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Effect of Electroshock and Pentamethylenetetrazol on Serum Cholesterol Levels in Monkeys* (33479)

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Apparent increases in serum constituents including cholesterol were previously reported to follow immediately after electroshock treatment (1, 2). A change in circulating blood volume was postulated as an explanation for these phenomena.

In previous studies we found that pentamethylenetetrazol (Metrazol) can lower serum cholesterol levels in mice (3) and in monkeys (4) as well as inhibit cholesterol synthesis in isolated mouse cells *in vitro* (5). In untreated monkeys weekly fluctuations in cholesterol expressed as mg/100 ml of serum were greatly minimized when recalculated as mg/10 g of serum solids, thereby eliminating apparent changes which were due to variations in serum water content (6).

In the present study convulsive seizures were induced in monkeys by electroshock as well as by parenteral Metrazol and changes in serum solids and cholesterol were compared to ascertain whether these altered values were due to *bona fide* changes in cholesterol metabolism in the organism or merely to changes in serum water content.

Methods. Twenty-two *M. mulatta* 3–6 kg, including 19 males and 3 females, were maintained on a standard diet consisting of bread, lettuce, and bananas offered at 4 p.m. Animals were caught by net and 1.5 ml of blood was obtained from the saphenous vein. All bleedings took place prior to feeding. Total and free serum cholesterol, serum solids, hematocrit, and proteins were determined as previously described (4). Animals were grouped according to body weight, and processed without knowledge of their initial cholesterol levels until after termination of the experiment.

For inducing electroshock seizures unilateral rectangular pulses of 0.3-msec duration, pulse repetition frequency of 120/sec for 1.3 sec were used. The preset current varied from 10mA average at 170 mA peak to 18 mA average at 300 mA peak. Convulsive or subconvulsive doses of pentamethylenetetrazol were administered as 10% Metrazol intramuscularly in dosages of 40–48 mg/kg.

In the first group of 7 monkeys, 2 received electroshock seizures, one received a convulsive dose of Metrazol (48 mg/kg), one received a subconvulsive dose of Metrazol (40 mg/kg), and 3 were injected with physiological saline i.m. to serve as controls. All monkeys were bled immediately prior to treatment. In the 3 monkeys in which seizures occurred, the first posttreatment sample was drawn 3 min after termination of the convulsion (which was 10 min after injection).

In a second group of 14 monkeys, 7 received a single subconvulsive dose of Metra-

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TABLE I. Fluctuations in Various Serum Constituents after Electroshock-Induced Convulsive Seizures.[a]

Time (min)	Cholesterol (mg/100 ml)	Solids (g/100 ml)	Proteins (g/100 ml)	Hematocrit (%)	Cholesterol (mg/10 g of solids)
0	143.0	10.42	6.75	45.4	137.2
3	146.5	10.73	7.15	46.4	136.5
13	149.2	11.03	7.45	48.6	135.3
23	144.6	10.73	7.00	46.6	134.8
33	143.2	10.81	6.70	46.3	138.6
(hr)					
24	142.9	10.31	6.64	46.3	138.6

[a] Average values from 2 monkeys, 1 male, 1 female, after a generalized seizure, 15–20-sec duration, followed by tremors and rigidity.

zol (40 mg/kg) and 7 physiological saline. Samples were taken prior to injection and at 24 hr.

For comparative purposes, the response of one additional, seizure-prone monkey with chronic experimental epilepsy, induced by disc application of alumina cream to one sensorimotor cortex (7), was also tested.[1] A convulsive seizure was induced in this animal by injection of Metrazol (20 mg/kg) i.m. Two months later an electroshock seizure was administered (10 mA av current, 170 mA peak). In both instances blood samples were obtained at 10-min intervals during the first hour and again at 6 and 24 hr following treatment.

Results. A significant increase in serum cholesterol (expressed in mg/100 ml) was found in normal monkeys following convulsive seizures induced by electroshock or Metrazol injection (Tables I, II). Increases appeared within 3 min after termination of seizure, and reached maximum values approximately 13 min later. The cholesterol levels returned gradually to normal in about 1 hr. Parallel changes were found in total serum proteins, total serum solids and hematocrit values, which increased to approximately the same extent, reached a peak 13 min after the end of observable convulsive activity and returned gradually to normal levels at 1 hr. When recalculated as mg/10 g of serum solids, cholesterol values showed practically no variations during the 1-hr test period. No increases were observed in the animal given a subconvulsive dose of Metrazol (Table III), or in those injected with saline.

In contrast to the results obtained at 1 hr, a significant decrease in serum cholesterol was observed 24 hr following administration of Metrazol whether in convulsive or subconvulsive doses. This decrease was not due to

TABLE II. Fluctuations in Various Serum Constituents after a Metrazol-Induced Seizure.[a]

Time (min)	Total cholesterol (mg/100 ml)	Solids (g/100 ml)	Proteins (g/100 ml)	Hematocrit (%)	Total cholesterol (mg/10 g of solids)
0	192.0	10.60	7.47	46.0	181.1
3	196.0	11.16	7.76	46.9	175.6
13	209.0	11.54	7.93	49.5	181.1
23	204.0	10.80	7.35	48.0	188.9
33	200.7	10.72	7.11	47.9	187.2
53	193.2	10.24	7.74	46.7	188.7
(hr)					
24	147.6	10.11	7.61	46.3	145.9

[a] Values from one monkey, given Metrazol 48 mg/kg, i.m., inducing in 6 min a generalized seizure, lasting 50 sec.

TABLE III. Fluctuations in Various Serum Constituents after Treatment with a Subconvulsive Dose of Metrazol.[a]

Time (min)	Total cholesterol (mg/100 ml)	Solids (g/100 ml)	Total cholesterol (mg/10 g of solids)
0	143.1	10.62	134.7
3	142.1	10.70	132.7
13	142.4	10.68	133.3
23	140.8	10.83	129.9
33	139.2	10.74	129.6
53	141.4	10.77	131.2
(hr)			
24	133.3	10.75	124.0

[a] Values from one monkey given a subconvulsive dose of Metrazol (40 mg/kg).

water fluctuation, inasmuch as serum solids, proteins, and hematocrit values had returned to near-normal levels, while cholesterol values recalculated as mg/kg of serum solids were approximately 20% below pretreatment levels (Table II). When a subconvulsive dose was used, there were no fluctuations in serum concentration of cholesterol, proteins or serum solids immediately after treatment. However, 6 hr later total serum cholesterol began to drop significantly and in 24 hr had decreased 7.9% in mg/10 g of serum solids (Table III). A summary of results obtained 24 hr after treatment (Table IV) indicated that an electroshock-induced seizure did not alter the level of serum cholesterol, while treatment with both convulsive and high subconvulsive doses of Metrazol caused a significant decrease. The latter was independent of serum water content inasmuch as recalculation from mg/100 ml to mg/10 g of serum solids did not materially affect the results.

"Constitutional" serum cholesterol levels varied greatly from animal to animal. The lowest value obtained in this series was 89 mg/100 ml; the highest, 192 mg/100 ml. Even the larger groups of animals (7 and 8) were not large enough to average out differences of this magnitude. However, individual values clearly indicated that all animals within each group reacted in a similar manner to Metrazol treatment or to placebo. Every animal treated with the drug showed a decrease of 8–18% in total serum cholesterol 24 hr after injection of 40 mg/kg. One typical placebo monkey showed 87 mg/10 g before and 87 mg/10 g after. Metrazol-treated animals showed 140 mg/10 g before and 101 mg/10 g after. In no instance was the decrease after Metrazol treatment less than 13 mg/10 g, with usual drops of 18 mg/10 g or greater. In no case was the change following placebo, up or down, greater than ± 6 mg/10 g, usually within the range of ± 3 mg/10 g of solids.

Similar results were obtained in the alumina-treated chronically epileptic monkey. Recalculation of cholesterol from the conventional mg/100 ml to mg/10 g of serum solids indicated that the immediate effect of a convulsion was a general increase in concentration of serum constituents as exemplified by cholesterol (Table V). At 24 hr the cholesterol concentration following an electrically-induced seizure had returned to its preconvulsive level, while that following a Metrazol-induced seizure had fallen below the starting level.

Discussion. Previous reports in the literature have indicated that convulsive seizures induced by electroshock in humans (1) and in dogs (2) were followed immediately by a

TABLE IV. A Summary of Changes in Total Serum Cholesterol 24 hr after Treatment.

Treatment	No. of monkeys	Seizure	Cholesterol (mg/100 ml) Before	Cholesterol (mg/100 ml) After	Cholesterol (mg/10 g of solids) Before	Cholesterol (mg/10 g of solids) After
Electroshock	2	Yes	143.0	142.9	135.1	138.6
Metrazol (48 mg/kg)	1	Yes	192.0	147.6	181.2	145.9
(40 mg/kg)	8	No	150.8	129.2	148.4	127.4
Saline	7	No	122.7	122.4	118.1	118.9

TABLE V. Changes in Total Serum Cholesterol in an Alumina-Treated "Epileptic" Monkey.

Time (min)	Total serum cholesterol			
	Metrazol (20 mg/kg)		Electroshock	
	(mg/100 ml)	(mg/10 g of solids)	(mg/100 ml)	(mg/10 g of solids)
0	139.3	137.4	151.1	137.4
3	140.2	137.6	162.1	142.2
13	143.0	137.2	166.5	143.0
23	133.6	132.2	162.7	144.7
53	133.0	135.8	160.5	140.0
(hr)				
3	132.5	139.4	158.3	139.6
6	130.4	131.0	151.0	138.6
24	127.0	126.7	150.6	138.2

decrease in circulating blood volume. Swingle and Swingle (2) reported a 20–30% decrease in plasma volume within 3 min after electroshock treatment. They also found small but consistent increases in concentration of plasma electrolytes, hemoglobin, and hematocrit, and large (20–100%) increases in blood sugar. In humans after electroshock treatment, Horwitz and Sperry (1) observed increases in serum cholesterol with concomitant increases in serum proteins and other constituents. In the course of our investigation of the effects of chemically-induced seizures on blood volume and concentration of serum constituents, we included for comparison some monkeys in which seizures were induced by electroshock. From the results obtained in 2 of them, and confirmed in a third which was an alumina cream-treated chronic epileptic monkey with a low Metrazol threshold, it appeared that electroshock seizures were accompanied by an immediate decrease in blood water content and a corresponding increase in total proteins and cholesterol comparable to those observed in other species (1, 2).

A rapid mobilization of water from circulation appears to have taken place within minutes after convulsive seizures, whether induced by electroshock or by an injected chemical agent (Metrazol). Total proteins, total serum solids, and total serum cholesterol increased, and this increase was also reflected in the hematocrit determination. Inasmuch as serum solids and cholesterol increased to approximately the same extent, their proportions remained unchanged and the value of cholesterol expressed per unit weight of dry serum solids showed no increase. This would indicate that no significant change in the metabolic pattern of cholesterol took place during the first hour after seizure. The observed changes in concentration per unit volume of serum were therefore due largely, if not entirely, to rapid withdrawal of water from circulation and not to changes in metabolic pattern of serum constituents other than water.

On the other hand, the decrease in serum cholesterol level observed 24 hr after treatment with Metrazol represents an example of a *bona fide* metabolic effect on cholesterol biogenesis, inasmuch as serum solids, proteins, and hematocrit values had returned to pretreatment levels. There is no indication in our data that this decrease in circulating cholesterol observed 24 hr after treatment represents an indirect result of water changes due to convulsions. Presumably, the homeostatic inhibition of biosynthesis of a metabolite is triggered by the presence of its excess in circulation. Therefore, changes due to water withdrawal if sufficiently high or prolonged could have accounted for the observed drop in serum cholesterol in 24 hr. However, it appears from our electroshock data that the observed changes in hemoconcentration which were of the same magnitude as those produced by Metrazol were insufficient to cause a delayed effect on cholesterol metabol-

ism. Furthermore, even subconvulsive doses of Metrazol which produced no immediate fluctuations in hemoconcentration led to a decrease in serum cholesterol in 24 hr, thus indicating that the effect was due to a specific action of Metrazol on cholesterol metabolism. While the number of animals used in this study is insufficient to provide a statistically significant dose–response relationship, a progressive decrease in serum cholesterol (in mg/10 g) was observed in 24 hr with increasing doses of Metrazol. Thus, treatment with 20 mg/kg produced a drop of 8.0%, while 40 and 48 mg/kg produced drops of 14.1% and 19.4%, respectively (Tables IV and V).

The effect of unavoidable stress in our experimental animals during treatment was evident. The animals were caught at weekly intervals and bled up to 7 times to establish base levels of serum cholesterol prior to the beginning of the experiment proper. The early bleedings, when the animals appeared quite agitated, fluctuated greatly. As the bleedings progressed, animals appeared less disturbed, and the fluctuations in serum cholesterol levels decreased. Inasmuch as all animals were treated in a similar manner throughout, those animals which received saline were considered to have served as controls for the stress factor.

The mechanism of the decrease in serum cholesterol following Metrazol treatment is being investigated. It is possible but unlikely that a decrease of the magnitude observed was due merely to greater activity of treated animals following injection of drug with a concomitant loss of weight and mobilization of fat deposits. We failed to observe any difference in weight pattern between the Metrazol-treated and control groups during the 24-hr posttreatment period. Studies in mice have indicated that sterol biosynthesis is inhibited by Metrazol (3). This effect of the drug on biosynthesis was examined with the aid of radioisotopes. Inhibition appeared to be dose-related. Studies in yeast indicated that a blocking of sterol synthesis took place mainly in the utilization of mevalonic acid, and to a lesser extent, squalene (8). This inhibition occurred in the 3-enzyme segment of the acetate–sterol pathway between mevalonate and isopentenylpyrophosphate and CO_2 (9). It is of interest that feedback inhibition of cholesterol biosynthesis by the sterol itself is said to take place between hydroxymethylglutaric acid and mevalonic acid, one step earlier than the apparent inhibition due to Metrazol (10).

Summary. An examination of the level of serum cholesterol in monkeys following convulsive seizures induced by electroshock or pentamethylenetetrazol (Metrazol) indicated that no significant changes in the metabolic pattern took place within the first hour. Observed increases in total serum solids, proteins, and cholesterol were due largely, if not entirely, to withdrawal of water from circulation. Metrazol, but not electroshock, caused a significant decrease in serum cholesterol 24 hr after a single treatment. This decrease was apparently due to a specific inhibition of sterol biosynthesis.

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