Choline (I). The presence of choline among the hydrolysis products of murexine was ascertained chemically (preparation of a reineckate identical to that of choline), by paper chromatography (*5*), and pharmacologically (the acetylation of the murexine hydrolysis products gives a substance which cannot be distinguished from acetylcholine).

Choline was determined quantitatively by direct weighing, following its precipitation as Reinecke salt. Aliquots of 200 mg of murexine picrate were used in the determinations.

$C_5H_{14}ON \cdot C_4H_7N_6S_4Cr$	from UCD	Calc: 61.97%
	" murexine picrate	Found: 62.80%–62.85%
$C_5H_{14}ON \cdot C_4H_7N_6S_4Cr$		Calc: Cr 12.26%
Reineckate from murexine hydrolysis products		Found: 12.46%
" " choline chloride		" 12.44%

Choline esterifying acid (II). Having demonstrated that murexine picrate contained 2 molecules of picric acid and 1 of choline, we were compelled, on the basis of the elementary analyses, to acknowledge the presence of 2 nitrogen atoms in II. Owing to the positivity of the Feigl reaction, also, the presence of 2 oxygen atoms became extremely probable.

A decisive step forward in the identification of product II was made by observing that it gave, in alkaline medium, an intense coupling reaction with diazonium salts, above all with the Pauly reagent. The color reactions of phenols, indoles, purines, and pyrimidines were, on the contrary, negative (these substances were also excluded through the ultraviolet spectrophotometric analysis). On the basis of these findings our orientation toward an imidazole derivation of the substance became more and more justified.

Chromatography on paper enabled us to discard the identification of product II with β-[imidazolyl-(4)]-propionic acid, and definitely to establish its identity with β-[imidazolyl-(4)]-acrylic acid, a possible intermediate product of the histidine metabolism.

A complete and easy separation of the choline chloride from the hydrochloride of the β-[imidazolyl-(4)]-acrylic acid was brought about by chromatographing the murexine hydrolysis products on a column of Whatman cellulose powder. *n*-Butanol saturated with 1 *N* HCl was used as solvent.

The hydrochloride of the β-[imidazolyl-(4)]-acrylic acid was finally transformed into the β-[imidazolyl-(4)]-acrylic acid, which is much less soluble in water and therefore more easily crystallizable and purifiable.

Needles, mp 217°–218° C, mixed mp with synthetic urocanic acid 217°–218° C.

$C_6H_6O_2N_2 \cdot 2H_2O$ (174)	Calc:	41.17	H 5.88	N 16.47%
Hydrolysis product II	Found:	41.25	5.93	16.41

β-[Imidazolyl-(4)]-acryl-choline dipictrate has been recently obtained by synthesis in our laboratories (*6*). It is identical with murexine picrate, both from the chemical and the pharmacological point of view.

Extensive pharmacological researches are now in progress on murexine and similar synthetic substances.

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The Effect of Estrogen on the Isometric Tension of Rabbit Uterine Strips

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It has been shown in our laboratory (*1*) that the concentration of uterine actomyosin, the contractile protein of the myometrium, is under the control of the ovarian estrogenic hormones. When rabbits in natural estrus were subjected to bilateral ovariectomy the concentration of uterine actomyosin decreased to about one fifth the original estrous value. This extreme condition could be reversed, however, by the administration of estrogen to the living animal, which brought about gradual increase in actomyosin concentration toward the estrous value.

Previous observations (*2, 3*) also indicated that similar changes in the concentration of uterine actomyosin occur under physiological conditions (pregnancy, labor, and menopause) that involve alterations in the estrogen output of the animal's own ovaries.

It has been generally accepted and has become a textbook statement that the fibrous protein actomyosin is the final contractile substance of muscle. The direct evidence in support of this conclusion—namely, Szent-Györgyi's actomyosin-thread "contraction" phenomenon—has not been accepted by some authors (*5, 6*). Evidence, however, may be obtained indirectly by utilizing the above-mentioned experimental variability of actomyosin concentration in uterine muscle. One would expect that the concentration of the final contractile substance would determine the maximum force the muscle is able to develop, provided that conditions are otherwise optimal. The existence of such a relation between actomyosin concentration and maximum tension will be demonstrated if the former is varied in a given muscle and proportionate alterations in maximum tension are then observed.

In accordance with this postulate we have measured the maximum tension developed by freshly excised uterine strips *in vitro* under controlled hormonal conditions in which the concentration of uterine actomyosin had previously been determined—i.e., in natural estrus, after ovariectomy, and ovariectomy followed by estrogen treatment.

Since previous experiments have clearly shown that in these hormonal conditions the spontaneous rhythmicity of myometrial contraction varies greatly *in vivo* (*7*), as well as *in vitro* (*8*), owing to the effect of estrogen, optimal electrical stimulation was used in our experiments in order to secure comparable conditions.

Purebred mature virgin female New Zealand white rabbits were used. Under Nembutal anesthesia the uterine horns were rapidly removed and placed for 10 min in oxygenated mammalian Krebs solution at 0° C. During this time the muscle fully relaxes and remains relaxed. Longitudinal strips of standard length (25 mm) and about 12 mm wide, were cut and placed in a glass tube filled with Krebs solution between two platinum hooks, by which they were attached to the writing lever of an isometric tension recorder. The temperature (37.5° C), the oxygen concentration (2.0 vol%), and the pH (7.4) were kept constant. A previous study with respect to optimum electrical stimulation taught us that stimuli of 5-sec duration, 24 v, 60-c AC, should be used (*9*). Alterations in the voltage (25%) or in the duration resulted in submaximal tension. Moreover, this type of stimulation at optimal frequency of one or two stimuli per minute produced contractions of uniform maximum tension for any desirable length of time.

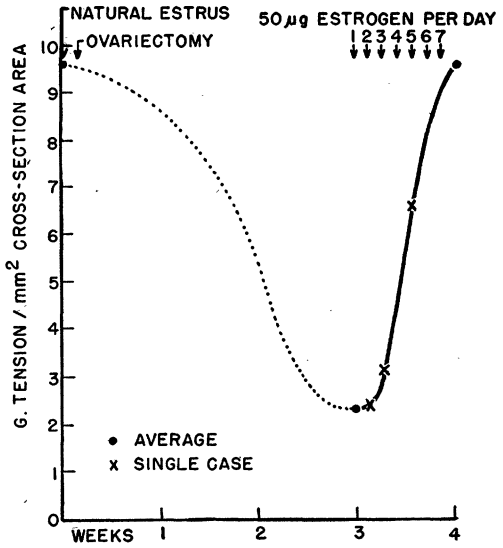

FIG. 1. The effect of estrogen on the maximum isometric tension of rabbit uterine strips *in vitro*.

After the maximum tension was determined the effects of ovariectomy and of estrogen administration were recorded from histological sections, and the cross-section area of muscle used was measured. Thus each value reported represents maximum tension per square millimeter of cross-section area.

As shown in Table 1, the average maximum tension of uterine strips from estrous rabbits is 9.6 (± 1.11 SE)/mm² cross-section area. The muscle, as reported previously, contains 7.6 mg actomyosin/g tissue in this condition. Three weeks after bilateral ovariectomy the tension drops to an average figure of 2.3 g (± 0.41 SE). The actomyosin concentration was found to be 1.7 mg/g tissue in this condition. Taking the estrous and the castrate conditions as extremes, the ratio of tension in the two contrasting states is 9.6/2.3 = 4.2, and that of actomyosin is 7.6/1.7 = 4.5. Considering the great difference between the two kinds of measurements, the two figures appear to be in good agreement.

TABLE 1

THE EFFECT OF ESTROGEN ON THE MAXIMUM ISOMETRIC TENSION OF RABBIT UTERINE STRIPS *in Vitro**

No.	Treatment	Tension	No.	Treatment	Tension	No.	Treatment	Tension
22	Natural estrus	8.00	2	Ovariectomy	2.00	6	540 μg E/ 7D	5.65
23	" "	7.80	4	"	0.70	7	560 μg E/ 8D	6.70
24	" "	14.20	12	"	4.30	8	600 μg E/ 9D	6.72
25	" "	10.03	13	"	1.00	17	450 μg E/ 9D	12.40
43	" "	7.70	14	"	1.18	18	500 μg E/10D	7.35
Av		9.60	15	"	1.49	19	600 μg E/12D	14.70
			37	"	3.40	40	350 μg E/ 7D	12.82
			38	"	3.10	41	350 μg E/ 7D	13.40
			39	"	3.60	42	350 μg E/ 7D	6.30
			Av		2.30	Av		9.55
			28	5000 μg P/5D	2.96			

* Tension calculated in g/mm² cross-section area of uterine muscle used for determination. E = estrogen, P = progesterone, D = day.

If, 3 weeks after castration, estrogen is administered in 50 μg daily doses for at least 7 days, the original estrous value of maximum tension is obtained (9.55 g ± 1.11 SE). As already mentioned, actomyosin increases in a similar fashion following estrogen treatment.

If, instead of estrogen, the other ovarian hormone, progesterone, is administered to the ovariectomized

rabbits, no significant change occurs in tension. Similarly, if estrogen and progesterone treatments are combined, the maximum tension of the uterus will not be significantly different from that in animals which were treated with estrogen only. This observation is in agreement with previous findings that progesterone domination does not significantly alter the actomyosin concentration (*10*).

Fig. 1 indicates the course of gradual increase in maximum tension as a result of the administration of estrogen. The curve is S-shaped. The increase in actomyosin concentration is also slight during the first few days of estrogen treatment (observations on this point were carried out only up to 4 days of estrogen treatment).

These observations indicate that the maximum isometric tension developed by the uterus depends in a final sense on the concentration of actomyosin, and thus the term "contractile protein" seems to be adequate.

In conclusion, we wish to emphasize the singular behavior of uterine muscle with respect to estrogen, which is characteristic, so far as known, of no other muscle. The final contractile system being dependent upon this specific hormone, we are able to make it disappear and reappear again in a fully grown animal as many times as the experiments require. Changes in actomyosin concentration in skeletal muscle related to hormone levels or other physiological conditions have not as yet been observed, except during the process of embryonic development, and no means is known of causing regeneration of the final contractile system in skeletal muscle once it has degenerated. It is a noteworthy fact, therefore, that we have in the uterus a tissue in which the actomyosin concentration can be changed at will for the purpose of observing simultaneous alterations in function. This tissue may be a useful experimental material for more general studies than ours. It can be considered, for example, for studies in growth and protein synthesis.

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Comments and Communications

Un-American Activity

WE HAVE noted with misgiving the continuation of attacks by a Congressional committee, individual members of Congress, and certain journalists upon the reputation of the new President of the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, E. U. Condon, and indeed upon the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE itself. May we take this opportunity to express our vigorous disapproval of such attacks, and our concern about the political climate which makes them possible?

We wish also to commend your organization and its officers for their long history of active championship of the cause of scientific freedom.

THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC.
Carl C. Pfeiffer, *Secretary*

Chicago, Illinois

Pain—Controlled and Uncontrolled

THE stimulating article by Henry K. Beecher, entitled "Experimental Pharmacology and Measurement of Subjective Responses" (SCIENCE, **116**, 157 [1952]), seems to us to require some extension. It is important to avoid overemphasis upon a single aspect of the study of pain; to wit, action of analgesic agents. Such studies, although of recognized practical importance, are not broad enough to encompass the analysis of the total pain experience.

We enthusiastically endorse Beecher's thesis that man's *subjective response must be used* for the study and the evaluation of pain sensation. It is perhaps a truism to say that one cannot study pain by avoiding it, yet methods of study that are directed at obtaining "objective data" about pain are nearly always experiments that attempt to avoid the *sine qua non* for pain studies—the sensation of pain itself. Beecher unfortunately falls into this trap and contradicts himself by saying in his article that "the chief field of usefulness for experimental pain methods may be in animals," after having emphasized that pain must be evaluated by the subjective response in man.

We consider arbitrary and confusing Beecher's emphasis on the dichotomy "experimental" pain—that produced by measured noxious stimuli in the laboratory—and "real" or "pathologic" pain—such as postoperative wound pain. He states that he has developed a successful method for the study of the latter, but implies that no one has been able to study "experimental" pain successfully and to relate such studies to the suffering patient.

In our monograph *Pain Sensations and Reactions* (Baltimore: Williams & Wilkins), we define the "pain