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ACTION OF THE OVARIAN HORMONES ON UTERINE MUSCLE*

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In 1907 Sir Henry Dale reported from his laboratory at Herne Hill the curious discovery that the uterus of the cat does not always react in the same way when its muscular tissue is stimulated through the hypogastric nerves or by intravenous injection of adrenaline. If the cat is not pregnant these stimuli cause relaxation of the uterus, but if she is pregnant they cause contraction. Before Dale's paper was printed Professor A. R. Cushny (1907) made identically the same observation at University College and reported it to the Physiological Society. Although it probably did not occur to either of these eminent pharmacologists that the reversal of muscular response which they had noticed was caused by action of the ovary, and although in fact "pregnancy reversal" still awaits detailed explanation, it was this observation that opened the way to study of the relation between the ovary and the behaviour of the uterine muscle.

In that same year the Heidelberg gynaecologist Erwin Kehrer (1907) was making his pioneer studies of uterine muscle *in vitro*, in which he applied the then very recent discovery of Rudolf Magnus that strips of involuntary muscle cut from the intestine will long remain active if immersed in warm oxygenated physiological salt solution. By attaching such a strip to a lever, the spontaneous isotonic contractions of the muscle and its response to stimulation can be registered. This beautifully simple method began at once to be used in every pharmacological laboratory where oxytocic drugs were studied, Kehrer himself leading the way. In 1908, reporting his first studies on the action of ergot derivatives, he recommended the use of young virgin cats, for he had found that uterine tissue from adult animals taken during the breeding season was often less active and less predictable in its responses. This precautionary specification of the virgin uterus for tests of oxytocic drugs was written into all the pharmacopoeias of the world long before it could be understood in the light of endocrinology.

In 1913 Max Stickel noticed that the movements of the rabbit's uterus *in situ* are greatly reduced by ablation of the ovaries, and a few years later (1919) Athias, of Lisbon, confirmed this fundamental observation by *in vitro* experiments on guinea-pigs' uteri by

the Magnus-Kehrer method. Castration, he wrote, causes a gradual weakening of the automatic contractions to the point of disappearance. If, however, ovarian tissue is regrafted into the animal at the time of castration the uterus retains full power to contract spontaneously *in vitro*. Evidently uterine contractility depends upon an internal secretion of the ovaries. We shall be able, later in this lecture, to explain this remarkable fact, in part at least, on biochemical grounds. Thus, before the two ovarian hormones were isolated or even clearly foreshadowed, characteristic differences in the behaviour of uterine muscle were observed which we can now see were related to the action of progesterone and oestrogen respectively.

• Shrewd Conjecture

One of us (G. W. C.) was fortunate in having in his laboratory, about the years 1921-2, a group of medical students doing research on various aspects of the ovarian cycle. Two of these young men, the twin brothers Guttmacher, happened to read Dale's paper of 1907, and made the suggestion that the changes in uterine response to adrenaline observed during pregnancy may be due to the action of the corpus luteum, and hence might be expected to occur also in non-pregnant animals during the corpus luteum phase of the reproductive cycle. This was an extraordinarily shrewd conjecture to be made by medical students. Going on to other studies, the Guttmachers were unable to test their conjecture, and with their consent it was turned over to another student, John D. Keye.

Keye was taught to utilize for this purpose the uteri of swine, which were readily available at an abattoir near the Johns Hopkins Medical School. The sow has a clearly defined reproductive cycle consisting of a follicular phase lasting one week, which culminates in oestrus and ovulation, during which oestrogen is the dominant hormone, and a corpus luteum phase about two weeks in length, during which progesterone is dominant. Such a sequence of events occurs, with various modifications, in all mammals that have been studied. In the rabbit, which is the chief subject of our contribution, oestrus is not periodic and ovulation does not occur spontaneously. Mature does will accept mating at almost any time, and then only do the follicles mature and rupture. The

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follicular phase of the cycle (if we may speak of a cycle in this case) is therefore of indefinite length. The corpus luteum phase is ordinarily associated only with pregnancy, but an infertile mating (which may be arranged by the experimenter) gives rise to a corpus luteum phase or "pseudo-pregnancy" comparable in all respects to the corpus luteum phase of the sow's or the human cycle.

Uterine Contraction Pattern

For the purpose of Keye's investigation, strips from sows' uteri at various stages of the cycle were suspended in warm oxygenated salt solution by the Magnus-Kehrer technique, and were to be treated with adrenaline and other oxytocics. But Keye (1923) never arrived at that stage of the experiment, for we were amazed to find that the pattern of spontaneous contraction of the muscle, before treatment with oxytocic drugs, varies in a dramatic way throughout the cycle. At oestrus, when ripe follicles are present in the ovaries, the contractions are large and infrequent, occurring once every one or two minutes; during the corpus luteum phase they are small but very frequent, at the rate of five to eight a minute. By observing these changes and the transitions between them it was possible to characterize the contraction pattern almost from day to day of the cycle. Simultaneously with Keye's study, a student of H. M. Evans—Blair (1923)—found something of the same sort in rats, but the shortness of the cycle in that species obscures the pattern of uterine contraction and no further work was done on the rat.

When the ovarian hormones became available they were promptly put to use in following up the earlier observations on uterine contractility. In 1929 Van Dyke and Gustavson reproduced the Dale-Cushny "pregnancy reversal" in kittens treated with crude lipoidal corpus luteum extracts. In the same year S. R. M. Reynolds introduced his ingenious method of recording uterine contractility *in situ* by a small balloon inserted into the rabbit's uterus through a vaginal fistula. He showed (Reynolds, 1931) that spontaneous contractility is almost abolished by castration but is quickly restored by subcutaneous injections of oestrogenic hormone.

Interest in the relation between the hormones and uterine motility was now greatly stimulated by a series of contributions from Herman Knaus (1930). This investigator noticed that uteri of pregnant and pseudo-pregnant rabbits studied *in vitro* do not respond to "pituin" by vigorous contraction as do the uteri of virgin rabbits. Learning, as had Van Dyke and Gustavson, of the corpus luteum hormone recently isolated by Corner and W. M. Allen, Knaus made the first crude progesterone produced in Europe, and with it induced in the virgin uterus an altered response to pituitrin resembling that seen in his earlier experiments on pregnant and pseudo-pregnant rabbits. As soon as crystalline progesterone was available, Makepeace, Corner, and Allen (1936) obtained the same result with the pure hormone. Reynolds and Allen (1932) found also that the large, regular, relatively infrequent contractions observed by the Reynolds fistula technique in oestrous rabbits or after oestrogen treatment are almost totally suppressed for hours by a single subcutaneous injection of progesterone. J. M. Robson (1936) added much to our detailed knowledge of the action of oxytocic hormones under varying hormonal conditions.

Beyond this point there has been no significant progress. Summarizing what has been learned, we see that (1) oestrogen is necessary for spontaneous motility; (2) the pattern of spontaneous contraction differs greatly according to the dominant hormone—namely, oestrogen in the unmated and oestrous rabbit, progesterone in pregnancy and in the corpus luteum phase of the cycle (pseudo-pregnancy); (3) the response to stimulation by certain oxytocic drugs may also differ under different hormone domination.

The observed facts cannot all be fitted together, nor do they permit, as yet, effective theorizing about their functional

significance for the behaviour of the uterus as a whole organ. They do, however, provide the basis for a new effort to discover, at the level of the muscle cell, and in biochemical terms, the mechanisms by which the ovarian hormones actually control uterine motility.

Molecular Physiology

For a clearer insight into the myometrial cell we must turn to molecular physiology. One single contraction-relaxation cycle of the uterus, observed in the obstetrical ward as a labour pain, or registered *in vivo* or *in vitro* by the techniques of classical physiology, is clearly a complex phenomenon involving a whole series of elementary events. In 1946 one of us (A. C.), a clinical obstetrician at the time, became convinced, after reading the articles of Szent-Györgyi and his associates, that the function of the myometrium, like that of the voluntary muscle which they were studying, could be understood only if the complexity of the contractile system were broken into simpler component elements and analysed step by step with biochemical and physico-chemical techniques. It was to be hoped that the system thus taken apart can gradually be put together again.

Muscle contraction involves basically a contractile substance, a supply of energy, a stimulus to initiate the contraction, and a means of conducting the stimulus to the contractile elements. Study of the process must be directed toward these four basic systems. The research which we here summarize began with the final contractile substance because, thanks to the efforts of the biochemists, it was far better characterized than any of the other systems.

Szent-Györgyi (1945) greatly simplified the problem by showing that "contraction" can be produced *in vitro* by merely bringing together the isolated constituents of the process—namely, the fibrous protein complex actomyosin (A.M.), the energy-rich nucleotide adenosine triphosphate (A.T.P.), and ions. Thus he claimed that muscle contraction in a final sense is an interreaction between A.M., A.T.P., and ions. His observation was the climax of a long extensive study conducted in several laboratories of different countries and led by outstanding biochemists and physical chemists (see for literature Szent-Györgyi, 1951).

Actomyosin

It is now a textbook statement that the final contractile substance of skeletal muscle is A.M. This complex, first recognized by Banga and Szent-Györgyi (see Szent-Györgyi, 1945), was decomposed in the latter's laboratory into its two component proteins, myosin (M.) and actin (A.). Actin-free myosin was prepared in pure ("crystalline") form by Szent-Györgyi himself, and actin was purified by his pupil Straub. A.T.P., the compound containing high-energy phosphate bonds ($\sim$ ph) was first demonstrated in muscle by Lohmann (1938). Myosin, as Engelhardt and Ljubimowa (1939) have shown, has the property of acting as an enzyme (more specifically, as an adenosine triphosphatase) which can break the high-energy linkage of the terminal phosphate of A.T.P. and release its free energy, calculated by Meyerhof to be 11,000 calories.

Weber (1935), who extracted from muscle a "myosin gel" (now known to consist of actomyosin), was able to form it into fibres similar in appearance to fine cotton threads. Szent-Györgyi (1945) showed that such threads of A.M. will shorten when A.T.P. is added, if they are suspended in a salt solution similar in ionic strength to serum. Objections were raised in various laboratories against the idea of analogy between this thread-contraction phenomenon and muscle contraction. This criticism led to the effort to make the molecular structure of the A.M. thread more like the structure of muscle. This was done by stretching the A.M. fibres, so that the A.M. micelles become parallel as they are in muscle. Such threads shorten and become thicker without changing volume. This effort culminated in Szent-Györgyi's preparation of glycerin-treated psoas muscle from the rabbit (Szent-Györgyi, 1947,

1951) in which the muscle-cell membrane is destroyed (in a functional sense as indicated by the loss of electrical excitability) and the water-soluble components are removed, but the contractile proteins preserve their architecture. When A.T.P. is added, such fibres develop tension similar to that of intact muscle. Taking all the opinions into account, Mommaerts (1950) wrote in his recent survey: "It seems justified to conclude that Szent-Györgyi actually succeeded in a dissolution, separation, and resynthesis of the contractile system."

With the information thus briefly outlined in hand, let us turn to the uterus and ask whether the final contractile system in the myometrium is fundamentally the same as that of skeletal muscle. With modifications required by the smaller amount of material available and the difference in structure, Csapo (1948, 1950a) succeeded in isolating A.M. from human, rabbit, rat, and cow uteri.

All tests performed so far on the A.M. of the uterus support the conclusion that it is fundamentally the same as that of skeletal muscle. It is true that slight differences have been observed between the two substances, which may be partly responsible for the differences between the two kinds of muscle long known to physiologists. First, however, let us consider the similarities.

Characteristics of Actomyosin

What are the characteristics of actomyosin? (see literature, Szent-Györgyi, 1951). A.M. can be extracted from fresh muscle by 0.5 mol KCl, and can be purified simply by precipitation by dilution. If redissolved in 0.5 mol KCl it has a high anomalous viscosity, which indicates that it is made up of highly asymmetric particles. If the purified extract is studied in the Svedberg ultracentrifuge or the Tiselius electrophoretic apparatus it tends to be distributed into two portions, yielding curves with a larger peak which represent A.M. and a smaller peak representing free M. uncombined with A. (Snellman and Erdős, 1984a, 1948b).

If A.T.P. is added to A.M. in the viscosimeter an immediate drop in viscosity will be observed. The viscosity of the component proteins, A. and M., being less than that of the complex, A.M., this drop in viscosity indicates the dissociation of the complex into its component parts. M. being an A.T.P.-ase, A.T.P. will be split and its dissociating effect on A.M. will be terminated; the A. and M. will then reassociate, combining to form A.M., as indicated by the gradual rise in viscosity. Actomyosin threads can be prepared from uterine muscle and will contract energetically when A.T.P. is added.

All the properties just mentioned are characteristic of uterine A.M. as well as that from skeletal muscle. As for the differences, observations by viscosimetry show that the amount of A.M. is much less in the uterus than in skeletal muscle (about one-seventh) and, moreover, A.M. forms only about one-third of the total amount of the contractile proteins, whereas in skeletal muscle extracts two-thirds is A.M. (Csapo, 1948, 1949, 1950a, 1950b).

By following the change of viscosity when A.T.P. is added to identical amounts of the two kinds of contractile proteins it is found (Csapo, 1949) that the recombination of uterine A.M. indicated by increase in viscosity does not begin as soon and does not proceed as fast as does that of skeletal muscle. This can be explained either by a lower A.T.P.-ase activity of the uterine extract or else by the lower activity of the myokinase system resulting in the accumulation of A.T.P. which keeps A.M. dissociated.

When the "contraction" of threads of the two kinds of actomyosin is compared (Csapo, 1950a), it is found that uterine A.M. contracts much more slowly than that from skeletal muscle, and with a longer "latent period" after the addition of A.T.P. Moreover, uterine A.M. threads contract only below the A.T.P. concentration of 4×10^{-3} mol. At this critical concentration the threads first lengthen before they contract, whereas above this concentration they

completely dissolve. In contrast threads of similar A.M. concentration (obtained by dilution) from skeletal muscle readily contract even in a twenty-times higher concentration of A.T.P. solution (Csapo, unpublished).

Uterine A.M., unlike that of skeletal muscle, practically loses its ability to contract (Csapo, 1949) if precipitated only once (by dilution). This process is reversible, however, and the precipitated A.M. can be reactivated if fresh watery extract is added to the precipitate or to the salt solution in which the thread is suspended. The speed of thread contraction thus depends on a factor (or factors) present in the watery extract of the uterus, and the speed of contraction is directly related to the concentration of this factor, which we will call "X." Skeletal muscle also contains X-factor in a much higher concentration or else in a more active form (Csapo, 1949). This matter of the X-factor is mentioned to emphasize the possibility that the differences between skeletal and uterine muscle extracts are not all necessarily due to differences of the A.M. itself, but may well be due to differences in their prosthetic groups or other active substances attached to the A.M. framework. When highly purified actin and myosin from the uterus become available this question can be further studied.

In summary, the final contractile substance in uterine muscle is actomyosin, generally resembling the characteristics of the contractile protein-complex of skeletal muscle, but present in much lower concentration and forming a smaller fraction of the total proteins.

Effect of Ovarian Hormones on Contractile Proteins of Myometrium

Next let us examine the effect of the ovarian hormones upon the contractile proteins of the myometrium. In skeletal muscle the concentration of A.M. and the ratio of A.M. to free M. are found to be constant. In uterine muscle, however, the concentration of A.M. is significantly different under varying hormonal conditions of the ovaries. When a rabbit is castrated the concentration of A.M. drops to about one-fifth that found in the oestrous animal. In late pregnancy, on the other hand, the concentration is about twice as much as in oestrus. In oestrus, moreover, two-thirds of the contractile protein is free M. and only one-third is A.M., whereas in late pregnancy the ratio is reversed, as viscosimetry has shown. The same holds for the human as demonstrated by ultracentrifugation and electrophoresis. These observations already suggest that the concentration of uterine A.M. is under the control of the ovaries (Csapo, 1948, 1950a, 1950b; Csapo, Erdős, Naeslund, and Snellman, 1950).

Studying the course of the change in A.M. concentration during pregnancy in rabbits, we observed a slight drop in the first third, followed by a sharp increase in the second third. A high plateau is maintained in the last third of pregnancy with a climax at labour, after which the concentration of A.M. drops rapidly to the non-pregnant (oestrous) level. This curve gives a further indication that oestrogen is responsible for the changes in A.M. concentration of the uterus (Csapo, unpublished). Final evidence for this conclusion was given by the following experiments.

Rabbits were bilaterally ovariectomized and a few weeks later the previously mentioned great decrease in A.M. concentration of the uterus was observed as a result of the castration. Oestrogen was then administered, and a gradual increase in the A.M. concentration caused by the removal of both ovaries can be experimentally reversed by supplying oestrogen to the animal (Csapo, 1950b).

In another set of experiments it was found that progesterone does not have any such effect as oestrogen upon concentration of A.M. in the uterus (Csapo and Corner, 1951). We believe that the slight drop of A.M. concentration during the first week of rabbit pregnancy mentioned above is due to the decrease or disappearance of tissue oestrogen and not to the increasing action of progesterone.

How oestrogen controls A.M. concentration in the uterus we do not know as yet. As pointed out by D. Needham (1952), it is possible that oestrogen affects the formation of A.M. by providing a means by which the "building stones" or precursors (of smaller molecular weight) are bound together to form longer fibrous units. This mechanism cannot, however, be the only one involved in A.M. formation, since in an oestrogen-treated castrate rabbit the uterine weight increases ten to twenty times while the protein concentration per unit of weight is also increasing. Thus large amounts of proteins must be synthesized as a result of the action of oestrogen upon the uterus. It is worth emphasizing that in the uterus we possess a tissue in which the effect of oestrogen on protein synthesis has been experimentally established. In skeletal muscle similar changes have been observed only during embryonic and post-natal development (Csapo and Herrmann, 1951); once sexual maturation is complete the contractile proteins of skeletal muscle are not subject to hormonal control.

The concentration of A.M. in uterine muscle is, however, not alone affected by oestrogen; the properties of extracted A.M. are also changed. With the viscosimeter we find that the "splitting-time" of A.T.P. is reduced by oestrogen treatment and the recombination of dissociated A.M. after A.T.P. has been split becomes faster (Csapo, 1949). The speed of A.M. thread contraction is also increased, as shown in specimens from late pregnancy (Csapo, 1948, 1950a). We assume tentatively that the concentration or activity of the X-factor is also affected by oestrogen.

Relation of Ovarian Hormones to the Energy-yielding System of the Uterus

With this we conclude our brief summary of the facts known at present about uterine A.M. and its dependence upon oestrogen. The next inquiry concerns the relation of the ovarian hormones to the energy-yielding system of the uterus. Before proceeding any further we must recall Szent-Györgyi's thread-contraction experiments, from which it is evident that not A.M. but A.M.-A.T.P. is the final contractile substance. Experiments in our laboratory have shown (Csapo and Gergely, 1950) that the total high-energy phosphate content of the uterus is only about one-seventh that of skeletal muscle, and recent measurements by means of Kalckar's differential spectrophotometric technique show that the same ratio holds for A.T.P. in particular (Menkes and Csapo, unpublished). Others have observed similar or lower values for the $\sim$ ph content of the uterus (see for literature Walaas and Walaas, 1950a). Since the concentration of actomyosin in uterine muscle was also found to be one-seventh that of the skeletal muscle a stoichiometric relationship may be assumed to exist between A.M. and A.T.P. The amount of A.T.P. present is much greater than is used in one contraction cycle, and since resynthesis of A.T.P. goes on with great speed the surplus may serve other purposes, such as to make the system complete and reactive. We calculated the amount of A.T.P. used in one contraction cycle and arrived at the figure of 0.25 micromols $\sim$ ph bond energy per gramme uterus per contraction (Csapo and Gergely, 1950). This value is identical with that calculated by A. V. Hill for skeletal muscle.

When the uterus is not under the influence of oestrogen its high-energy phosphate store is at a low ebb. In immature rabbits and also in adults after castration we found (Menkes and Csapo, 1952) a low concentration of A.T.P. and absence of creatine phosphate (C.P., another energy-rich phosphate ester in muscle from which the depleted A.T.P. is immediately resynthesized through the Lohmann reaction). Since the A.M. concentration is also very low in these conditions and there is no significant muscle function, A.T.P. is split only slightly and can be resynthesized slowly by other means than through C.P. The presence of a supply of A.T.P., thus maintained, without appreciable contractile activity indicates that this substance is essential not only for the contractile function but also for the

"private life," so to speak, of the myometrium. A certain amount of it is obviously required merely to keep the tissue alive.

When during sexual maturation the ovaries become functional and oestrogen production is increased, A.T.P. begins to increase and C.P. appears in significant quantities (Menkes and Csapo, 1952). The same occurs when oestrogen is administered experimentally to ovariectomized animals (Menkes and Csapo, unpublished). The increase of high-energy phosphates in response to oestrogen treatment is much quicker than that of A.M., and their concentration in the muscle reaches a climax while A.M. concentration is still not maximal and function is not yet fully restored. One possible explanation of the early rise of A.T.P. is that it may be involved in the resynthesis of the contractile proteins.

If oestrogen administration is continued, the $\sim$ ph will not increase further. These observations seem to be in contradiction to what one would expect when function is maximal. There is, however, another mechanism which takes care of the energy supply of increased function without increase of the concentration of A.T.P., by controlling the turnover of $\sim$ ph. As shown by Walaas and Walaas (1950b) and by Borell (1951), using P^{32} , oestrogen increases the turnover of the easily hydrolysable phosphate esters of the uterus. Thus we assume that in the castrated or in the immature animal—that is, in the non-functional uterus—both the final contractile system and the energy-yielding system are quite incomplete. If oestrogen is administered, A.T.P. and the turnover of the high $\sim$ ph increase with the simultaneous appearance of C.P. The increase in A.T.P. concentration soon becomes constant while function is still increasing, the extra amount of energy used being compensated for by the increased turnover of $\sim$ ph. A similar phenomenon has been observed in our laboratory to occur in the uterus during pregnancy (Menkes and Csapo, 1952). Until the twentieth day of pregnancy in rabbits the easily hydrolysable phosphate fraction of the uterus gradually increases. No further increase, but rather a decrease, can be observed after this period, which coincides with the beginning of a gradual increase in muscle activity and pharmacological reactivity. Thus we must assume that the low concentration of $\sim$ ph found after the twentieth day of pregnancy or in oestrus is optimal for maximal function, and the increased use of energy is taken care of by increased turnover of $\sim$ ph.

Progesterone, unlike oestrogen, has no activating effect on the uterus of castrates nor a regenerating effect on the final contractile system (Csapo and Corner, 1951). It increases the concentration of the easily hydrolysable phosphate fraction without influencing the A.T.P. concentration. It results, as observed so far, in the accumulation of an unknown compound in this fraction which is neither A.T.P. nor A.D.P. (Menkes and Csapo, unpublished). This effect can be evoked by injected progesterone or by mating (which results in natural progesterone domination through ovulation and corpus luteum formation). It has been shown recently by Walaas (1952) that oestrogens selectively increase the glycogen (and thus the potential $\sim$ ph) content of the myometrium of rats and rabbits. The effect is absent in the endometrium and in skeletal muscle and is independent from the blood-sugar level. He concludes that an increased rate of the hexokinase reaction is responsible for increased glycogen formation resulting from oestrogen effect. He found progesterone to be ineffective on the glycogen concentration of the uterus. All these observations indicate that the ovarian hormones affect the energy-yielding system of the uterus, but much more needs to be done before a more complete understanding can be achieved.

Considering the varying concentrations of actomyosin and of high-energy phosphates in various hormonal conditions of the reproductive tract, we ask how these changes alter myometrial function. For the answer we must turn to living muscle and try to explain its behaviour with the

data obtained at the molecular level. A first step in this direction is to observe the relation between actomyosin concentration in the myometrium and the working capacity of the muscle. The most adequate measure of this function is the isometric tension; that is to say, the force developed by contraction of an electrically stimulated strip of muscle that is not allowed to shorten. Thermodynamic studies of Szent-Györgyi (1951) and his associates on frog skeletal muscle have shown that for adequate information on working capacity isometric tension must be studied rather than isotonic amplitude.

One would expect that under well-standardized and optimal conditions the maximum isometric tension which the muscle is able to develop will depend on its actomyosin concentration. Experiments confirm this. After castration the actomyosin concentration and the maximum isometric tension both fall practically to zero and both are restored in similar fashion by administration of oestrogen. The ratio of actomyosin concentration in the oestrous uterus to that of the castrate is 4.6:1; the ratio of maximum isometric tension in the two conditions is 4.2:1. Progesterone, on the other hand, does not affect either actomyosin concentration or maximum tension (Csapo and Corner, 1953).

An Unexpected Observation

During the course of our work on isometric tension we made an unexpected observation which we think will help in understanding just how the ovarian hormones act upon the tissue which they affect. The answer to this question being of extreme importance in the physiology of reproduction, we summarize here what we have discovered, although the final proofs are still not available.

It is well established that the function, not only of the myometrium but of all other parts of the reproductive tract, is determined in a final sense by the two ovarian hormones, oestrogen and progesterone (see for literature Reynolds, 1949). Removal of both ovaries results in partial atrophy of the whole reproductive tract, including the myometrium. Castrate atrophy can be avoided, or, if it occurs, the original condition can be restored by the administration of oestrogen to the castrated animal. Special histological and physiological changes functionally adapted to pregnancy are brought about by progesterone.

How the ovarian hormones act upon the tissues to produce these conditions is unknown, and, indeed, the hope of understanding their basic action has been slight because of the great variety of their effects upon various elements of the reproductive tract. It has, however, proved helpful to assume that each ovarian hormone has but one type of effect, which shows itself in different ways in various parts of the reproductive tract because the structure and function of the component cellular elements are different. Moreover, the action of oestrogen and progesterone on a specific tissue, often seemingly opposite or antagonistic, may be fundamentally similar, differing only in the direction of the effect produced. This conjecture rests on a more general assumption about hormone action developed by Dr. Albert Szent-Györgyi (personal communication) from studies of the isolated frog heart, which postulates that hormonal effects are exerted upon the ionic balance of the living cell, and thus the same hormone may similarly affect tissues which are quite different in their organized structure and functions.

Maximum Isometric Tension in Relation to Stimuli

Although in our experiments with the final contractile system we followed the effect of the ovarian hormones down to the molecular level, we do not suppose that their effect

actually occurs at this low level of organization and that the hormone directly participates in the individual reactions of which myometrial function is composed. No one, indeed, has ever demonstrated an effect of the sex-gland hormones on any isolated enzyme system, a negative fact which makes it probable that these hormones act at the cellular level, and that their action is to produce favourable or unfavourable conditions for a set of reactions taking place in the cell. Our experiments so far indicate, though they do not

FIG. 1.—Dependence of maximum isometric tension on frequency of stimulation. Electrical stimulation at intervals of $\frac{1}{2}$, 1, 2, and 4 minutes. A, oestrogen-treated castrate; B, castrate treated with oestrogen plus progesterone; C, untreated castrate; D, natural oestrus; E, seven-days pregnancy.

as yet completely prove, that the effect of the ovarian hormones on the myometrium is accomplished by alteration of the potassium balance of the myometrial cells. The new observation from which this conclusion stems is that the maximum isometric tension which a strip of uterine muscle can develop depends on the rate at which successive stimuli are applied. If the rate of stimulation is varied from once every half-minute to once in four minutes, the oestrogen-dominated uterus exhibits a decrease in tension with decreasing rate of stimulation (Fig. 1), whereas the progesterone-dominated uterus increases its tension as the intervals become longer. This difference occurs not only in castrates treated with the respective hormones but also in intact animals under natural conditions—that is, in oestrus and pregnancy. Whether the hormone is supplied by the animal's own ovaries or by infection, the effect is the same. It should be added that the castrate uterus is insensitive to changes in rate, its weak isometric contractions developing the same tension regardless of the intervals.

It is surprising to see that the progesterone-dominated uterus, which completes a single isometric contraction cycle in shorter time than the oestrogen-dominated muscle, requires more rest between two stimuli in order to develop maximum tension. Return to zero tension, therefore, does not indicate complete recovery of the contractile system as a whole in the progesterone uterus. We first believed that the incomplete resynthesis of $\sim$ ph was responsible for the unique behaviour in this hormonal condition. This possibility can still not be completely disregarded in view of the active sodium transport, which may overlast the contraction cycle and which uses a considerable amount of energy (Steinbach, 1952). The suggestion of Ling (1952) also has to be considered, that high-energy phosphates influence muscle function not only because of their stored energy but also because of their high-valency negative charges, which participate in linking cations to the contractile proteins. Their concentration, therefore, will partly determine the cation gradient between the outside and the inside of the cell on which the resting potential depends. A more direct explanation results from the realization that the effect we have been discussing is quite similar to the well-known staircase phenomenon of heart muscle.

Hajdu and Szent-Györgyi (1952) have recently studied the Bowditch phenomenon of the frog heart under isometric conditions, varying the frequency of stimulation in a "steady state." They find that the steep slope of the staircase that develops in regular Ringer-Conway solution can be completely abolished if the potassium concentration of the perfusion fluid is decreased to one-eighth of the usual level.

Referring to the data of Fenn (1937) and Fenn and Cobb (1936), who found that contraction entails a loss of potassium, they assumed that the resting frog heart contains more intracellular potassium than is optimal for the development of maximum tension, and therefore the heart must be stimulated very frequently to exclude the supra-optimal amount of potassium and keep it out. If stimulation is discontinued, intracellular potassium accumulates, the staircase reoccurs, and the tension drops. This effect can be abolished by decreasing the potassium outside, the increasing gradient being unfavourable to the return of potassium. They have also demonstrated that the staircase disappears at 0° C., whereas its slope becomes steeper with increasing temperature. Serum, desoxycorticosterone, and digitalis glucosides have the same effect as the decrease of potassium in the perfusion Ringer. They believe that these substances inhibit the return of potassium to the cell.

The Uterine Staircase

We have shown that the uterus of a castrate rabbit exhibits no staircase if stimulated with constant frequency after a period of rest (Fig. 2). This suggests that the phenomenon

FIG. 2.—Uterine staircase. Maximum isometric tension developed upon stimulation at intervals of 1 minute, beginning after 10 minutes' rest. Castrated mature virgin rabbits; A, treated with oestrogen; B, oestrogen plus progesterone; C, untreated.

is not a fixed characteristic of the myometrium, but rather an expression of the dominant hormonal effect. The oestrogen-dominated uterus has a similar staircase to that of the frog heart, as first observed by Bowditch, whereas the progesterone-dominated uterus behaves in an opposite manner—that is, it has a staircase sloping

in the reverse direction, which will be designated as "negative staircase" in contrast to the regular or "positive staircase" (Csapo and Corner, 1952).

One would expect to see slopes intermediate between the extreme positive and negative staircase if the two hormones are simultaneously administered in various proportions. Experiments have shown this to be the case. For this and similar studies in which quantitative precision is important the frequency of stimulation has to be varied in an otherwise steady state.

The staircase was found to be most negative in early pregnancy. This condition can be imitated in the castrate only if after three days' oestrogen treatment progesterone is administered without oestrogen, a fact which suggests that the latter is not present during the first few days of pregnancy. By the administration of oestrogen during early pregnancy we expected, therefore, to produce a levelling effect on the negative slope of the staircase and also a disturbance of pregnancy. We found that a single dose of 5 μ g. of oestrogen per 4 kg. body weight, administered between the second and fifth days of pregnancy, significantly reduces the slope of the negative staircase and completely abolishes pregnancy (Csapo, 1953).

Effect of Temperature and the Potassium-sodium Ratio

Inasmuch as the staircase so accurately reveals the delicate balance of the ovarian hormones, it is important to learn its nature. In agreement with Hadju and Szent-Györgyi's observation (1952), we found that the positive staircase of the oestrogen uterus disappears at low temperature (Fig. 3), whereas its slope increases with increasing temperature. On the other hand, the negative slope of the

FIG. 3.—Temperature dependence of slope of staircase and negative staircase.

staircase of the progesterone uterus decreases with increasing temperature. Like the frog heart, the oestrogen uterus exhibits an increase in tension within about two minutes if the potassium-sodium ratio is decreased in the bath. The staircase does not disappear, however; its slope is only slightly reduced. If the tissue is subjected to repeated washing for half-hour periods in potassium-free Krebs solution, a gradual decrease in the slope of the staircase can be observed, with a complete disappearance of the staircase after about two hours' treatment, but at the same time the tension also decreases gradually (Csapo, 1953).

If the oestrogen uterus is kept in potassium-free Krebs solution as before and the perfusion fluid is then changed to normal Krebs solution, the potassium-sodium ratio thus being suddenly increased, and if the muscle is stimulated once every half-minute, the tension drops and remains at the low level for about 20 minutes, increasing slowly thereafter. If during this short period of time the staircase is observed a negative slope appears. Thus this rather drastic treatment results in behaviour of the oestrogen uterus similar to that of the progesterone uterus. It is a transitory effect, however, and after the 20 minutes the staircase is positive again (Csapo, 1953).

The effect of alterations in temperature and potassium-sodium ratio can be more easily observed with the progesterone-dominated uterus (Fig. 4). Increase of either

FIG. 4.—Dependence of slope of negative staircase upon potassium concentration and temperature.

temperature or the external potassium-sodium ratio results in gradual disappearance of the negative staircase. At sufficiently high potassium-sodium ratio outside (about fourfold increase in potassium concentration of the original Krebs solution), the negative slope of the staircase with the progesterone uterus not only disappears but there is a slight positive slope, such as is characteristic of the oestrogen uterus. Thus by alterations in the potassium concentration of the perfusion fluid the characteristic behaviour of oestrogen and progesterone uteri with respect to the slopes of their respective staircases can be more or less completely reversed (Csapo, 1953).

A striking difference in the behaviour of oestrogen and progesterone uteri with respect to maximum tension also appears if the potassium-sodium ratio outside is gradually increased (Fig. 5). The oestrogen uterus exhibits a gradual

FIG. 5.—Dependence of maximum isometric tension upon potassium concentration. Each bar represents a group of four contractions in response to stimulation at 1-minute intervals. The K concentration is raised in each group from left to right, from standard or half-standard K concentration of Krebs solution, to 2×, 3×, . . . 10×K. A, castrate treated with oestrogen; B, castrate treated with oestrogen plus progesterone; C, as A; D, strip from same uterus as C, but after 24 hours' storage at 0° C. in Krebs solution of standard K concentration. Note resemblance to B.

decrease in maximum tension with increasing potassium-sodium ratio, whereas the progesterone uterus shows the opposite behaviour up to a threefold increase in the potassium concentration over the original Krebs solution. If the oestrogen uterus is subjected to storage it loses its characteristic behaviour and exhibits a pattern similar to that of the progesterone uterus (Csapo, 1953).

The duration of a single isometric contraction cycle is also greatly dependent on temperature and on the external potassium-sodium ratio. In general, a decrease in temperature increases the duration of both oestrogen and progesterone uteri, but the former has a much greater temperature dependence. Decrease in the potassium-sodium ratio outside increases the duration of the contraction of the oestrogen uterus and steepens the ascending phase of the isometric contraction cycle. The progesterone uterus has no change in duration under similar experimental conditions (Csapo, 1953).

Conclusions

From these experiments one can conclude that oestrogen and progesterone alter myometrial function in opposite directions, as revealed by measurements of the maximum isometric tension. Their effect can be counteracted or even reversed *in vitro* by changing the temperature or the potassium concentration of the perfusion Krebs solution. Whether a different intracellular potassium concentration exists in the uterus *in vivo* in the two contrasting hormonal conditions or as a result of the alteration of the potassium concentration in the perfusion fluid, we cannot say as yet, because we do not know the extent of the extracellular space in the uterus. It is possible that not the absolute quantities of the respective ions, but rather the dynamics of their movements, are changed. If the intracellular potassium concentration is changed as a result of hormonal action, the question ought to be considered whether a change in the distribution of other ions occurs simultaneously as a consequence of this alteration or as an independent effect.

The demonstrated *in vitro* effect of the potassium ion on the myometrial cell cannot be discussed in any detail at present, because the system in which the experiments have been performed is too complex. Hajdu and Szent-Györgyi (1952) proposed that alterations in the potassium balance of the muscle cell directly affect the contractile protein, actomyosin. The effect of potassium is, however, probably more complex. Its extreme importance in activating enzyme reactions, especially those involved in the glycolytic sequence of rabbit skeletal, cardiac, and uterine muscle, has been recently emphasized by Boyer (1953). We know from Walaas's work (1952) that oestrogen controls glycogen formation in the myometrium, perhaps through the hexokinase system.

Thus we have to consider, as one possibility, that the ovarian hormones, through their alteration of the potassium balance of the myometrium, will modify the activity of specific enzyme systems, especially those involved in cellular energetics. Ling (1952) has shown that A.T.P., C.P., and hexosephosphates occupy key positions in muscle function not only because of their stored energy but also because of their high-valency negative charges which link cations (especially potassium) to the contractile and other proteins of the muscle cell. Their concentration therefore determines not only the amount of energy which can be spent immediately during activity but also the gradient of cations between the outside and the inside of the cell, which in turn determines the resting potential of the cell. Thus we see that a change in the potassium balance of the myometrium evoked by the action of the ovarian hormones might easily modify a whole series of intracellular reactions, thus producing great variations in function of the organ as a whole.

This concept may ultimately not only explain the "pregnancy reversal" described 45 years ago by Sir Henry Dale but also provide a key to many other problems of uterine physiology and hormone action.

REFERENCES

- Athias, M. (1919). *J. Physiol. Path. gén.*, **18**, 731.
 Blair, E. (1923). *Amer. J. Physiol.*, **65**, 223.
 Borell, U. (1951). *Acta endocrinol.*, **Kbh.**, **8**, 131.
 Boyer, P. D. (1953). *Potassium Symposium*, Minneapolis. In press.
 Corner, G. W. (1923). *Amer. J. Anat.*, **32**, 345.
 Csapo, A. (1948). *Nature, Lond.*, **162**, 218.
 — (1949). *Acta physiol. scand.*, **19**, 100.
 — (1950a). *Amer. J. Physiol.*, **160**, 46.
 — (1950b). *Ibid.*, **162**, 406.
 — (1953). *Potassium Symposium*, Minneapolis. In press.
 — and Corner, G. W. (1951). *Endocrinology*, **49**, 349.
 — (1952). *Endocrinology*, **51**, 378.
 — (1953). *Science*, **117**, 162.
 Erdős, T., Naesslund, J., and Snellman, O. (1950). *Biochim. biophys. Acta*, **5**, 53.
 — and Gergely, J. (1950). *Nature, Lond.*, **166**, 1078.
 — and Herrmann, H. (1951). *Amer. J. Physiol.*, **165**, 701.
 Cushny, A. R. (1907). *J. Physiol.*, **35**, 1.
 Dale, H. H. (1907). *Ibid.*, **34**, 163.
 Engelhardt, W. A., and Ljubimowa, M. N. (1939). *Nature, Lond.*, **144**, 668.
 Fenn, W. O. (1937). *Amer. J. Physiol.*, **119**, 307.
 — and Cobb, D. M. (1936). *Ibid.*, **115**, 345.
 Hajdu, S., and Szent-Györgyi, A. (1952). *Ibid.*, **168**, 159.
 Kehrer, E. (1907). *Arch. Gynäk.*, **81**, 160.
 — (1908). *Arch. exp. Path. Pharmacol.*, **58**, 366.
 Keye, J. D. (1923). *Bull. Johns Hopk. Hosp.*, **34**, 60.
 Knaus, H. (1930). *Arch. Gynäk.*, **141**, 374. (See for references to a series of papers by this author.)
 Ling, G. N. (1952). *Phosphorus Metabolism*, **2**, 748. Baltimore.
 Lohmann, K. (1938). *Ann. Rev. Biochem.*, **7**, 125.
 Makepeace, A. W., Corner, G. W., and Allen, W. M. (1936). *Amer. J. Physiol.*, **118**, 376.
 Menkes, J. H., and Csapo, A. (1952). *Endocrinology*, **50**, 37.
 Mommaerts, W. F. H. M. (1950). *Muscular Contraction*. New York.
 Needham, D. (1952). *Advanc. Enzymol.*, **13**, 151.
 Reynolds, S. R.-M. (1931). *Amer. J. Physiol.*, **97**, 706.
 — (1949). *Physiology of the Uterus*, 2nd ed. New York.
 — and Allen, W. M. (1932). *Amer. J. Physiol.*, **102**, 39.
 Robson, J. M. (1936). *J. Physiol.*, **88**, 100. (See for references to a series of papers by this author.)
 Snellman, O., and Erdős, T. (1948a). *Biochim. biophys. Acta*, **2**, 650.
 — (1948b). *Ibid.*, **2**, 660.
 Steinbach, H. B. (1952). *Proc. nat. Acad. Sci., Wash.*, **38**, 451.
 Stickel, M. (1913). *Arch. Physiol.*, p. 259.
 Szent-Györgyi, A. (1945). *Acta physiol. scand.*, Suppl. **25**, 9, 1.
 — (1947). *The Chemistry of Muscular Contraction*. New York.
 — (1951). *Ibid.*, 2nd ed. New York.
 Van Dyke, H. B., and Gustavson, R. G. (1929). *J. Pharmacol.*, **37**, 379.
 Walaas, O. (1952). *Acta endocrinol.*, **Kbh.**, **10**, 175.
 — and Walaas, E. (1950a). *Acta physiol. scand.*, **21**, 1.
 — (1950b). *Ibid.*, **21**, 18.
 Weber, H. H. (1935). *Pflügers Arch. ges. Physiol.*, **235**, 205.

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