

Farrant(4), in the capsule. In the sections of the organisms in mitosis an occasional spindle-like structure can be observed, as well as what appears to be a double membrane at points of division.

Discussion. Use of electron-dense antibody conjugates presents a new method of observing immune reactions at subcellular level in formalin-fixed preparations. Heretofore formalin fixation has been used infrequently in electron microscopy, as it was believed to reduce ability to obtain good resolution of cellular material. However, the electron-dense material, in what is considered as nuclear region, is well demonstrated in our sections. It has the further advantage of rapidly inactivating any infectious agents used. Although formalin fixation may not be as satisfactory for other tissues as it apparently is for staphylococci, it appears warranted to further investigate its usage in electron microscopy. As unbound ferritin conjugate was completely removed by simple washing, the problem of nonspecific reactions with the immunofluorescent technic was not encountered. This will permit a more critical evaluation of specificity of immune reactions, a problem which has been most troublesome in formalin-

fixed tissues. It should now be possible to localize definitely the sites of antigen production as well as viral aggregates in tissues that have been properly fixed with formalin as well as other material at the subcellular level. The number of ferritin molecules around bacteria could probably be increased by changing ratio of antigen-antibody in the reaction.

Summary. A method is described by which immune systems can be studied at subcellular level with the electron microscope, using a ferritin-antibody conjugate. A conjugated immune serum to *Staphylococcus aureus* was applied to homologous organisms, embedded in methacrylate with uranium and thin sections cut. An antigen-antibody reaction was detected by attachment of the ferritin molecule in the capsule-like material. Use of formalin in primary fixation for electron microscopy as well as nonspecific immunofluorescence is discussed.

1. Singer, S. J., *Nature*, 1959, v183, 1523.
2. Hcbs, B. C., *J. Hyg.*, 1948, v46, 222.
3. Ward, R. T., *J. Histochem. and Cytochem.*, 1958, v6, 398.
4. Farrant, J. L., *Bioclinica et Biophysica acta*, 1954, v13, 569.

Received April 1, 1960. P.S.E.B.M., 1960, v104.

Effect of 5-Hydroxytryptamine on Phosphorylase and Glycogen Levels in Muscle Tissue.* (25828)

SAMUEL L. LEONARD AND HOLLIDAY T. DAY
Dept. of Zoology, Cornell University, Ithaca, N. Y.

Although the isolated uterus of the estrous rat is frequently employed for bio-assay of certain psychopharmacological agents and hormones, little is known concerning the effects of these substances, particularly the former, on glycogen stores and enzyme phosphorylase in uterine muscle. Epinephrine and oxytocin, however, induce a rapid decrease in both uterine glycogen(1) and phosphorylase activity(2). The effects of serotonin (5-OH-

tryptamine), lysergic acid and reserpine on these 2 constituents in uterine smooth muscle were investigated. Since serotonin alone produced changes in smooth muscle, its effect was studied also on skeletal, cardiac, and diaphragm muscle.

Methods. Adult female rats were spayed, 7-9 days later injected with 50 μ g of estradiol benzoate (Schering Co.) subcutaneously and 48-56 hours later given an intraperitoneal injection of the test compound. Uteri were removed 30-60 minutes later from the anesthetized rats and were frozen and stored as de-

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

TABLE I. Phosphorylase Activity in Uterine Smooth Muscle, Diaphragm and Heart.

Treatment*		Exp.			Control		
		P-lase <i>a</i>	P-lase <i>t</i>	<i>a/t</i> × 100	P-lase <i>a</i>	P-lase <i>t</i>	<i>a/t</i> × 100
Uterus							
Serotonin	(12)	35 ± 2	96 ± 3	36.4 ± 1.0	74 ± 2	116 ± 2	64.0 ± 1.1
Reserpine	(9)	44 ± 2	136 ± 7	33.6 ± .8	46 ± 3	134 ± 5	34.7 ± 2.1
LSD	(12)	62 ± 3	130 ± 2	47.9 ± 2.9	69 ± 3	128 ± 4	54.3 ± 1.7
Diaphragm							
Serotonin	(10)	119 ± 5	150 ± 3	79.4 ± 1.8	119 ± 3	164 ± 7	72.9 ± 1.4
" & electr. stim.	(8)	33 ± 6	94 ± 7	35.0 ± 5.5	36 ± 4	94 ± 4	39.2 ± 3.6
Epinephr. & " "	(8)	58 ± 5	96 ± 5	60.7 ± 2.7	44 ± 3	108 ± 5	40.5 ± 1.2
Heart							
Serotonin	(16)	126 ± 9	194 ± 4	64.6 ± 3.2	126 ± 7	189 ± 6	66.4 ± 2.0

* Details in text.

Figures in parentheses = No. of rats.

scribed(2). Diaphragms were obtained from male rats, cardiac muscle from both sexes, and all tissues were quickly frozen upon removal. To produce tetanic contraction of the diaphragms prior to removal, electrodes were placed on posterior surface of half of exposed diaphragm of the anesthetized rat while passing a current from electronic stimulator for 20 seconds (frequency 60/sec, duration 1 msec, 10 volts). Serotonin creatine sulfate (California Corp. for Biochem. Res), reserpine (Ciba Pharm. Co.), lysergic acid diethylamide (LSD, Sandoz Pharm. Co.), and l-epinephrine-HCl (Parke Davis Co.), were diluted in water and given in 1 ml volume, intraperitoneally, in doses/100 g body weight. Details of methods of analysis are given; for glycogen(1,3), for uterine (smooth muscle) phosphorylase(2) and for striated muscle phosphorylase(4). Active phosphorylase *a* and total phosphorylase *t* (with added adenylic acid) are reported as $\mu\text{g P}$ liberated from glucose-1- PO_4 in 10 minutes at 37°C by 40 mg of uterine tissue, 2.5 mg of diaphragm or 10 mg of cardiac tissue. Results are presented as means $\pm$ standard error.

Results. Doses of serotonin sufficient to alter either glycogen or phosphorylase levels were considerably in excess of those used for *in vitro* pharmacological testing. Groups of 4 female rats, pretreated as described, were injected with the following doses of serotonin: 0.1, 0.25, 0.5, 1.0 mg and, after 30 minutes, uterine glycogen levels in mg % were respectively, 291 $\pm$ 15, 217 $\pm$ 16, 207 $\pm$ 17 and 153 $\pm$ 40 as compared to 307 $\pm$ 7 mg % in

controls. To determine if a change in phosphorylase activity would accompany this decrease in glycogen, similarly prepared rats were given 0.5 mg of serotonin and, after 30 minutes, the tissues assayed. Table I shows that a marked decrease in active phosphorylase *a* and activity ratios occurred. When reserpine and LSD (0.5 mg dose each) were injected into the prepared female rats, within 1 hour, uterine glycogen level was unaltered and phosphorylase activity was unchanged as noted (Table I). The relatively low activity ratios in controls of experiments with reserpine indicate again the variability encountered between successive experiments(2). Phosphorylase *a* levels in these controls are about twice that found if estrogen had not been given and sufficiently high to permit a lowering of enzyme activity under the proper conditions.

Examination of leg muscles in several rats for changes in either glycogen or phosphorylase revealed that these 3 substances were without effect. A report that serotonin increased uptake of P^{32} in the rat diaphragm (5) suggested a study of this muscle. Serotonin (0.5 mg dose) lowered glycogen levels of diaphragms in male rats from 314 $\pm$ 18 to 246 $\pm$ 19 mg % in 30 minutes (12 rats, previously starved for a day). When 1 mg doses of serotonin were injected into fed male rats and diaphragms assayed 1 hour later, glycogen decreased from control level of 626 $\pm$ 28 to 391 $\pm$ 16 mg % (12 rats). There was no change in active phosphorylase and only a

slight rise in activity ratios in diaphragms of these rats (Table I).

Except for negative results on phosphorylase levels in the diaphragm, the effects of serotonin were suggestive of those of epinephrine. A more sensitive method for demonstrating epinephrine effects on skeletal muscle phosphorylase requires that muscles of experimental and control animals be electrically stimulated prior to removal(6). To further ascertain that serotonin had no effect on phosphorylase in the diaphragm, the muscle was subjected to this more sensitive assay procedure. Preliminary experiments were made in which doses of 20 μ g of epinephrine were given to rats 30 minutes before placing the electrode on the center of the right half of the diaphragm and stimulating for 20 seconds. While the right half was in tetanic contraction, the left half almost always continued its normal respiratory movements (only the right portion assayed). Control rats were treated similarly but without epinephrine. Results in Table I show that epinephrine inhibited the expected decrease in active phosphorylase and maintained activity ratios 50% higher than in controls. When rats were injected with 1 mg doses of serotonin and stimulated as above, phosphorylase activity was not maintained at a level higher than that in controls.

In studying heart muscle, 1 mg doses of serotonin were given to 8 fed rats (4 of each sex) and after 1 hour, the rats were autopsied. An equal number of controls of both sexes were used in the determinations. No sex difference was noted and the results were pooled. Table I shows that serotonin did not alter phosphorylase activity but, surprisingly, heart glycogen increased from a control level of 465 ± 27 to 728 ± 47 mg %.

Discussion. Serotonin, but not reserpine or LSD, lowered uterine glycogen and phosphorylase, both of which are located essentially in the myometrium(7). Although LSD *in vitro* antagonizes serotonin induced uterine contractions(8) and produces overt behavior in the rat, neither smooth nor skeletal muscles were affected. LSD affects carbohydrate metabolism in other ways and in other target organs(9) but there is little in-

formation on *in vivo* effects in muscle. The same may be said for serotonin(10) although it produces hyperglycemia without affecting skeletal muscle glycogen(11). High levels of serotonin in the uterus have been produced *in vivo* by administering the precursor, 5-hydroxytryptophan, without inducing abortion or altering uterine activity(12) but it is likely that much higher levels of serotonin are required to affect the glycogen.

Induced changes in uterine smooth muscle glycogen and phosphorylase have so far always been positively correlated(1,2). Acute changes in glycogen in other tissue, (liver, skeletal muscle, heart) have been correlated with changes in phosphorylase but not necessarily in the same direction(6,13,14,15). However, the effect of serotonin was unique, it lowered diaphragm glycogen, elevated cardiac glycogen and in both instances failed to alter phosphorylase activity. Phosphorylase activity may not be rate limiting in all instances of acute changes in tissue glycogen(13). The increase in heart glycogen following serotonin may be the result of increased mobilization and utilization of fatty acids. Preferential utilization of fatty acids over glucose would permit rapid accumulation of glycogen without necessarily requiring a concurrent increase in phosphorylase activity (summary ref. 16). Epinephrine increases cardiac glycogen and phosphorylase activity(14) thus differing from serotonin. Serotonin is also reported to increase phosphorylase and glycogenolysis in the sheep liver fluke(17).

Summary. Serotonin, but not reserpine or lysergic acid (LSD), when given intraperitoneally to rats, decreased uterine (smooth muscle) glycogen levels and phosphorylase activity. Serotonin rapidly decreased glycogen concentration in the diaphragm, rapidly elevated glycogen in heart muscle and in neither instance altered phosphorylase activity. Thus it is possible to produce acute changes in glycogen stores in some tissues without evoking alterations in phosphorylase activity.

1. Kostyo, J. L., Leonard, S. L., *Endocrinology*, 1955, v56, 616.

2. Leonard, S. L., *ibid.*, 1958, v63, 853.

3. ———, *ibid.*, 1952, v51, 293.

4. ———, *PROC. SOC. EXP. BIOL. AND MED.*, 1957, v96, 720.
5. Lingjaerde, P., Skaug, O. E., *J. Biol. Chem.*, 1957, v226, 33.
6. Leonard, S. L., *Endocrinology*, 1957, v60, 619.
7. Bo, W. J., *Anat. Rec.*, 1959, v133, 252.
8. Gaddum, J. H., *Ann. N. Y. Acad. Sci.*, 1957, v66, 643.
9. Bain, J. A., *ibid.*, 459.
10. Page, I. H., *Physiol. Rev.*, 1958, v38, 277.
11. Gordon, P., Morris, L., *The Physiologist*, 1959, v2, 47.
12. Davidson, J., Sjoerdsma, A., Loomis, L. N., Udenfriend, S., *J. Clin. Invest.*, 1957, v36, 1594.
13. Sutherland, E. W., *Ann. N. Y. Acad. Sci.*, 1951, v54, 693.
14. Hess, M. E., Haugaard, N., *J. Pharm. Exp. Therap.*, 1958, v122, 169.
15. Leonard, S. L., Wimsatt, W. A., *Am. J. Physiol.*, 1959, v197, 1059.
16. Bowman, R. H., *ibid.*, 1959, v197, 1017.
17. Mansour, T. E., *Sympos. on Catecholamines, Am. Soc. Pharm. Exp. Therap.*, 1959, pg. 465.

Received April 5, 1960. P.S.E.B.M., 1960, v104.

Role of the Pulsatile Thrust of the Aorta in Genesis of Gastric Ulcer.* (25829)

WARD O. GRIFFEN, JR., DEMETRE NICOLOFF, ALDO CASTANEDA, NELSON H. STONE
AND OWEN H. WANGENSTEEN

Dept. of Surgery, University of Minnesota Medical Center, Minneapolis

A jet stream of gastric juice obtained by overnight aspiration from the stomach of patients with duodenal ulcer, projected intermittently over a period of hours, at an ejection pressure of 30 cm of H₂O will produce ulceration in the duodenum of rabbits quite regularly(1). Even perforation of the duodenum has been observed as early as 1.5 hours following commencement of the jet perfusion. On the contrary, a constant flow at the same pressure produces little injury to the duodenum. During the past year, bleeding was observed from the stomach of a patient in whom that viscus had been elevated into the thorax 15 years previously as replacement of the esophagus, for a high-lying cancer. At the recent operation, the pulsatile thrust of the aorta upon the lesser curvature of the stomach, site of origin of the gastric bleeding, was very obvious. This observation suggests the need of assessing the role of the pulsatile aortic thrust in genesis of gastric ulcer.

Methods and materials. Adult mongrel dogs were operated upon under pentobarbital anesthesia. In Group I, through a left subcostal incision, the postero-lateral aspect of the diaphragm was incised exposing the aorta

which was dissected free for 3 cm cephalad to the celiac artery. The anterior wall of the stomach was placed behind this segment of the aorta and either sutured back onto itself or to the left lateral abdominal wall (Fig. 1A). In another group of dogs (1A), 30 mg of histamine in beeswax was given intramuscularly once daily to determine whether augmentation of gastric secretion would accelerate ulcer production under these circumstances. In Group II, the stomach was placed behind the aorta of dogs in the same location, with this difference: the aorta in the chest was divided and aortic continuity re-established by a Teflon graft (Fig. 1B). In consequence, there was no forward blood flow through the segment of the aorta, behind which the fundus of the stomach was placed. To insure absence of pulsations in the portion of the aorta overriding the stomach, an aortic ligature was placed just proximal to the celiac artery. There were 12 dogs each in Group I and Group II, and 6 dogs in Group IA. Upon recovery from operation, all the dogs were fed *ad libitum*.

Results. In Group I, 9 of 12 dogs (75%) died as a result of development of ulceration at the area subjected to the thrust of aortic pulsations. Of these 9 dogs, 5 succumbed

* Supported by U.S.P.H.S. Peptic Ulcer Grant and Donald J. Cowling Fund for Surgical Research.