Biochemical Studies on Blood of Guinea Pigs Treated with Pituitary Adrenocorticotrophic Hormone and Cortisone. (18770)

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In the course of investigating the effect of the pituitary adrenocorticotrophic hormone (ACTH) and cortisone on anaphylaxis in the guinea pig(1), the opportunity presented itself of studying the effect of these hormones on certain of the constituents of guinea pig blood. This was deemed worthwhile because of the paucity of findings on the normal guinea pig and because available findings are seldom grouped together in a single publication. This report presents averages of about 65 determinations taken from apparently healthy animals, also values obtained on the effect of the above hormones on the blood cell picture, serum proteins, cholesterol, chloride, uric acid, creatine, creatinine and glutathione.

Materials and methods. ACTH and cortisone: The preparations of ACTH used in these experiments were Armour Lot 77-S having a potency of 41% of Armour Standard La-1-A and Lot 128-105 with a potency equivalent to 160% of the standard.* The cortisone was used in the form of the acetate marketed by Merck and Co. under the name, "Cortone Acetate." *Antibodies, antigens and sensitization technics*: The antiserum used for passive sensitization was rabbit anti-egg white. The guinea pigs were inoculated by the intravenous (i.v.) injection of 5 ml/kg of this serum. After 2 hr, they were challenged by the i.v. injection of 0.4 ml of a dilute fresh egg white solution (1:8 in physiological saline). Active sensitization to horse serum was accomplished by injecting subcutaneously 1.0 ml of the antigen diluted 1:10 with saline. Challenge was performed on about the 13th day following sensitization by the i.v. injection of 1.0 ml of undiluted antigen.

Histamine shock: Histamine shock was effected by the i.v. injection of histamine, 0.4 mg/kg, as free base. This has been determined(2) as being the MLD_{100} dose. *Blood smear studies*: Cell counts of the mononuclear and polymorphonuclear series were carried out by the routine differential count of a peripheral blood smear stained either by the Wright or Giemsa technic. *Chemical determinations*: Cardiac blood was withdrawn from the guinea pigs for the chemical determinations; the animals in the treated groups were in a state of anaphylactic shock. *Uric acid*: The method of Brown(3) was used as described except that ½ of the original quantities were taken in order to conserve blood serum. *Creatine and creatinine*: The Folin and Wu(4) procedure was utilized for these determinations except that they were carried out on 1/5 the original quantities. *Glutathione*: Determinations were carried out on serum according to the procedure of Benedict and Gottschall(5), as described by Snell and Snell(6), except that it was adapted for the photoelectric colorimeter, the degree of absorption being read at 580 mμ. *Chloride*: This procedure was essentially a modification of that of Snell and Snell(6) in which the chloride determination was adapted as a chromate determination to permit reading in the photoelectric colorimeter. To one ml of a Folin-Wu(4) blood tungstic acid filtrate in a 15 ml centrifuge tube were added about 10 mg of magnesium carbonate to neutralize any possible trace of excess acidity, followed by about 5 mg of dry silver chromate and 9 ml of distilled water. After centrifuging 10-15 min at 1500 RPM 1

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* Supplied through the cooperation of Armour and Co., Chemical Research and Development Department, Chicago, Ill.

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TABLE I. Mean Values for Determinations on Blood of Normal and Hormone Treated Guinea Pigs.

Determination		Experimental groups: Normal controls	Untreated anaphylactic shock	Untreated histamine shock	ACTH treated anaphylactic shock	Cortisone treated anaphylactic shock
Glutathione	No. det.	61	15	9	35	10
	mg %	42.6 (19.4–64.2)*	46	62.6	41.4	43.2
	t†		.8	4	.4	.1
Creatine	No. det.	45	15	9	34	10
	mg %	1.14 (.19–2.73)	1.80	1.41	1.60	.87
	t		3	1.1	1.9	1.2
Creatinine	No. det.	65	26	9	49	10
	mg %	1.53 (1.10–2.14)	1.49	1.36	1.61	1.39
	t		.6	1.7	.8	1.6
Uric acid	No. det.	67	26	9	48	10
	mg %	2.53 (1.38–3.88)	3	3.77	4	3.23
	t		2.9	5.4	7.5	3
Uric acid/ creatinine	No. det.	64	26	9	48	10
		1.62	2.02	2.94	3.03	2.38
	t		4.7	8.8	6.7	5.8
Cholesterol	No. det.	48	14	8	49	10
	mg %	41.2 (20 -81.5)	44.3	32	45.5	54.2
	t		.7	1.6	1.3	2.3
Total serum protein	No. det.	57	23	9	49	9
	g %	6.02 (4.5–8)	6.10	6.81	7.29	6.07
	t		.3	2.5	4.2	.2
Albumin/ globulin	No. det.	53	23	9	43	10
		1.73	3.06	2.90	2.63	2.23
	t		3.7	5.6	3.7	2.2
Serum chloride	No. det.		11		14	
	mg %		454 (412–490)		480	
	t				4.7	
Mononuclear leucocytes	No. det.	33		10	47	10
	%	82.6 (34–91)		52.1	56.4	57
	t			6.1	5.8	6.4
Polymorphonuclear leucocytes	No. det.	33		10	47	10
	%	16.1 (9–65)		47.1	42.5	42.7
	t			6.3	5.7	6.7
Eosinophilic leucocytes	No. det.	33		10	47	10
	%	1.3 (0–5)		.8	1.2	.3
	t			.6	.2	1.3

* The values in parentheses indicate the range.
† A value, $t > 3$, indicates significant difference based on 98% confidence limit from normal mean.

ml of the supernatant liquid was removed and placed in a 50 ml volumetric flask. To this were added 40 ml of water, 1 ml of 40% acetic acid, 1 ml of s-diphenylcarbazide reagent(6), and finally water to dilute to 50 ml. The solution was well mixed and read in the photoelectric colorimeter at 545 mμ 15-20 min after adding the reagents. Macro cuvettes were used and the amounts of chloride were translated from a standard curve. *Serum proteins*: Albumin and globulin were determined by the sodium sulfate salting out procedure of Greenberg(7). *Cholesterol*: The technic of Leiboff (8,9) was carried out essentially as described, except for minor modifications in apparatus and volumes. For the extractions a Folin-Wu blood sugar tube was used with a by-pass connecting the constriction, at about the 6 ml mark, with the upper portion at about the 12.5 ml mark. Blood serum, 0.2 ml, was placed on a small square of chloroform-washed filter paper, allowed to dry, then

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placed in a chloroform-washed 10 x 50 mm Whatman extraction thimble caught and held in place in the sugar tube constriction. About 3.5 ml of chloroform were added and the cholesterol was extracted by refluxing for 30 min. After cooling, chloroform was added to the 4 ml mark, 2 ml of a fresh mixture of 1 part of conc. sulfuric acid and 20 parts of pure, dry acetic anhydride were added, the contents mixed well and the color read in the photoelectric colorimeter at 575 mμ after standing in the dark for exactly 10 min. The cholesterol content was translated from a standard curve. The results of the various determinations are summarized in Table I.

Results and discussion. Histamine shock, passive and active anaphylaxis: Neither ACTH nor cortisone had any effect on the fatal outcome of the reaction, regardless of dosage, number of injections and total dose, time relationship of the challenge dose to the pretreatment, etc. The details are published elsewhere(1).

Chemical and cellular studies: The figure listed as normal for most of the values was the average of a number of determinations taken from apparently healthy guinea pigs. Some values were also obtained from guinea pigs shocked passively and in histamine shock, neither group having had any previous ACTH or cortisone therapy. The statistical evaluation of a significant difference for the results obtained indicates that the difference between the normal and the experimental means varies within at least a 98% confidence limit.

Summary. Normal values for the following constituents of the blood of guinea pigs are presented: blood cell count, serum proteins, cholesterol, chloride, uric acid, creatine, creatinine and glutathione. Physiological alterations were produced by the administration of the adrenocorticotrophic hormone and cortisone as evidenced by an increase in the uric acid/creatinine and albumin/globulin ratios, a decrease in the relative mononuclear and an increase in the polymorphonuclear leucocyte counts.

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Rate of the Enzymatic Breakdown of Digitoxin.* (18771)

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The breakdown of digitoxin by strong acids to form digitoxigenin and digitose has been described by Stoll(1) and others. A similar effect by heart and skeletal muscle was described by Fischer(2). However, in neither of these cases was any attempt made to study the reaction rates of these changes. The development of a polarographic method for the analysis of digitoxin (Hilton)(3), in very small quantities has made it possible to study the breakdown of digitoxin and to determine the reaction rate constants for these reactions in dilute acid and in extracts of heart, liver, kidney, and yeast. In the case of the heart extract attempts were made to purify the essential enzymatic component which was responsible for the reaction.

Methods. The breakdown of digitoxin by 0.85% hydrochloric acid was carried out as follows: 9.0 ml of 0.85% hydrochloric acid were placed in each of six 50 ml Erlenmeyer flasks which were shaken in a constant tem-

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