TABLE II. Passive Transfer of Anaphylactic Sensitivity to Guinea Pigs Using Anti-Bovine Gamma Globulin Rabbit Serum.

Rabbit No.	Optimal ratio	No. of guinea pigs sensitized	Anaphylactic shock			
			Fatal	Severe	Mild	None
I-8	1/128	5	5	0	0	0
I-11	1/64	5	5	0	0	0
I-18	1/128	5	4	0	0	1
I-10	1/64	5	2	1	1	1
H-72	1/128	5	3	0	0	2
P-90	Normal Control Serum	5	0	0	0	5

TABLE III. Passive Transfer of Anaphylactic Sensitivity to Guinea Pigs Using Anti-Horse Rabbit Serum.

Rabbit No.	Optimal ratio	No. of guinea pigs sensitized	Anaphylactic shock		
			Fatal	Severe	None
I-7	1/12	7	5	1	1
I-3	1/16	6	4	0	2
I-17	1/16	6	6	0	0
I-20	1/16	6	5	0	1
H-71	1/16	6	5	0	1
L-22 to L-65	Normal Rabbit Serum	7	0	0	7

or severe anaphylactic shock when challenged with an appropriate intravenous injection of the antigen.

Summary and conclusions. By the use of 3 criteria, namely: the presence of circulating antibodies in the serum; the mode of development of the skin reactions to intradermal injections of antigen, and the passive transfer of anaphylactic sensitivity to guinea pigs; it has been demonstrated that the sensitivity induced in rabbits to foreign proteins while incorporated in Freund's adjuvant is of the usual anaphylactic or foreign protein type. The animals simultaneously develop a tuberculin sensitivity due to the *M. tuberculosis* organisms injected but this in no way alters the quality of the sensitivity developed to the foreign proteins. The persistence of the skin reactions to the higher concentrations of the serum antigens appears to be a reaction to the marked necrosis of tissue that occurs in these highly sensitized animals to the concentrated antigens rather than to any fundamental qualitative difference in the type of reactivity induced in the animals.

Received June 25, 1951. P.S.E.B.M., 1951, v77.

Effects of Oral and Intrasplenic Administration of Cortisone.* (18898)

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It has been found in our laboratories that

* The cortisone acetate suspension was generously supplied by the Merck Corp. through the courtesy of Dr. A. Gibson. We are indebted to Dr. J. J. Schneider, Division of Endocrine and Cancer Research, Jefferson Medical College, for preparing the cortisone hemisuccinate used in this work. The technical assistance of Mrs. M. Sammaritano is gratefully acknowledged.

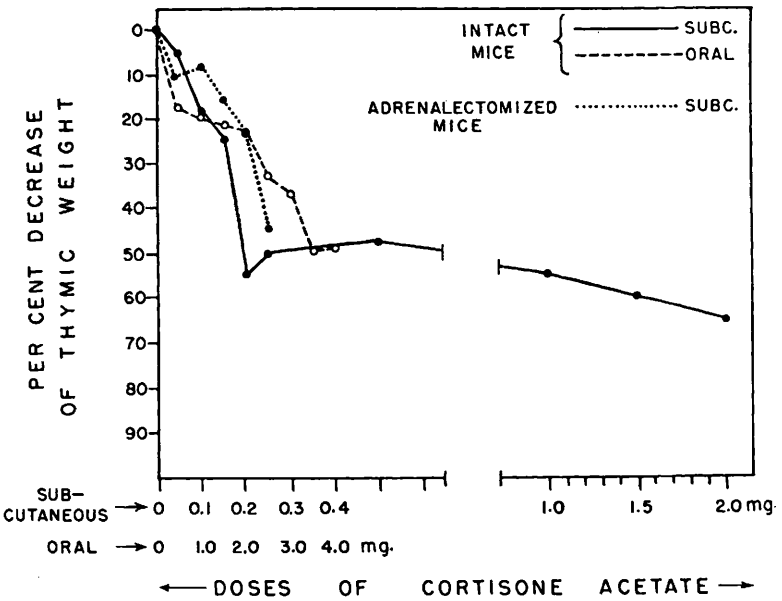

FIG. 1. Effect of a single dose of cortisone acetate on thymic weight of the mouse.

liver(1,2) and other tissues(1) inactivate cortisone *in vitro*. On the other hand, good therapeutic effects have been obtained with oral administration of cortisone in the treatment of rheumatoid arthritis(3-6). It was therefore deemed of interest to compare the effects of subcutaneous, intrasplenic and oral administration of cortisone in the mouse, in order to evaluate the role of the liver in inactivating this compound *in vivo*.

Methods. The effect of commercial cortisone acetate and of cortisone hemisuccinate upon the thymic weight of intact and adrenalectomized male mice (5 to 6 weeks old) was studied. The cortisone hemisuccinate was dissolved in a saline phosphate buffer solution at a pH of 7.4, 0.1 ml of the solution containing 3.2 mg of cortisone hemisuccinate (2.5 mg free cortisone). A comparison was made of (a) intrasplenic and subcutaneous routes and (b) oral (stomach tube) and subcutaneous routes. The weight loss of the thymus offered an accurate quantitative measurable endpoint.

For intrasplenic injection, the mice were anesthetized with ether, and the spleen was exposed by laparotomy. Oral administration was by stomach tube using either a piece of No. 4 ureteral catheter, or polyethylene tubing (.034 inch inside diameter). Because in the intact animal the operation and the passing of the stomach tube respectively could cause an alarm reaction, and a decrease of thymic weight through endogenous cortical hormones, all control animals were subjected to the same procedures receiving 0.85% NaCl intrasplenically, subcutaneously or by stomach tube respectively. The animals were autopsied at the end of 14 hours or at the termination of the experiment, and the wet weight of the thymus

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TABLE I. Effects of Repeated Daily Administration of Oral and Subcutaneous Cortisone Acetate on Thymic Weight of Mice.

Procedure	No. of animals	Body wt (g)	Thymic wt (mg)	Thymic wt (mg/100 g)	% change (thymic wt)	"P"*
A. Control I (0.85% NaCl 0.1 ml S.C. daily × 5 days)	10	18.8	50.9	272.9 ± 20.90	—	—
B. Cortisone acetate (0.25 mg S.C. daily × 5 days)	10	17	6.9	41.1 ± 3.55	-84.9	<.001 (B-A)
C. Control II (0.85% NaCl 0.1 ml daily × 5 days orally)	10	19.3	48.1	247.4 ± 18.14	-9.5	<.4 (C-A)
D. Cortisone acetate (0.25 mg orally daily × 5 days)	10	17.8	20.8	116.4 ± 9.53	-53	<.001 (D-C)

* Students Method, Fisher, R. A., Statistical Methods for Research Work, 4th ed., Edinburgh 1932.

was determined on a Roller-Smith torsion balance. The comparative effects of a single dose and of repeated daily doses when given by the oral and subcutaneous routes were studied.

Results. A. *Oral and subcutaneous administration of cortisone acetate.* Fig. 1 is a dose-response curve of single subcutaneous and oral doses of cortisone acetate upon the thymic weight of male mice at the end of 14 hours. Ten animals were used with each dose. The minimal single subcutaneous dose of cortisone acetate which caused a statistically significant drop in thymic weight was found to be 0.2 mg as compared to 3.0 mg when the compound was administered by the oral route. Thus, under the conditions of our experiment, a single dose of cortisone acetate was found to be about 10 to 15 times as effective when administered by the subcutaneous as compared with the oral route.

The sensitivity of adrenalectomized mice to a single subcutaneous dose of cortisone acetate did not differ from that of intact mice.

The effects of administration of daily oral and subcutaneous doses of cortisone acetate for a period of 5 days are illustrated in Table I. Whereas cortisone acetate was effective when administered by either route, the thymic involution was more marked in those animals receiving cortisone by subcutaneous injection. It is interesting to note that the total oral cortisone (1.25 mg) administered in this experiment was not effective when given as a single dose.

B. *Oral and intrasplenic administration of cortisone acetate and cortisone hemisuccinate.* (Table II). A single intrasplenic injection of 2.5 mg of cortisone acetate (= 2.21 mg cortisone) was as effective as the same dose given subcutaneously. Histological examination of the livers of the animals receiving this large dose of cortisone acetate intrasplenically revealed extensive focal necrosis. Therefore the effect of a buffered saline solution of cortisone hemisuccinate containing 3.2 mg of cortisone hemisuccinate (= 2.5 mg cortisone) per 0.1 ml of solution was studied. There was no difference in the thymic response following the intrasplenic administration of this compound as compared with the subcutaneous route. When a smaller dose of cortisone acetate (0.25 mg) was used, no effect on the thymic weight was observed following the intrasplenic administration, but a significant drop followed the subcutaneous injection of the same dose. Histological studies of the livers of these animals failed to reveal any pathological changes.

Discussion. Early experimental work by Heilman and Kendall(7) indicated that cortisone extracted from adrenal cortical tissue was physiologically active when fed to small laboratory animals. These investigators found that lymphosarcoma tumors failed to develop and well-developed tumors regressed rapidly in mice receiving 0.3 mg of Compound E daily in their drinking water. Recently, Emerson (8) has reported the effects of oral and subcutaneous cortisone on the development of experimental lymphosarcoma, the volume of the tumor following the administration of a single dose by either route being used as an

7. Heilman, F. R., and Kendall, E. C., *Endocrinology*, 1944, v34, 416.

8. Emerson, G. A., *Trans. N. Y. Acad. Sciences*, 1951, v13, 92.

TABLE II. Effects of Subcutaneous, Intrasplenic and Oral Administration of Cortisone (Single Dose) on Thymic Wt of Mice (14 Hr).

Exp No.	Procedure	No. of animals	Body wt (g)	Thymic wt (mg)	Relative Thymic wt (mg/100 g body wt)	% change of relative thymic wt	"p"*	
1.	A. 0.85% NaCl 0.1 ml subcutaneously (control 1)	10	18.6	73.2	393 ± 20.37			
	B. Cortisone Ac. (2.5 mg)† subcutaneously	10	19.6	43.8	222.1 ± 18.59	−43.4	<.05	(A-B)
	C. 0.85% NaCl 0.1 ml intrasplenically (control 2)	10	16.4	55.7	337.5 ± 33.41	−9	<.2	(A-C)
	D. Cortisone Ac. (2.5 mg)† intrasplenically	10	17.3	35.8	210.4 ± 29.05	−37.6	<.01	(C-D)
2.	A. 0.85% NaCl 0.1 ml subcutaneously	10	19.7	55.6	280.6 ± 19.67			
	B. 0.85% NaCl 0.1 ml intrasplenically (control)	10	18.5	51.2	277.3 ± 14.49		>.9	(A-B)
	C. Cortisone hemisucc. (3.2 mg)‡ subcutaneously + 0.85% NaCl 0.1 ml intrasplenically	10	18.3	38.9	211.8 ± 25.05	−23.6	<.05	(B-C)
	D. Cortisone hemisucc. (3.2 mg)‡ intrasplenically 0.85 % NaCl 0.1 ml subcutaneously	10	19	38.7	202.7 ± 23.21	−26.9	<.02	(B-D)
3.	A. 0.85% NaCl 0.1 ml subcutaneously	10	19.5	60.9	313.1 ± 22.50			
	B. Cortisone Ac. (0.25 mg) subcutaneously	9	20.6	38	183.3 ± 9.96	−41.4	<.001	(A-B)
	C. Cortisone Ac. (0.25 mg) orally	10	18.2	51.6	287.2 ± 30.09	−8.2	<.2	(A-C)
	D. Cortisone Ac. (0.25 mg) intrasplenically	10	19.7	66.5	339.1 ± 30.53	+8.5	<.5	(A-D)

* Student's Method, Fisher, R. A., Statistical Methods for Research Work, 4th ed., Edinburgh 1932.
† Equivalent to 2.21 mg free cortisone.
‡ Cortisone hemisuccinate solution 0.125 cc = 3.2 mg of cortisone hemisuccinate = 2.5 mg of free cortisone (phosphate buffer pH = 7.4).

index of cortisone activity. She found cortisone to be 5 to 10 times more active by the subcutaneous than by the oral route. Our results are in agreement with her findings, in as much as we found the minimal effective oral dose to be about 10 to 15 times that of the parenteral.

In our experiments small doses of cortisone, which exert a significant thymolytic effect when given by subcutaneous route, are ineffective either by intrasplenic or oral route. This suggests that the inactivation of cortisone by liver tissue previously observed *in vitro*(1,2) also occurs *in vivo*. With larger doses of cortisone, limitations of the capacity of the liver to inactivate the compound may permit enough to pass into the systemic circulation to produce a maximal effect upon the thymus.

The possible role of extrahepatic tissues in the inactivation of cortisone *in vivo* cannot be evaluated at present. The occurrence of this phenomenon in *in vitro* experiments has been demonstrated(1).

Clinical observations with orally administered cortisone have been reported by Freyberg(3), Hench(4), Boland and Headley(5), and Engleman *et al.*(6). In some cases the oral dose necessary to obtain a clinical response appeared to equal the intramuscular dose, whereas in others oral doses had to be up to 20 to 30% larger than intramuscular doses. Our studies on the effects of small daily doses of cortisone upon thymic weight duplicate clinical methods of administration. The animals receiving the drug subcutane-

ously showed a higher degree of thymic involution but oral administration was also quite effective. The fact that the total amount of orally administered cortisone was ineffective when given as a single dose suggests that a cumulative effect may be involved in the physiological action of cortisone.

Summary. (1) Thymic weight loss in 14 hours offers an accurate quantitative endpoint by means of which to measure the action of cortisone when administered by different routes. (2) Under conditions of our experiment, cortisone was much more effective when administered by subcutaneous route as compared with the oral or intrasplenic route. The subcutaneous:oral ratio is about 1:15. (3) Sensitivity of adrenalectomized mice to the thymolytic action of cortisone did not differ from that of intact animals. (4) Administration of small doses of cortisone, which caused thymic involution when given by subcutaneous route, failed to do so when given by intrasplenic or oral route. This suggests that inactivation by the liver, previously found in *in vitro* experiments, also plays a role *in vivo*. If the dose administered through the portal routes is large, sufficient amounts escape inactivation to produce a maximal effect upon the thymus.

Received June 25, 1951. P.S.E.B.M., 1951, v77.

Effect of ACTH and Cortisone on Phagocytosis. (18899)

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The experiments to be reported represent an attempt to determine the effect of adrenocorticotrophic hormone (ACTH) or cortisone treatment on the activity of the polymorphonuclear neutrophilic leukocyte in man. This study was prompted by the occasional progression of infection, possibly due to impaired phagocytic function, in patients undergoing treatment with these agents. For example, bacteremia with extensive abscess formation, without the usual systemic and local reactions, has been observed here and elsewhere. Some of these occurrences are cited by Mote(1). The general plan of the investigation was to use whole defibrinated blood from patients before and after treatment as a source of leukocytes, combined with virulent type I pneumococci and anti-type I pneumococcus rabbit serum. The organisms and antiserum were presumably unvarying, and any differences in phagocytic activity could be attributed to an effect, either direct or indirect, of the drugs on the leukocytes.

Methods and materials. The patients under examination were suffering from a variety of conditions which are indicated in Table I. A blood sample was obtained, defibrinated with glass beads, and tested before treatment was begun, and again after 4 to 21 days of therapy. Blood was taken at the same time each day; the patient was still under treatment when the last specimen was taken. The type I pneumococcus was maintained in cooked meat medium in the refrigerator for the duration of the experiment and no mouse or other passage of the organism took place. Ten to 12 hour cultures were made from this stock in 2% Bacto-tryptose, 0.5% glucose, 0.5% NaCl, 10% horse serum broth. The organisms were then neutralized to about pH 7.6, sedimented and suspended in 0.85% saline. The turbidity of the suspension in a 13 mm inside diameter cuvette was adjusted to 35% transmission at a wave-length of 620 Å in a Coleman Junior spectrophotometer. The antiserum stored in the frozen state was all of the same lot, small portions being thawed and diluted in 0.85% saline as needed.

Screw-cap vials, 6 x 1 cm, were loaded with 0.5 ml of blood, 0.1 ml of organism suspension and 0.1 ml of varying 2-fold dilutions of anti-

1. Proc. 1st Clin. ACTH Conf., edited by John R. Mote, Blakiston Co., 1950.