his serum cholesterol reached levels of 520 mg % and 536 mg %.

Since life itself is stressful and since this

experiment was conducted during the regular school term it is obvious that these control data cannot represent truly an unstressed level of serum cholesterol. It does seem likely, however, that the periods of observation do coincide with periods of relatively lesser and greater degrees of mental and emotional stress.

As one might expect, these individuals vary greatly in their clinical and biochemical responses to stress. It is apparent that the situational stress of examination week must have different meaning and hence a differing stress value for the subjects. Further investigation is needed to establish whether this increase in serum cholesterol is harmful or simply part of a normal and desirable bodily adjustment to mental and emotional stress.

The difference between these results and those of Beischer(6) may be due, as he has suggested, to his use of fewer subjects and of a particularly and relatively transient stress experience. Our data would coincide with changes which Selye(5) has described in connection with the "resistance phase" of the general adaptive response to various forms of systemic stress.

Summary. A significant increase in mean value for serum cholesterol is shown to accompany the mental and emotional stress of examination week in a group of 44 apparently healthy, male, medical students. The mean value for serum cholesterol increased to 238 mg %, which represents an 11% increase over the mean control value of 214 mg % ($P < 0.001$).

1. Keys, A., *Minnesota Med.,* 1955, v38, 758.
2. Katz, L. N., Stamler, J., Pick, R., *J.A.M.A.,* 1956, v161, 25.
3. Selye, H., *The Physiology and Pathology of Exposure to Stress,* Acta Inc., Medical Publishers, Montreal, Canada, 1950.
4. Kuhl, Jr., W. J., Beck, E. M., Gershberg, H., Street, E., Ralli, E. P., *Metabolism,* 1955, v4, 143.
5. Selye, H., *ibid.,* 3, p278.
6. Beischer, D. E., *J. Av. Med.,* 1955, v27, 260.
7. Pearson, S., Stern, S., McGavack, T. H., *Anal. Chem.,* 1953, v27, 260.
8. Friedman, M., Rosenman, R. H., *Circulation,* 1957, v16, 339.

Received October 29, 1957. P.S.E.B.M., 1958, v97.

Hormonal Regulation of Pituitary Adrenocorticotrophin.* (23677)

JULIAN I. KITAY,† DONALD A. HOLUB,‡ AND JOSEPH W. JAILER
Departments of Medicine, Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, and Presbyterian Hospital, N. Y. City

Most of the information available concerning regulation of pituitary secretion of adrenocorticotrophin (ACTH) has been derived from indirect indices of pituitary function, primarily depletion of adrenal ascorbic acid in the intact rat. On the other hand, direct determinations of the ACTH content of pituitary tissue under various experimental conditions have been reported infrequently, and, when attempted, have often been conflicting or inconclusive(1-11).

Recently, Saffran and Schally(12) have described a technic of biological assay for ACTH based upon steroidogenesis by adrenal slices *in vitro.* Birmingham *et al.*(13) have reported a new method of extraction of pituitary ACTH. Assay of their extracts by the *in vitro* technic yielded values considerably higher than those obtained previously. These latter procedures have been employed in the following study, which was undertaken to define more clearly the effects of adrenalectomy and injection of ACTH, epinephrine, and cortisone upon pituitary ACTH content.

Materials and methods. All animals were kept in a constant temperature room for at least one week prior to use and maintained on a diet consisting of Purina Laboratory Chow and water *ad libitum.* Adrenalecto-

* This study was supported by grants from USPHS, Nat. Inst. Health.

† Commonwealth Fund Fellow in Medicine.

‡ Fellow of the National Foundation for Infantile Paralysis.