

synthesis<sup>12</sup>, and both effects are known to influence erythropoiesis in opposite ways. By combination of both at the highest dosage, the bone marrow could be stimulated in the presence of an impairment of its function with the resulting development of non-viable red cells. A suppressive effect of adrenal cortical steroids on erythropoiesis has already been reported<sup>13,14</sup>. Thus, GORDON et al.<sup>14</sup> have shown that high doses of methyl prednisolone depresses erythropoiesis in normal mice as the result of a decreased erythroid responsiveness to erythropoietin and a depression of erythropoietin formation. However, these mechanisms do not appear to be similar to those which are responsible for the results reported here<sup>15</sup>.

**Resumen.** La administración de 2 mg/kg/día de hidrocortisona durante 40 días en perros adrenalectomizados 4 meses antes produjo elevación del volumen de la masa roja circulante y de la síntesis de hemoglobina. El tratamiento con la hormona en la dosis de 4 mg/kg/día durante los siguientes 40 días produjo más aumento de la actividad eritropoyética de la médula ósea, una gran proporción de la cuál fue inefectiva en términos de producción de eritro-

citos, produciéndose un descenso de la masa roja circulante.

C. E. BOZZINI<sup>16</sup>, M. E. BARRIO RENDO<sup>16</sup>,  
J. A. KOFOED<sup>16</sup> and G. O. FREY

*Cátedra de Fisiología, Facultad de Odontología,  
Universidad de Buenos Aires and Departamento  
de Aplicaciones, Comisión Nacional de Energía  
Atómica, Buenos Aires (Argentina),  
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## The Effect of Conflicting Unconditioned Reflexes on Serum Cholesterol Level in the Rat

Plasma lipids, particularly cholesterol, are thought to be involved in numerous pathological conditions, hence, their regulation in the body may bear some clinical importance. That the functional state of the central nervous system (CNS) affects serum cholesterol level has been demonstrated by many investigators. Some have found an increase in serum cholesterol following augmented strain on the central nervous system<sup>1,2</sup>, whereas others have revealed a diminution<sup>3</sup> or phasic changes<sup>4</sup> in the cholesterol concentration of blood plasma. There is reason to believe that the discrepancy between these results was due to the differences in the method employed, species, and in previous condition of the nervous system. The present work was undertaken to clarify whether and to what extent the activity level of the CNS interferes with the response in serum cholesterol level to a nervous strain if other variables, i.e. species and experimental conditions, are kept constant. Also, several endocrine aspects of the problem have been investigated.

**Methods.** Experiments were made on a total of 538 Wistar inbred male rats weighing 180–220 g. The rats were fed a standardized semisynthetic diet, 15 g/day. Conflicting of the thirst and escape reflexes was undertaken after water deprivation for 40 h, in a special cage shown in Figure 1, over periods of 45 min. When the animals attempted to drink, they received an electric shock, 40–50 V<sup>5</sup>. After 45 min of conflicting, the rats were transferred into their boxes, where they were given water ad libitum. Spontaneous intake of water was observed for periods of 15–30 min. 90 min after conflicting of the reflexes had been started, the animals were decapitated, and total cholesterol in blood serum was determined according to the method of BLOOR<sup>6</sup>, using a Beckman DU-G 2400 spectrophotometer. About half of the animals served as controls in each group; these were treated similarly, except that they were not subjected to the conflict situation.

In some of the animals, the above treatment was preceded by similar conflicting for 1 week, 2 or 3 weeks, 3

times every week. Another group of rats was subjected only to 1 conflict situation, but methimazol (1-methyl-5-mercaptoimidazol) was given for 5 days before decapitation, in daily doses of 2.5 mg/100 g body weight, dissolved in 0.5 ml of physiological saline; the controls were given the same amount of 0.9% saline, s.c.

The effect of 40–50 V electric shocks alone was checked also in a group of rats not subjected to water deprivation and conflict situation. The shocks were applied as many times as the number of voluntary drinking attempts had been observed in the experimental group.

In some of the rats, attempts were made by a paper chromatographic method<sup>7</sup> to establish whether the factors causing statistically significant changes in blood cholesterol levels also affected corticosterone in the serum.

Statistical analysis of the results was undertaken by using Student's *t*-test.

**Results.** (1) Single conflicting resulted in a statistically significant reduction of serum cholesterol by 15 mg% (column C in the Table). Previous administration of the thyroid inhibiting agent prevented this diminution to occur (E), while physiological saline injection did not (D). Electric shocks, in the absence of conflicting, failed to affect serum cholesterol level (B).

(2) If conflicting was preceded by similar conflict situations for 3 weeks, 3 times a week, serum cholesterol

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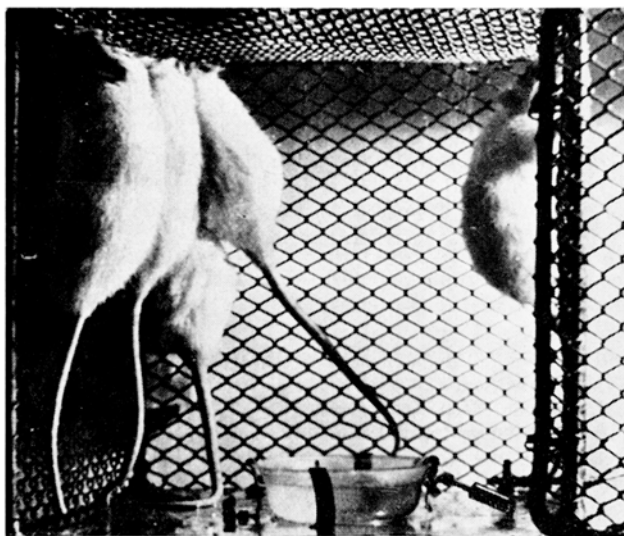


Fig. 1. The behaviour of the rats during reflex confliction.

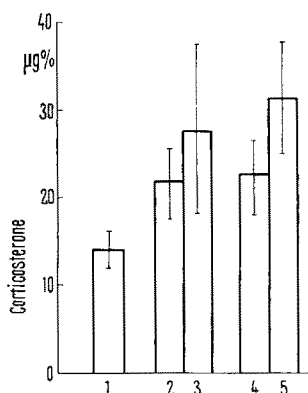


Fig. 2. Changes in serum corticosterone levels in response to varying stimuli. Height of the blocks represents corticosterone concentration ( $\mu\text{g}\%$ ), the experimental procedures were the following (according to the order of blocks 1-5): control, water deprivation for a single occasion, water deprivation and conflicting for a single occasion, water deprivation over periods of 3 weeks, water deprivation and repeated conflicting over periods of 3 weeks, respectively.

concentration rose by 19  $\text{mg}\%$  (H). Similar pre-treatment for 1 week (F) or 2 weeks (G) did not alter serum cholesterol level significantly.

(3) Following water deprivation for 40 h, serum corticosterone was found elevated ( $0.02 < P < 0.01$ ). This was not further altered by single conflicting, however, when the animals had been subjected to repeated conflict situation, 3 times a week, for 3 weeks, a statistically highly significant further increase occurred in serum corticosterone level (Figure 2,  $P < 0.001$ ).

**Discussion and conclusions.** Conflicting of unconditioned reflexes for 45 min for a single occasion resulted in a reduction of serum cholesterol concentration. When the rats had been given the same treatment for 3 weeks, cholesterol level increased. Thus, whether serum cholesterol increases or decreases in response to the conflict situation depends on previous activity level of the nervous system. This observation provides an explanation for the conflicting results obtained by others with respect to the effects on cholesterol level of nervous strain<sup>1-4</sup>.

Changes in serum cholesterol level in various experimental conditions

	Mean $\pm$ S.E.	Statistical probability
Control		
(A)	109.0 $\pm$ 3.41 (20)	0.7 $< P < 0.8$
Electric shock without water deprivation		
(B)	107.8 $\pm$ 3.68 (30)	
Water deprivation for 40 h on one occasion		
(C)	112.2 $\pm$ 3.12 (28) 97.8 $\pm$ 3.21 (30) <sup>a</sup>	0.001 $< P < 0.01$
(D) <sup>b</sup>	119.8 $\pm$ 3.58 (23) 106.3 $\pm$ 3.18 (26) <sup>a</sup>	0.001 $< P < 0.01$
(E) <sup>c</sup>	125.6 $\pm$ 3.78 (23) 125.5 $\pm$ 4.18 (28) <sup>a</sup>	0.9 $< P$
Water deprivation for 40 h, 3 times a week		
(F)	114.3 $\pm$ 3.68 (25) 110.0 $\pm$ 3.41 (26) <sup>a</sup>	0.3 $< P < 0.4$
Water deprivation for 40 h, 3 times a week, for 2 weeks		
(G)	109.4 $\pm$ 6.02 (12) 106.7 $\pm$ 4.22 (13) <sup>a</sup>	0.2 $< P < 0.3$
Water deprivation for 40 h, 3 times a week, for 3 weeks		
(H)	107.9 $\pm$ 6.12 (27) 126.7 $\pm$ 5.63 (29) <sup>a</sup>	0.02 $< P < 0.05$

No. of rats in brackets. <sup>a</sup> Plus conflicting. <sup>b</sup> Pretreatment with placebo. <sup>c</sup> Pretreatment with metothylin for 5 days.

FALCONER and associates<sup>8</sup> observed thyroid overproduction for about 2 h after an acute stressful stimulus in sheep; if the stress was repeated several times, however, the enhancement of thyroid activity did not occur. In the present experiments, the diminution in serum cholesterol concentration in response to a single conflict situation could be prevented by pre-treatment with a thyroid inhibiting agent. This observation indicates that a transitory increase in thyroid activity might have played a role in blood cholesterol changes.

When the conflict situation was applied repeatedly over periods of 3 weeks, the rise in serum cholesterol was associated with an increase of blood corticosterone level. In fact that glucocorticoids tend to increase cholesterol in the blood<sup>9,10</sup>, it may be assumed that enhanced adrenocortical activity was in some way involved in the hypercholesterolaemia occurring in response to repeated stimuli.

**Zusammenfassung.** Einmalige nervöse Belastung führte zur Abnahme des Serumcholesterinspiegels, die durch Methimazol-Dosierung behoben werden konnte. Nach mehrmaligen Belastungen erfolgte eine Zunahme des Serum-Cholesterin-, bzw. -Corticosteronspiegels.

T. SZAMOSI, J. I. SZÉKELY,  
A. BÁLINT<sup>11</sup> and J. ZOMBORY<sup>12</sup>

*Institute of Biochemistry and Institute of Physiology,  
University Medical School, Budapest (Hungary),  
8 March 1968.*

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<sup>11</sup> Present address: Second Department of Pathologic Anatomy, University Medical School, Budapest.

<sup>12</sup> Present address: Institute of Geriatrics, University Medical School, Budapest.