isms, whereas, diethylaminoethanol involved only the second.

Summary. In the isolated frog heart balanced responses to mixtures of epinephrine and acetylcholine were converted to positive inotropic responses by atropine, hexamethonium and tetraethylammonium. These drugs specifically and competitively antagonized acetylcholine depression when present with the latter. Prior perfusion with them as well as with diethylaminoethanol effectively blocked the positive inotropic action of epinephrine. This antagonism is believed to be non-specific and related to changes which raise the threshold to epinephrine.

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Received July 6, 1954. P.S.E.B.M., 1954, v86.

Life Maintaining Action of 9 Alpha Chlorohydrocortisone Acetate in Adrenalectomized Rats.* (21213)

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Chlorohydrocortisone acetate is 4 times more active than cortisone acetate in promoting deposition of liver glycogen(1). Furthermore, chloro-F-acetate is more active than hydrocortisone or cortisone acetate in extending survival of adrenalectomized rats given one injection(2). Since single injection experiments appraise duration of action rather than daily hormone requirements, a comparison was made of the daily requirements for chloro-F-acetate and cortisone acetate in adrenalectomized rats.

Methods. Immature male rats (Long-Evans strain) were adrenalectomized when 60-65 g. All rats were fed *ad libitum* a diet consisting of 20% casein, 22% dextrose, 25% dextrin, 25% Crisco, 2% corn oil, 2% cellulo flour and 4% Wesson's salt mixture. Each kg of diet was supplemented with 2 g choline chloride and vitamins (Table I). The hormones used were chloro-F-acetate (Squibb)† and cortisone acetate (Merck) in aqueous suspension.

Results. Chloro-F-acetate provided 100% survival for 20 days when given to adrenalectomized rats in 15 μg amounts daily whereas 150 μg of cortisone acetate was required to provide similar protection (Table II). Ten μg and 100 μg daily of the 2 steroids proved inadequate for 100% survival of the adrenalectomized rats suggesting a 10:1 ratio of activity. Preliminary studies indicate that 15-20 μg of desoxycorticosterone acetate† (DCA in oil) will provide 100% survival of adrenal-

TABLE I. Vitamin Supplements/100 g of Diet.

Alpha tocopherol	4.0 mg
Vit. A	900 U.S.P. units
" D	180 *idem*
	mg
2-methyl-1,4 napth-quinone diacetate	1.0
Thiamin HCl	.8
Riboflavin	1.6
Pyridoxine HCl	.8
Niacin	4.0
Calcium pantothenate	4.4
Para aminobenzoic acid	4.0
Inositol	21.6

* Supported by U. S. Public Health Grant A-462.

† Grateful acknowledgement is made to E. R. Squibb & Sons for making the chlorohydrocortisone acetate available and to Ciba Pharmaceutical Products for the desoxycorticosterone acetate (percorten).

TABLE II. Survival of Adrenalectomized Immature Male Rats Treated with Chloro-F-Acetate or Cortisone Acetate.

Daily treatment, mg	No. of rats ADX	Rats alive after 20 days	Avg body wt gain in 20 days, g
	Cortisone acetate		
.10	15	7	2
.15	10	10	13
	Chloro-F-acetate		
.010	11	7	41
.015	10	10	56
.025	10	10	63
.00	20	0	—

ectomized rats on the same diet. Thus, chloro-F-acetate simulated DCA in potency.

Body weight gain exhibited by cortisone acetate-treated rats was only 13 g as compared to the 56 g body weight gain attained on chloro-F-acetate. These data emphasize the lack of correlation between survival and body weight gain.

Longevity of steroid action can be tested by the survival of rats following one injection given the day of adrenalectomy. Previous studies revealed that 1 mg of cortisone acetate had little effect on survival of adrenalectomized immature rats whereas 5 mg doubled the life span(3). For the current study, groups of 10 immature male rats were adrenalectomized and fed the purified diet. Untreated rats survived an average of 7 days whereas one subcutaneous injection of 2.5 mg of cortisone acetate extended survival to 10.5 days. Considering chloro-F-acetate as 10 times more active than cortisone acetate, a .25 mg dosage was used. Chloro-F-acetate provided an 18.2 day average survival indicating a more prolonged action than cortisone acetate and on this basis a greater than 10:1 ratio of activity. One mg of DCA (in oil) had no effect on survival and single injections did not simulate chloro-F-acetate activity as observed with daily injections.

The influence of dietary protein on hormone action was observed in 12 adrenalectomized immature male rats. Rats receiving .25 mg of chloro-F-acetate and fed a protein-free diet survived 12.2 days; 6 days less than rats fed casein but significantly longer than untreated controls. Normal rats readily survive 20 days on the protein free diet.

Hydrocortisone will inhibit the uterine weight increasing action of estradiol(4). Preliminary studies using 1 μg of estradiol benzoate and 2.5 mg of chloro-F-acetate revealed no antagonism.

Summary. The daily dosage requirement of 9 alpha chlorohydrocortisone acetate to permit survival of adrenalectomized rats for 20 days indicates that this compound is 10 times more active than cortisone acetate. Activity was equal to or slightly greater than desoxycorticosterone acetate. A single injection of chloro-F-acetate (.25 mg) provided a longer survival of adrenalectomized rats than cortisone acetate (2.5 mg). Hormone effectiveness was influenced by diet.

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