about 3 times the dose (Fig. 3). This response may be blocked by atropine. Morphine sulphate produces no bronchoconstriction in doses of up to 250 γ.

Bronchodilators have little or no action on the standard preparation, presumably because the bronchi are maintained maximally dilated by the epinephrine. Even epinephrine, in doses up to 5 γ, produces only a slight and very temporary effect. When superimposed on a typical response to histamine, however, epinephrine has a marked effect in accelerating recovery (Fig. 2); papaverine and theophylline also show some dilator activity in rather large doses (250 γ and 500 γ respectively). As mentioned earlier, epinephrine also produces a well marked effect on a preparation newly set up, and if epinephrine is omitted from the perfusion fluid, marked sensitivity is shown to bronchodilators.

Summary and Conclusions. A technic for the observation and measurement of the effects of drugs on bronchial resistance in an isolated perfused lung preparation is described. This preparation shows an absolute sensitivity to histamine comparable to commonly used biological assay objects, and shows a reproducibility adequate for assay purposes. Its simplicity makes it suitable also for teaching demonstrations of the effect of drugs on the bronchioles.

1. Bayliss, L. E., and Ogden, E., *J. Physiol.*, 1933, v77, 34P.

2. Zucker, M. B., *Am. J. Physiol.*, 1944, v142, 12.

3. Gaddum, J. H., Hebb, C. O., Silver, Ann and Swan, A. A. B., *Quart. J. Exp. Physiol.*, 1953, v38, 255.

4. Daly, I. deB., *J. Physiol.*, 1928, v65, 422.

5. ———, *ibid.*, 1930, v69, 238.

6. Berry, J. L., and Daly, I. deB., *Proc. Roy. Soc.*, 1931, v109B, 319.

7. Alcock, P., Berry, J. L., and Daly, I. deB., *Quart. J. Exp. Physiol.*, 1936-7, v26, 13.

8. Dale, A. S., and Narayana, P., *ibid.*, 1935, v25, 85.

9. Binet, L., and Bargeton, D., *J. Physiol. Path. Gen.*, 1939-40, v37, 1217.

10. Nisell, O. I., *Acta Physiol. Scand.*, 1950, v21, Supp. 73.

11. Sollmann, T. and von Oettingen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1927, v25, 692.

12. Seibert, R. A., and Handley, C. A., *J. Pharmacol.*, 1954, v110, 304.

Received January 13, 1956. P.S.E.B.M., 1956, v91.

Potentiation of Glycogenolytic Effect of Epinephrine by Desoxycorticosterone in Certain Skeletal Muscles.* (22237)

JOAN N. TAYLOR AND SAMUEL L. LEONARD.

Department of Zoology, Cornell University, Ithaca, N. Y.

The glycogenolytic effect of epinephrine on skeletal muscle (hind limb) is inhibited by cortisone acetate in normal rats(1), by adrenal cortical extract in adrenalectomized rats(2), and by growth hormone in hypophysectomized rats(3). On the other hand, adrenalectomy(2,4) or the injection of thyroxin(3,4) potentiates the glycogenolytic action of epinephrine and thyroidectomy depresses it(4). Those hormones which inhibit this effect of epinephrine also induce a storage of glycogen in these skeletal muscles (glycopexic effect)(1,2,3,5). While it is usually stated that desoxycorticosterone does not stimulate glycogen storage in muscle(5-8), there is no information on whether this mineral-corticoid influences epinephrine effects on carbohydrate metabolism in the intact animal. This report will show that desoxycorticosterone acetate (DOCA) potentiates the glycogenolytic action of epinephrine in certain skeletal muscles.

* Aided by grant from Muscular Dystrophy Assn. of America, and Sage and Sackett Research Funds of Department of Zoology, Cornell University.

Materials and methods. Adult female rats of the Long-Evans strain were selected for uniformity of age and weight within each ex-

periment. The animals were fed a diet consisting of calf meal and dog pellets *ad libitum*. The adrenalectomized animals received 1% salt solution in addition to drinking water. Subcutaneous injections of 2 mg DOCA† per day in peanut oil were administered for 3 days prior to autopsy. Hormone treatment was begun one week after adrenalectomy and 3 weeks after thyroidectomy. Epinephrine was injected intraperitoneally in doses of 10 γ per 100 g body weight one hour prior to autopsy. In all experiments the rats were starved for 24 hours prior to the administration of epinephrine. The muscles used in this study were the *rectus femoris,* a portion of the abdominal muscles and the diaphragm. The method for anesthetizing the animals, removing the muscles, and determining glycogen has been previously described(9). The glycogen is reported as mg of glucose per 100 g of muscle, wet weight (averages with the standard error of the mean).

Results. The injection of DOCA into normal intact rats had no significant effect on the glycogen content of any of the muscles used in this study (Table I-A), which confirms earlier work(5-8). The injection of epinephrine into the normal rats decreased the glycogen content in all 3 muscles significantly. When the rats were pretreated with DOCA, however, epinephrine induced a greater loss of glycogen in the leg and abdominal muscles as compared to the loss observed in the untreated rats. There was no potentiation of the epinephrine effect in the diaphragm. If one compares the loss of glycogen expressed as a percentage of the respective initial levels in the DOCA-treated and control animals, it is noted that the greatest potentiation of epinephrine occurred in the leg muscle (44% *vs* 23% loss), with an intermediate response in the abdominal muscle (62% *vs* 46%).

In view of the previous finding that glucocorticoids inhibited the glycogenolytic action of epinephrine in some muscles(1,2), it was considered possible that the administration of DOCA suppressed the endogenous secretion of the gluco-corticoids via the pituitary(10) which would essentially remove an inhibitor to the action of epinephrine. To test this possibility, the effect of DOCA was studied in adrenalectomized rats.

The data in Table I-B show that adrenalectomy alone had no significant effect on the glycogen levels of the 3 muscles when compared to the intact controls. Following the administration of epinephrine, a significant decrease in muscle glycogen was exhibited by all 3 muscles. Furthermore, the loss of glycogen was greater in the adrenalectomized rats than in the intact rats but only in the leg muscle. Pretreatment of the adrenalectomized rats with DOCA caused a significant increase in glycogenolysis only in the leg muscle.

Collip *et al.*(4) and Leonard(3) have reported that thyroxin potentiates the glycogenolytic action of epinephrine in hypophysectomized animals. This finding introduces the possibility that the potentiating effect of DOCA might be conditioned by the thyroid hormone: accordingly, the effect of DOCA on glycogenolysis was investigated using thyroidectomized rats.

The data show (Table I-C) that thyroidectomy alone causes no appreciable decrease in muscle glycogen. Administration of epinephrine to the thyroidectomized rats caused no significant decrease in the glycogen levels of the *rectus femoris* or abdominal muscles; the diaphragm exhibited a significant decrease. Pretreatment of the thyroidectomized animals with DOCA failed to increase the glycogenolysis induced by epinephrine.

Progesterone is reported to have properties similar to DOCA in regard to salt and water retention(11) and also to its inability to stimulate the deposition of glycogen in muscle(12,13). Accordingly, an experiment was made to determine whether progesterone, like DOCA, would potentiate the glycogenolytic action of epinephrine.

The data in Table I-D show that intact rats pretreated with 2 mg progesterone per day for 3 days exhibited no significant increase in glycogenolysis above that obtained with epinephrine alone.

† Desoxycorticosterone acetate and progesterone were donated by Schering Corp., Bloomfield, N. J.

TABLE I. Effect of DOCA and Progesterone on Epinephrine Induced Glycogenolysis.

Exp.	No. of rats	Glucose (mg/100 g muscle) *Rectus femoris*	Abdominal	Diaphragm
Part A—Intact rats				
Control	16	333 ± 13	428 ± 11	285 ± 7
DOCA treated	6	321 ± 15	424 ± 17	306 ± 11
E† treated	15	256 ± 6	233 ± 10	107 ± 8
E & DOCA treated	10	178 ± 9*	160 ± 14*	111 ± 9
Part B—Adrenalectomized rats				
Control	10	339 ± 10	377 ± 12	313 ± 17
E treated	15	228 ± 5	203 ± 9	122 ± 8
E & DOCA treated	14	169 ± 9*	183 ± 10	96 ± 8
Part C—Thyroidectomized rats				
Control	4	309 ± 27	377 ± 21	269 ± 13
E treated	12	293 ± 15	281 ± 26	122 ± 16
E & DOCA treated	12	269 ± 17	291 ± 22	132 ± 20
Part D—Intact rats				
P† treated	5	352 ± 10	430 ± 13	246 ± 21
P & E treated	10	251 ± 9	198 ± 13	86 ± 21

* In all cases where DOCA increased glycogenolytic effect of epinephrine significantly ($P < .01$), results are marked with an *.
† E = Epinephrine; P = Progesterone.

Discussion. These results show that the mineral-corticoid, DOCA, in some manner enhances the glycogenolytic action of injected epinephrine in the *rectus femoris* and the abdominal muscles, but not in the diaphragm. In contrast, the gluco-corticoid, cortisone, inhibited this effect of epinephrine to the greatest extent in the leg muscle, less in the abdominal muscles and not at all in the diaphragm(1). There is a basic difference in sensitivity to epinephrine among these three muscles in the untreated rat; the diaphragm is the most sensitive and the *rectus femoris* the least.

The possibility that DOCA was repressing the secretion of the adrenal gluco-corticoids via pituitary ACTH, thus removing an inhibitor to the action of epinephrine, was discounted when it was observed that DOCA potentiated glycogenolysis in the adrenalectomized animal. This suggests that DOCA is acting directly on the muscle to potentiate epinephrine.

At first sight it appeared that some interrelationship with the thyroid gland was involved, since DOCA did not potentiate epinephrine in the thyroidectomized animal. However, the loss of thyroid activity in an animal is known to decrease the absorption of injected epinephrine(14) and this decrease could be of such magnitude that any effect of DOCA would be unobservable. It is reported that DOCA increases the absorption of electrolytes from the intestine in adrenalectomized rats(15) and increases the permeability of the skin(16). If DOCA, in potentiating epinephrine, acts by increasing the permeability of the target organ to the passage of epinephrine, adequate thyroid secretion may be essential for this effect. It seems more likely that thyroidectomy acts by preventing absorption of the epinephrine at the injection site.

A concept of mutual antagonism between the 2 types of adrenal cortical steroids on certain stress reactions and on the specificity of their antagonism to definite target organs has recently been discussed by Selye(17). It may be that the normal maintenance and regulation of glycogen storage in skeletal muscle is dependent in part on the relative endogenous production of the two types of adreno-corticoids and epinephrine. There is little evidence at present to indicate how much the normal output of epinephrine affects the level of muscle glycogen in the intact animal. Bowman(18) has shown that in the hypophysectomized animals which lack the glycopexic hormones, removal of the whole adrenal gland induces a rise in the glycogen level in fasting rats. The increase in muscle glycogen would seem to be caused by the elimination of endogenous epinephrine.

Finally, it was observed that progesterone was unable to potentiate the glycogenolytic action of epinephrine.

Summary. Desoxycorticosterone acetate increased the glycogenolytic action of epinephrine in the *rectus femoris* and abdominal muscles but not in the diaphragm in intact rats. In adrenalectomized rats, the effect was limited to the *rectus femoris*. Thyroidectomy decreased epinephrine induced glycogenolysis, and DOCA was unable to nullify this inhibi-

tory effect. Progesterone had no effect on the glycogenolytic action of epinephrine.

1. Wortman, L. C., and Leonard, S. L., *Endocrinology,* 1953, v53, 480.
2. Winternitz, W. W., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 683.
3. Leonard, S. L., and Ringler, I., *Endocrinology,* 1954, v55, 212.
4. Collip, J. B., Thomson, D. L., and Toby, G., *J. Physiol.,* 1936, v88, 191.
5. Leonard, S. L., *Endocrinology,* 1953, v53, 226.
6. Long, C. N. H., Katzin, B., and Fry, E. G., *ibid.,* 1940, v26, 309.
7. Reinecke, R. M., and Kendall, E. C., *ibid.,* 1943, v32, 505.
8. Winter, C. A., and Thomson, J. D., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 95.
9. Leonard, S. L., *Endocrinology,* 1952. v51, 293.
10. Cheng, C., and Sayers, G., *ibid.,* 1949, v44, 400.
11. Thorn, G. W., and Harrop, G. A., *Science,* 1937, v86, 40.
12. Corey, E. L., *Am. J. Physiol.,* 1941, v132, 446.
13. Unpublished data from this laboratory.
14. Russell, J. A., and Cori, G. T., *ibid.,* 1937, v119, 167.
15. Stein, L., and Wertheimer, E., PROC. SOC. EXP. BIOL. AND MED., 1942, v46, 172.
16. Opsahl, J. C., *Yale J. Biol. and Med.,* 1948, v21, 255.
17. Selye, H., *Science,* 1955, v122, 625.
18. Bowman, R. H., Doctoral Thesis. Cornell University, 1952.

Received January 16, 1956. P.S.E.B.M., 1956, v91.

Inhibition Studies with *Streptococcus faecalis.* Acridines and Acridones. (22238)

AARON ARNOLD AND SHIRLEY D. SOBELL. (Introduced by M. L. Tainter.)
Sterling-Winthrop Research Institute, Rensselaer, N. Y.

The investigations of Woods(1) led to the finding that the sulfonamides owed their chemotherapeutic activity to their property of interfering with the utilization of p-aminobenzoic acid by the microbial cell. This observation has stimulated the synthesis and testing of compounds related closely in structure to known metabolites for trial in the controlled interference with selected metabolic reactions. In the instance of folic acid, this has given rise to a series of folic acid blocking agents(2), some of which have proven usefulness(3), though limited, in a variety of ways. The interference with the utilization of folic acid was applied by Hitchings, among others, to develop compounds of biological interest. As a result Hitchings and his associates observed(4,5) that the rather active anti-folic, pyrimethamine, appeared also to possess some promise against the malarial parasite, though continued study may have modified this view somewhat(6,7).

In view of the findings that compounds which interfere with the utilization of folic acid may have application in other disorders for which agents are needed, a reinvestigation of 53 available acridines(8,9) and acridones (10), prepared initially for testing against the malarial parasite, was undertaken.

Methods. The folic acid assay medium utilized in this study was slightly modified from that of Teply and Elvehjem(11). The modifications consisted of omitting the xanthine and of utilizing "vitamin free" casein hydrolysate (Nutritional Biochemicals) in place of the charcoal treated hydrolysate originally suggested. When folic acid was added in amounts up to 1.0 mμg per ml of medium the acid production upon titration with N/10 alkali went up from blanks of about 0.5 ml of alkali per tube to 9.5 to 10 ml of alkali per tube at the higher levels of added folic acid. The test compounds were added to tubes containing 0.32 mμg of folic acid per ml of medium, a level designed to support half maximum growth as judged by acid production. This usually ranged about 4.5 to 5.0 ml of N/10 alkali per tube. For the assays, the test compounds were added to duplicate tubes usually at 4 levels of test: 1.6, 8,