acts primarily by increasing liver glycogenolysis(1). Unpublished data on 5 patients in this hospital‖ demonstrate that Nilevar therapy does not alter plasma gluco-corticoid levels or ACTH responsiveness of adrenal glands. The normal I^{131} uptakes of thyroids of patients in our study are also indirect evidence of lack of pituitary inhibition by this drug. However, this does not rule out the possibility of direct interference by Nilevar of gluco-corticoid or other hormonal enhancement of liver glycogen deposition.

Both mechanisms may contribute to our findings, *i.e.,* diminished liver glycogen and direct antagonism of anabolic and catabolic substance. Nilevar, by decreasing gluconeogenesis by glucagon or adrenal glucocorticoids may gradually deplete the liver of glycogen. There is the possibility that Nilevar is an enzyme poison or inhibitor. This is of course compatible with any of the above mentioned theoretical mechanisms. Finally, Nilevar may produce this alteration of liver function by causing actual liver cell damage. The rapid return to a normal glucagon response in all patients after Nilevar was discontinued, argues against this.

Whatever the mechanism(s) may be, there exists the intriguing thought that therein may lie a clue to the role of glucagon in normal body economy.

Summary. Sixteen patients were studied as to the effect of Nilevar on hyperglycemic response to glucagon. Nilevar abolished or profoundly diminished this response. This effect was reversible when Nilevar therapy was stopped. Nilevar had no apparent effect on the control of 3 diabetic patients nor on the 24-hour thyroid I^{131} uptakes of 8 patients. Possible mechanisms are discussed.

‖ Studied in collaboration with Dr. M. G. Goldner and Dr. A. Carter.

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Strain Gauge Measurement of Tension Changes in Rat Colon.* (23874)

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In both rat and dog, we have found a direct association between blood pressure changes and movements of sodium into and out of extracellular compartment(1,2). Our current interpretation of these and other results is that blood pressure regulation depends on sodium transfer systems, broadly defined, which govern rate of sodium exchange between vascular smooth muscle and the surrounding medium. Definitive proof of this theory depends on study of the response of smooth muscle (vascular and other) to acute alterations of the cationic medium, or on analysis of cationic changes in the medium during and after induction of contraction in the muscle strip.

Methods currently in use for study of smooth muscle *in vitro* are unfortunately not applicable to this problem, since they would not permit rapid analysis or rapid alteration of the medium and further, the high volume of the medium relative to tissue would tend to dilute sodium shifts beyond detection limits of current methods. Thus, we have prepared new instruments. These have turned out to be simple, rugged and highly sensitive; further, they seem to us to be generally useful for study of smooth muscle strips. The present

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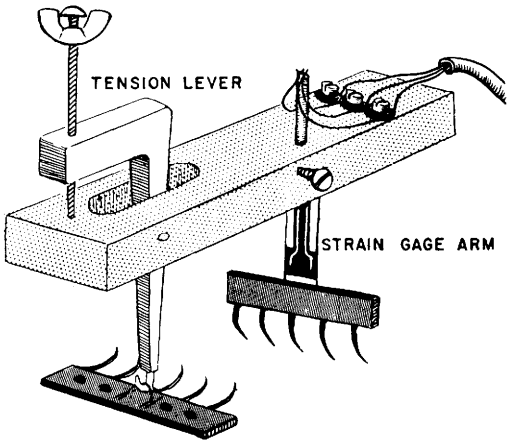

FIG. 1. Type I Tensometer. The body (10 × 2 × 1 cm) is lucite; other parts are brass, varnish-coated for insulation. Two strain gauges (Type A8, Baldwin, Lima, Hamilton) are mounted back-to-back on phosphor-bronze. The instrument is supported on a vertical rod with standard clamps for simple raising and lowering.

report deals with rat colon as test tissue and, as a by-product, the usefulness of this particular tissue is demonstrated. *Type I Tensometer* (Fig. 1) is a self-contained immersion unit for use with exchangeable baths, thus ensuring rapid exchange of medium. *Type II Tensometer* (Fig. 2) is an adaptation of the immersion unit featuring volume and temperature control of bath and a closed external pump circuit permitting rapid sampling of medium. This latter tensometer is also suitable for continuous recording of ion concentration using radioactive tracer technics. Both instruments feature back to back mounting of the strain gauges. These are connected into 2 arms of the bridge circuit of a Sanborn strain gauge amplifier recording through a Sanborn direct writing oscillograph. The double gauge feature results in high gain and low drift. *Basic sensitivity* trials reported below were carried out using the simpler Type I instrument with bath at room temperature. Test tissue was taken from distal 7 cm of colon from adult male albino rats of inbred Wistar strain. The colon was split longitudinally and placed in buffer solution for at least 30 minutes. Two strips approximately 3 cm in length were mounted (total weight approximately 0.5 g) and left immersed for 90 minutes more before use. A Krebs-Henseleit medium(3), modified for the rat, was used. Its composition in mM/liter is as follows: NaCl 129, KCl 5, $CaCl_2$ 2.1, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 200 mg %. The buffer was continuously aerated with carbogen to pH 7.4. The bath volume was 70 ml, mixed constantly by magnetic stirrer. *Tension* was measured as cm deflection of stylus calibrated as μin/in strain. For our Type I instrument used, 14 μin/in strain = 1 g tension on direct calibration. The attenuator built into the amplifier permits a rapid adjustment of amplification in steps for easy full-scale reading. Within the tension range the response was linear.

Results. a) Carbachol. Measured amounts of carbachol (carbamylcholine) dissolved in 0.2 ml of distilled water were added to the bath to cover a wide range of final concentrations and the isometric tension response was measured. The threshold dose for 8 separate pieces of tissue ranged from 3 to 4 mμg (mμg = 10^{-9} g) of drug/ml of medium. The dose response curve for 3 typical runs is shown in Fig. 3A. Responsiveness of tissue was increased if, within limits, a small basal tension was applied (up to 40 μin/in). This is shown for 3 separate pieces of tissue in Fig. 3B, indicating the necessity for standardizing the basal tension in quantitative studies. Unless otherwise noted the basal tension in these studies was 10 μin/in applied

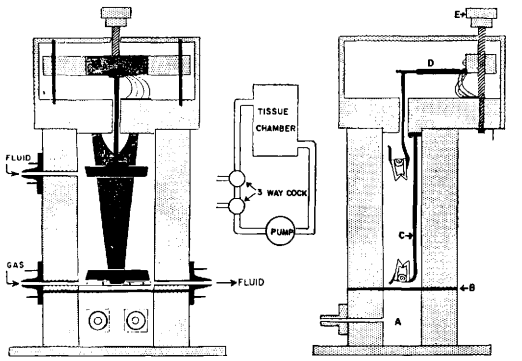

FIG. 2. Type II Tensometer showing external closed circulating system with a small centrifugal pump as driver. Stippled parts are lucite; other parts are metal, varnish or plastic-coated for insulation. A, heat exchange chamber; B, copper heat exchange plate fully insulated from upper chamber; C, rigid support arm; D, strain gauge arm; E, basal tension adjustment.

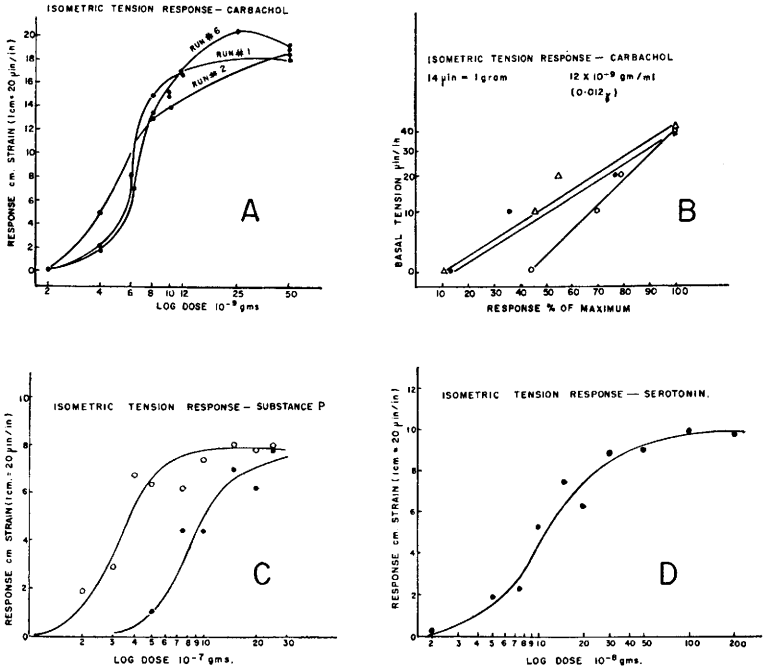

FIG. 3. A. Dose response, carbachol. B. Effect of altered basal tension on response to carbachol. C. Dose response, Substance P. D. Dose response, serotonin creatinine sulfate.

at start of experiment and left unchanged.

b) *Substance P* (*vonEuler*). An amount of this material sufficient for 2 trials was available. The dose response curves are shown in Fig. 3C. Potency of the material was approximately 60 vonEuler units/mg so that the threshold response ranged from 0.01 to 0.03 unit. The latent period, as shown in tracings of Fig. 4, was as described by Pernow for this substance(4).

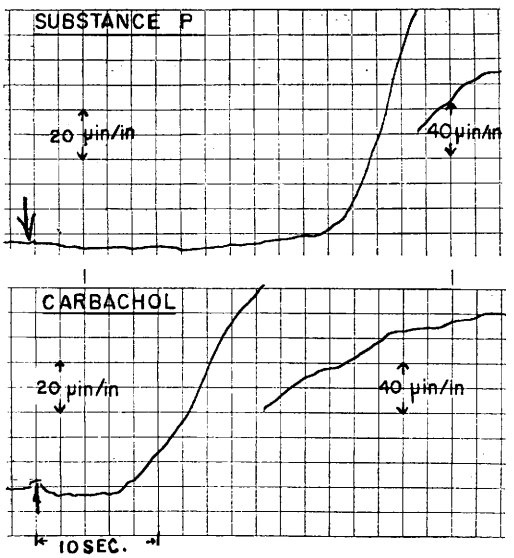

FIG. 4. Comparable responses to Substance P and carbachol showing latency periods.

c) *Serotonin.* This material was used as serotonin creatinine sulfate and doses are expressed in terms of the salt. The results for several separate pieces of tissue with replicate runs in each were quite stable. A typical dose response curve is shown in Fig. 3D.

Discussion. Sensitivity data for the strain gauge tensometer using rat colon strips have been presented. Absolute comparisons are difficult since rat colon has not been much used for this type of work. Some figures, however, are of interest. Thus, Innes *et al.* (5) found that 5 to 13 mµg/ml of carbachol produced tension changes of 1 to 5 g in guinea pig ileum; in our work, the threshold for rat colon was 4 mµg/ml while 13 mµg/ml produced strain in the range of 15 g tension. For Substance P and rat duodenum Pernow(4) reported that the threshold was 2 units/ml and for guinea pig ileum 0.1 unit/ml; we found the threshold for rat colon to be 0.01 to 0.03 unit/ml. For serotonin (measured as base) and atropinized rat colon the reported threshold

was 0.01 to 0.02 μg/ml and with time the threshold decreased 10-fold(6); in our work the threshold for non-atropinized rat colon was 0.02 μg/ml of salt (approximately 0.01 μg/ml as base).

It should be noted that our measurements were obtained at room temperature, a point of considerable convenience, although presumably the sensitivity would be greater at 37°. Rat colon is thus a useful tissue for such studies.

Summary. Strain gauge tensometers applicable to the study of ionic or metabolic exchanges in smooth muscle strips have been developed. Sensitivity was assessed using rat colon with carbachol, Substance P, and serotonin. The threshold for carbachol was 4 mμg/ml, for Substance P 0.01 to 0.03 unit/ml (vonEuler units) and for serotonin as salt 0.02 μg/ml.

We are indebted to Professor U. S. von Euler who graciously supplied Substance P.

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Effect of Malonate on Performance and Metabolism of Isolated Dog Heart.* (23875)

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Malonate is the classical inhibitor of the Krebs tricarboxylic acid cycle. Krebs and Eggleston(1), using pigeon breast muscle mince, found that 0.025 M malonate caused 92% inhibition of pyruvate oxidation. Smyth (2) used the same concentration of malonate in sheep heart mince and observed an 80% inhibition of pyruvate oxidation. If malonate were added in sufficient concentration to dog heart-lung preparation one would expect to produce heart insufficiency. For if it is assumed that oxidative metabolism in the heart is mainly channeled through the Krebs cycle, an interruption of the latter would lead not only to inhibition of oxidation, but also, according to current concepts, to cessation of production of high-energy phosphate bonds which, directly or indirectly, provide energy for muscle contraction. Early experiments with 0.03 M malonate produced a mild insufficiency lasting a few minutes, followed by a return to normal performance. In a few instances, there was even a "rebound" and a greater heart output was observed than before malonate addition. This unexpected result could have several explanations, 1) that malonate did not diffuse into heart muscle in sufficient concentration to inhibit succinate oxidation. Succinate determinations were then performed on malonate-treated hearts, an accumulation of succinate being interpreted as evidence for diffusibility of malonate.

Methods. Dog heart-lung preparations were performed under pentobarbital anesthesia but blood donors were anesthetized with chloroform. 5.6 g Na_2 malonate H_2O were dissolved in 50 cc H_2O and added to venous reservoir in 10 minutes. This brings molarity of the system to 0.03, if total weight of preparation is taken as 1 kg. Where double dose was used 5 minutes elapsed and another dose of 5.6 g malonate in 50 cc water was introduced in 20 minutes. Experiments were terminated 65 min. after onset of first malonate infusion. Phosphorus compounds were estimated as previously described(3) except

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