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Sulfhydryl Content of Failed and Strophanthin Poisoned Rabbit Hearts. (24289)

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Recently, Bertelli and Mussini(1) reported that the sulfhydryl content of hearts perfused with 1:100,000 strophanthin-g (ouabain) was markedly lowered. There was a decrease of approximately 50% of sulfhydryl content of hearts in complete systolic contracture induced by strophanthin-g, compared to unpoisoned, perfused rabbit hearts. This finding and observations in our laboratory, led us to study the sulfhydryl content of crude myosin extracts of hearts after the hearts had been subjected to various conditions.

Methods. Perfusion. Rabbits of the same strain, 3 to 4 months old, raised in air conditioned animal room on the same diet, were killed by blow on occiput, and their hearts removed as rapidly as possible. These hearts were perfused with the apparatus described by Anderson and Craver(2), available from Metro Industries, L. I. City, N. Y. The perfusion solution was described by Chenoweth and Koelle(3). This work was performed during the summer months. Immediately after removal, the hearts were perfused 30 minutes at 37 $\pm$ 0.5°C. The first 500 ml of perfusion solution passing through heart was not recirculated because it contained blood. To avoid possible injury to hearts by attaching a hook, kymograph recordings were not made. Hearts that appeared damaged or showed evidence of failure during this 30-minute period were not analyzed. After perfusion, the drug was added to a liter of solution in the aeration reservoir, and the heart perfused 15 minutes more. Each heart was perfused 45 minutes. A control group of hearts was perfused 45 minutes continuously. A second group was perfused with 1:100,000 strophanthin Kombé N. F. (S. B. Penick Co.) for 15 minutes. Then they were in complete systolic contracture. A third group was perfused with 0.04% sodium pentobarbital. A fourth group was perfused with 5% ethanol (v/v). A fifth group was failed by perfusing with a solution containing 25% of usual calcium ion concentration: 0.54 mM $CaCl_2$/l, or 0.079 g $CaCl_2 \cdot 2H_2O$/l. All hearts in the last 3 groups failed, stopping in diastole in 7 to 9 minutes; however, all hearts were perfused for the full 15 minute period. *Extraction.* The hearts were removed from apparatus and ventricles were immediately frozen in liquid nitrogen. While still frozen, the ventricles were crushed in stainless steel

TABLE I. Rabbit Heart Sulfhydryl Content (μg SH/mg N).

	Strophanthin-K poisoned	Untreated	Failed Pentobarbital	Failed Ca^{++} deficient
			13.95	
	17.	15.52	13.01	17.27
	18.05	16.	16.05	14.20
	18.06	16.58	14.15	14.04
	18.75	17.68	11.35	17.62
	18.52	16.26	11.85	17.61
Mean	18.08	16.41	13.39	16.15
S.D.	.06	.72	1.72	1.66
P	.008		.006	

cylinder by sledge-hammer driven piston. Then portions weighing 100 to 200 mg were suspended in 10 ml of 3.6% potassium chloride—0.4% potassium bicarbonate solution with a Potter-Elvejhem homogenizer equipped with Teflon® pestle. This procedure dissolves myosin and other water soluble fractions of heart but not fibrous tissue. This crude extract of heart muscle was centrifuged 30 minutes at 6,000 rpm at 5°C. The supernatant was immediately analyzed for sulfhydryl content by the method described by Ellman(4). Micro-Kjeldahl determinations for nitrogen were performed on an aliquot of this supernatant. *Sulfhydryl determinations*: Two ml of supernatant were diluted with 3 ml water and 2 ml 0.03 M potassium phosphate buffer at pH 8.0. To this solution was added 3 ml of 0.1% acetone solution of bis (*p*-nitrophenyl) disulfide. The mixture was then centrifuged 5 minutes at 6,000 rpm to remove precipitated protein. The yellow *p*-nitrobenzenethiol anion which was released by the sulfhydryl groups in the tissue was then measured with Beckman DU spectrophotometer at 412 mμ. These determinations were made against appropriate blank solutions. Amount of sulfhydryl present was determined and sulfhydryl content/mg of nitrogen calculated. The data were analyzed statistically with the "t" test according to Snedecor(5).

Results. Table I presents data. The significant changes are (1) depression of SH levels in pentobarbital failed hearts, and (2) elevation in Strophanthin K poisoned hearts. Failure caused by treatment with Ca deficient bath fluid failed to change SH levels. Measurements on hearts failed with ethanol are not given in the Table since variation in results is so large as to make comparison with other results difficult. [12.73 $\pm$ 5.4 (1 SD) mg SH/mg N]. We did not at any time observe detectable SH material in the perfusion fluid.

Discussion. These data are obviously in contradiction to data of Bertelli and Mussini, who reported a lowering of sulfhydryl concentration with strophanthin-g poisoning, and these data indicate a rise in sulfhydryl concentration with strophanthin Kombé poisoning. Since both of these drugs are very similar pharmacologically, it seems strange that they should produce opposite effects on sulfhydryl levels.

Our data also suggest that in some types of failure, sulfhydryl concentration of heart muscle is lowered. Since Bertelli and Mussini did not describe the condition of hearts before adding strophanthin-g, it is possible that the hearts were already in some degree of failure before the drug was added. These workers did not analyze hearts for sulfhydryl that had been failed. The methods of sulfhydryl determination in these experiments, although different, are both reliable methods; therefore, there should be no disagreement arising from actual analysis of sulfhydryl groups. It is more likely that a difference would arise from the methods in which tissues were handled before analysis. Bertelli and Mussini do not describe the procedure they followed in homogenizing their tissue samples, but care has been taken, in this case, to preserve the sulfhydryl content of tissues during analysis.

Summary. Perfused rabbit hearts were analyzed for sulfhydryl content. Unfailed hearts showed average concentration of 16.41 μg sulfhydryl/mg nitrogen. Hearts which had been put into systolic contracture with strophanthin-K showed a significant rise in sulfhydryl concentration, whereas hearts which were put into diastolic arrest with sodium pentobarbital showed a significant lowering in sulfhydryl concentration. Hearts which were put into diastolic arrest by lowering of calcium ion in the perfusion media showed an average sulfhydryl concentration

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Adrenocortical Function in Some Mental Diseases. (24290)

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Controversial reports have appeared regarding the functional state of the adrenal cortex in mental diseases. In schizophrenia, hypoactivity of the adrenal cortex has been claimed(1-6) and denied(7-10). One reason for this controversy might have been the different diagnostic criteria of the workers. The present investigation deals with functional activity of the adrenal cortex in patients suffering from schizophrenia, paranoia, depression and mania. Eosinopenic response to injected epinephrine was used as the test in all the diseases. In some of the schizophrenic patients eosinopenic response to injection of ACTH and urinary excretion of 17-ketosteroids were also determined.

Methods. Patients were selected from Lumbini Park Mental Hospital at Calcutta. Normal subjects were male and female nurses of the same hospital. Patients with composite diagnosis like depression with paranoia, patients complicated with arteriosclerosis, psychosis, general paralysis of the insane, thyrotoxicosis with psychosis were avoided. The 4 groups of patients were selected according to the following basic features: *Schizophrenia* starts insidiously at adolescence and progresses slowly. There is gradual personality deterioration with disorders of feeling, thought, conduct and memory, and withdrawal of interest from environment. *Paranoia* is a focal personality disorder with delusion formation without much deterioration of personality. Hallucination may be present. Power of reasoning remains relatively unimpaired, but does not accord with the existing surroundings and patients do not realize the absurdity of their delusions or hallucinations. *Mania* is associated with elated mood with emotional instability. There is flight of ideas and psychomotor hyperactivity. Megalomanic ideas may be present. *Depression.* Patients suffer from depressed mood and retardation of thinking process. Sluggishness of psychomotor activity, sense of guilt, self-reproach and self-depreciating ideas are often present. In the schizophrenic group stress was given to the basic features, and different sub-classes of this group were not included. In manic depressive psychosis, importance was given to the nature of manifestation at time of study and patients of this group were included in either mania or depression. .3 cc of epinephrine hydrochloride (1/1000) was injected subcutaneously in early hours of morning before breakfast and blood collected from anticubital vein in oxalated tubes both before and 4 hours after injection. Eosinophils in blood samples were counted by the method of Menneman *et al.* (11). In 8 schizophrenic patients, who did not improve with insulin shock and electric convulsion therapy, 25 mg ACTH was injected subcutaneously and eosinophils were counted in the blood samples collected before and 4 hours after the injection. In these patients 17-ketosteroids in 24 hour urine samples were extracted and estimated by the method of Davidson *et al.*(12).

Results. Tables I-III summarize the results. Eosinophils of patients suffering from schizophrenia and mania were significantly