# Ultrasonic Movement of Corticosteroids into Pig Tissues.* (27237)

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The experiments to be described here were performed to determine whether cortisol (Cortril®, Chas. Pfizer & Sons, Inc., New York) could be driven through pig skin *in situ* by ultrasonic energy and recovered from underlying soft tissue. The results reported here on the levels of cortisol in pig tissues exposed to sonation alone and to sonation plus cortisol, indicate this may be possible. If this is so, it might be a method of administering certain drugs without injection.

The fluorometric method of Sweat(1,2) as modified by McLaughlin *et al.*(3) was used in the present work with minor changes because skeletal muscle rather than plasma was extracted.

*Experimental procedure.* Eight boars (34-57 kg) were anesthetized with thiopental-sodium (1 ml of a 2.5% solution/kg) *via* the vena cava. The areas treated were paravertebral, lower cervical or lower lumbar. The skin in this area was washed with water to remove surface dirt. Hair was cut with scissors, care being taken to leave the skin intact. In the case of pigs exposed to sound plus cortisol, 1 g of ointment containing 25 mg of cortisol was applied over an area of 5 $cm^2$, equal to the radiating surface of the transducer (Birtcher Corp., Los Angeles, Model U-105). For pigs exposed only to sound, mineral oil or ointment base was the coupling agent. The transducer was held in a stationary position. An intensity of 3 watts per square centimeter of the sound head was given for 5 minutes. Immediately after removal of the sound head the animal was sacrificed by bleeding. A large area of skin and subcutaneous fat (amounting to 1 to 2 cm in thickness) surrounding the transducer was excised and a block of muscle approximately 2 × 2 × 5 cm underlying the transducer was removed and sectioned so as to yield 4 samples. Block size varied with pig size. Samples weighed from 2.6 to 24 g. Control samples were removed in a similar fashion from the opposite side of the pig. The samples were wrapped individually in aluminum foil, marked for identification, and frozen until extracted.

Each frozen sample was homogenized with 25 ml of water in a Waring Blendor and the slurry was extracted with chloroform. The chloroform extract was washed with 5% sodium bicarbonate solution, then washed with water, dried over sodium sulfate, filtered through glass wool, and completely evaporated *in vacuo*. The residue was partitioned between equal parts of 70% methanol and heptane. The heptane fraction was discarded; the methanol fraction was evaporated to dryness.

Standard cortisol was made in concentrated solution in ethanol. Immediately prior to use a portion of this standard was diluted to give 1 μg per ml. Two 1 ml portions of this standard were evaporated to dryness under nitrogen, one to serve as column reference, the other to serve as the known standard of cortisol.

Three silica gel micro-columns of the type described by Sweat(2) were set up, with stop-cocks closed. Five ml of chloroform was poured in, followed by 1 g of activated silica gel, followed by 15 ml of chloroform. Then the stop-cocks were opened. The tissue extract and the evaporated column reference were dissolved in 10 ml of chloroform and transferred to their respective columns, while 10 ml of chloroform was added to another (the blank) column. The columns were developed with two 15 ml portions each of 1,3, and 5% ethanol-chloroform solutions (made up directly before use), and the eluates were collected in corresponding 15 ml fractions. To effect quantitative transfer of steroids, the

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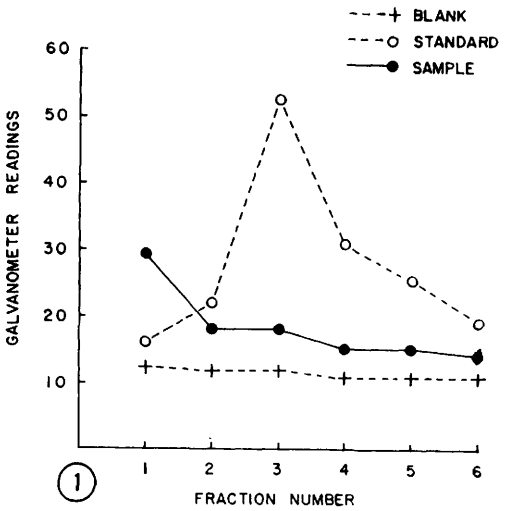


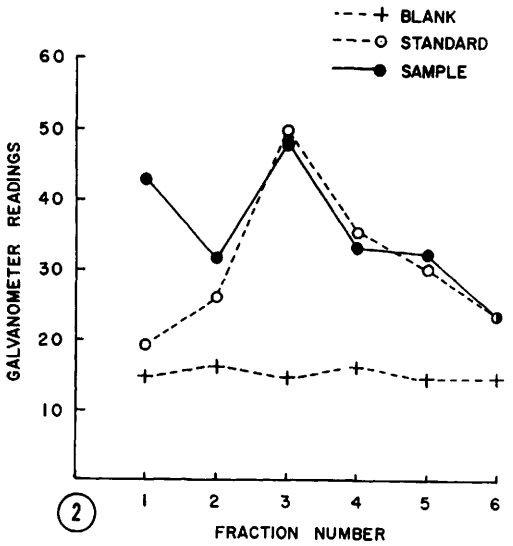


FIG. 1. Tissues exposed to sound without cortisol.

FIG. 2. Tissues exposed to sound plus cortisol.

extract and reference flasks were rinsed with each change of eluent used. As each elution was completed, 8 ml of sulfuric acid was added and the mixture was shaken in a separatory funnel. Fifteen ml of chloroform was added to the known standard of cortisol followed by sulfuric acid as with the column fractions. Fluorescence determinations were carried out with a Farrand Model A fluorometer. The fluorometer settings were adjusted so that the known standard always gave a reading of 100 units on the galvanometer. Acid alone usually gave a reading of 10 to 15 units. If the acid reading was over 25 when the known standard reading was 100, the acid was discarded and both known and column standards were freshly prepared.

The identity of the isolated compound was substantiated by spectrofluorometry, paper chromatography and correlation of the results with those obtained by silica gel chromatography. A sample of the tissue extract was chromatographed on Whatman #1 paper in a toluene propylene-glycol system. The zone corresponding to cortisol, located by comparison to the reference chromatogram, was eluted and rechromatographed on the silica gel column. The fluorescence and excitation spectrum of the cortisol fraction in sulfuric acid was the same as that shown by the reference cortisol.

*Results.* Fig. 1 represents the average galvanometer readings of 10 samples each of blank (no cortisol or tissue extract present), standard reference column (1 $\gamma$ of cortisol), and sample (tissue extract without cortisol added), chromatograms in 6 fractions. The standard column always showed maximum fluorescence in the third fraction. The first eluate of the tissue extracts contained material other than cortisol, as shown by a great deal of fluorescence in the first fraction. Fig. 1 indicates that tissues exposed only to sound contained minute amounts of cortisol, as little fluorescence was seen in the third or cortisol fraction.

Fig. 2 shows the sequence of fluorescence levels for the various fractions, namely, blank, standard reference columns, and sample (which in Fig. 2 means after cortisol ointment and sonation) of tissues exposed to sound plus cortisol. These tissue extracts always presented the greatest fluorescence in the third fraction. The second and fourth fractions showed much less fluorescence. Five and six were still lower. The fluorescence pattern for tissue extracts was thus similar to that of the column reference standards. Tissues exposed to a similar intensity of sound plus ointment without cortisol showed the same pattern as tissues exposed to sound with mineral oil as the coupling agent. Tissues exposed to ointment with cortisol but without sound showed the same pat-

TABLE I. Effect of Ultrasonic Energy on Movement of Cortisol through Pig Skin. (Values are expressed as μg/10 g of tissue.)

| Sample | Control | Cortisol ointment | Difference |
|---|---|---|---|
| 1 | .26 | 1.45 | +1.19 |
| 2 | .45 | .62 | + .17 |
| 3 | .19 | .89 | + .70 |
| 4 | .29 | 1.93 | +1.64 |
| 5 | .08 | .10 | + .02 |
| 6 | .10 | .17 | + .07 |
| 7 | .29 | .82 | + .53 |
| 8 | .18 | .68 | + .50 |
| 9 | .31 | .33 | + .02 |
| 10 | .91 | 1.17 | + .26 |
| Mean ± S.D. | .31 ±.24 | .82 ± 1.14 | + .51 ±.54 |

tern as those exposed to sound plus ointment without cortisol.

Calculation of the quantity of cortisol recovered from skeletal muscle (Table I) showed that tissues exposed to sound but not to cortisol had an average of 0.31, whereas tissues exposed to sound plus cortisol had an average of 0.82 γ of cortisol per 10 g of tissue. Using the method of least squares the standard deviation of the difference was 0.54, ($p = <0.02$).

*Summary.* 1. Pig muscle contained an average of 0.31 γ of cortisol per 10 g when analyzed by McLaughlin's modification of Sweat's fluorescence method. 2. After inunction of an ointment containing cortisol followed by exposure to ultrasonic energy an average of 0.82 γ per 10 g of muscle was found.

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## Serum Albumin and Gamma-Globulin in Normal Human Gastric Juice*† (27238)

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In 1934, one of us (FH) speculated on the possibility that the alkaline component of gastric juice includes a transudate of interstitial fluid, along with one or more mucinous secretions(1). It was not until 1953, however, that the first evidence to support this hypothesis was made available by the electrophoretic studies of Henning *et al.*(2), which revealed the presence of albumin in specimens of normal as well as pathological gastric juice from human subjects. In 1959 Citrin *et al.*(3) reported the occurrence of serum proteins in gastric juice of a patient with Menetrier's disease. The following year serum albumin was demonstrated in this laboratory(4) to be an important constituent of anacid gastric mucinous secretions obtained from dogs equipped with Heidenhain pouches. Gullberg and Olhagen(5), by instilling buffer into the stomach to prevent proteolysis, found albumin in normal human gastric juice. Holman *et al.*(6) used $I^{131}$-labeled albumin and γ-globulin to study serum proteins in small bowel secretions, and mentioned incidentally that serum proteins were found in saliva and gastric juice from normal human subjects. The current study was undertaken to confirm the presence and quantitate the amounts of albumin and γ-globulin in normal human gastric juice obtained by nasogastric intubation, and to evaluate the possible contribution of saliva.

*Materials and methods.* Only subjects without evidence of gastrointestinal disease and with normal serum proteins were studied.

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