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ON THE SPECIFIC VASO-DILATING AND PLAIN MUSCLE STIMULATING SUBSTANCES FROM ACCESSORY GENITAL GLANDS IN MAN AND CERTAIN ANIMALS (PROSTAGLANDIN AND VESIGLANDIN)

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Earlier investigations [Euler, 1934*a*, 1935*a*] have shown that extracts and secretions from the human prostate gland and seminal vesicles, as well as seminal fluid, greatly lower the blood pressure after injection into animals and, even in small amounts, stimulate the isolated intestine and the uterus. At about the same time Goldblatt [1933, 1935] independently observed similar actions for human seminal plasma. The active substance is different from hitherto known autopharmacologic active substances, including the "substance P" [Euler & Gaddum, 1931; Gaddum & Schild, 1934]. The latter substance, which is obtained from intestinal muscle and brain, stimulates the isolated intestine and lowers the blood pressure; it has recently been shown to be an albumose-like substance [Euler, 1936].

Biological actions of extracts from the accessory genital glands have been described several times before, but the older observations have received no great attention, probably because the experimental data do not permit of definite conclusions as regards the nature and the specificity of the active constituents.

Thus Japelli and Scafa found in 1906 that extracts of the prostate of the dog caused a rise in blood pressure in the dog on intravenous injection. This action is probably due to adrenaline, since Collip [1929] and Euler [1934*b*] have demonstrated the presence of this substance in considerable amounts in this and related glands. In rabbits a lowering of blood pressure was observed, but since knowledge of depressor substances was limited at that time, it is not possible to draw any conclusion with regard to the nature of the active substance or substances.

Thaon [1907] also found a rise in blood pressure, followed by a fall, in the rabbit, on injection of extracts from the prostate gland of the dog and the bull.

Camus & Gley [1907] found that intravenous injection of secretion from the internal prostate of the hedgehog (taken during a period of sexual activity) killed rabbits even in amounts of 0·3–0·4 c.c. per kg., the animals becoming severely dyspnœic. Biedl [1916] has remarked in this connexion that the toxic effects were probably caused by intravascular clotting, especially as such secretions are known greatly to promote coagulation. Whether or not other active substances took part in the effect is not clear.

Götzl [1910] reports that press juice from human prostate glands (obtained from autopsy material or during operations), after injection into the ear vein of the rabbit, will kill the animal even in amounts of 2 c.c. Intravascular clots were found in these cases, and the blood was afterwards found to be permanently fluid.

The following year Dubois & Boulet [1911] found that water or glycerine extracts of the prostate of the dog, the bull and the sheep caused in most cases—twenty-seven out of thirty—inhibition of the isolated intestine of the dog, cat, rabbit and sheep, and also of the intestine *in situ*. This effect may, so far as is known now, be due either to adrenaline or to adenylic compounds, most probably the former. The same authors found in 1912 that water extracts of the prostate of the dog promoted contraction of the urinary bladder of this animal *in situ*, and believed that the prostate gland produced a hormone with this action. This conception [Dubois & Boulet, 1919] has not yet been confirmed.

Experiments with extracts from a fresh human prostate gland were carried out by Battez & Boulet [1913]. The material was obtained from a man of 20 years who had been executed. After intravenous injection of the extract in the dog, in an amount corresponding to 0·125 g. per kg., a strong depressor action was observed, accompanied by contraction of the bladder.

The first communication on a pharmacodynamic action of human seminal fluid originates from Kurzrok & Lieb [1931]. These authors found, on adding 1 c.c. of seminal fluid to a strip of human uterus suspended in a 100 c.c. bath, either an increase or a decrease in spontaneous movement or tone. Later, Cockrill *et al.* [1935] reported that most specimens of human semen caused inhibition of the isolated human uterus strip (0·4 c.c. semen in 100 c.c.). Some of the specimens caused contraction, but after they had stood for half an hour at *p*H 10 the effect was inhibition. At *p*H 11 all specimens were without action, and this was the case after short boiling. According to these authors, the active substance is dialysable, soluble in alcohol, and biuret negative. After the addition of atropine, the effect is inhibited, whereas it is enhanced by eserine. The observed action corresponds with about 10γ acetylcholine per c.c. of semen, which is a remarkably high content. In support of the opinion of the authors that the active substance is acetylcholine, it is stated that an inhibitory as well as a stimulating effect may be obtained from acetylcholine on the isolated uterus strip, and that both substances show similar properties as regards solubility and stability (both are destroyed in 30 min. at *p*H 11).

The pharmacodynamic effects of human seminal fluid and of secretions from the prostate and the seminal vesicles, found by Goldblatt and by Euler, manifested themselves most clearly in a strong depressor action in various animals and in a stimulating action on certain plain muscle organs. Goldblatt [1935] announces, however, that in his experiments the effect of human seminal plasma on the isolated uterus is probably caused by a substance different from the depressor and gut-stimulating one. This is not in conformity with the results to be reported in the present

paper, but there seems to be no doubt that, in spite of this, both investigators have been dealing with the same active substance.

With regard to the nature of the active substance, it is dialysable and soluble in water, alcohol, acetone, ether and chloroform. The substance is unstable at high temperatures, more so in alkali than in acid. Cataphoresis experiments have shown that the active substance has acid properties. In order to make reference more convenient, and since it is clear that this substance is different from other autopharmacologic substances, it has been preliminarily called "prostaglandin" [Euler, 1935*a*].

In extracts from the vesicular gland of the monkey (*Macacus rhesus* and others) a substance producing a marked fall in blood pressure in the atropinized rabbit could be demonstrated [Euler, 1935*b*]. In certain respects, however, this substance differs distinctly from prostaglandin. It has preliminarily been called "vesiglandin".

In the present paper the occurrence, properties, and biological actions of these new pharmacologically active substances are described so far as they are at present known.

Experimental results

Prostaglandin

(1) *Occurrence and demonstration of prostaglandin.*

During an investigation of various organ extracts for the purpose of finding the distribution of substance P, the observation was made that extracts from the human prostate had a pressor as well as a depressor action on the blood pressure of the atropinized rabbit. The pressor action could be referred to adrenaline, the occurrence of which seems to be widely spread in the accessory genital organs [Euler, 1934*b*]. After inactivation of the adrenaline by means of iodine at neutral or slightly acid reaction, the depressor action was left almost pure, though some loss probably occurred. This treatment also changed the inhibitory action on the rabbit's isolated gut into stimulation. Thus an effect was observed which in the respects mentioned agreed with the action of substance P, and since inactivation experiments pointed in the same direction it was assumed that the biological action of the extracts was due to the presence of substance P. On electrodialysing the active solutions, part of the activity was recovered on the cathode side whereas the anode solution was inactive [Euler, 1934*a*]. Later experiments have indicated, however, that, though a part of the action described is most probably due to the substance P, the greater part of it is caused by another substance. This

substance is carried by the electric current to the anode, but, owing to the development of free halogens, it is there rapidly destroyed, a fact which could be demonstrated with the purified substance (cf. p. 218). Similar effects to those observed in the extracts mentioned, deprived of adrenaline, were found in much higher degree in human seminal fluid and in secretion from the prostate and seminal vesicles obtained from autopsy material. Also in a few cases where the prostates were removed by operation the active substance could be demonstrated in the extracts. The remarkable pharmacodynamic action of human semen is shown by the fact that even 0·05 c.c., injected intravenously in the rabbit after atropinization, causes a prolonged lowering of the blood pressure. An obvious difference was observed in the action of substance P on the blood pressure, the action here being of relatively short duration. The rabbit's isolated gut was stimulated in a similar way as by substance P, i.e. the action consisted in a gradually developing increase in tone, frequently accompanied by a greater amplitude. The assumption that the active principle in prostatic extracts and in human seminal fluid is identical with that of substance P [Euler, 1934*a*] must be abandoned, however, since it has emerged during the purification experiments that the first-mentioned substance is not only soluble in alcohol (like substance P) but also readily soluble in acetone, and that it is not precipitated by ether from an alcoholic solution. The water-soluble part of the residue from the alcohol-ether filtrate shows the same actions as native seminal fluid. From this it is permissible to conclude (1) that human semen contains an active substance not hitherto known, with strong pharmacodynamic actions (prostaglandin), and (2) that this substance is almost solely responsible for the biological actions on the preparations mentioned.

(2) *Purification experiments.*

In most of the experiments human seminal fluid from various sources was used for the purification and test experiments, since this material was very active, on the one hand, and practically free from disturbing and biologically active contamination, on the other.

After the addition of 3–5 volumes of alcohol or acetone and a little hydrochloric acid—in order to bring the fluid to a slightly acid reaction—and filtering, the filtrate was evaporated under low pressure. After drying in the desiccator, the residue was laked with absolute alcohol and the alcoholic solution mixed with 3–5 volumes of dry ether. A precipitate resulted, which was removed by centrifugation from the clear yellowish alcohol-ether solution. The precipitate consisted to a considerable extent of

choline. The solution was evaporated and the residue extracted with water, leaving lipoids behind. The clear watery solution, which contained only a small amount of dry substance, was acidified to *p*H 4, if necessary, in order to make it stable.

From solutions in water the prostaglandin could be extracted by ether or chloroform but only when the solution was distinctly acid (about *p*H 3–4). From an alkaline watery solution practically no activity could be extracted with ether or chloroform. The extraction of the concentrated solutions of prostaglandin with ether was carried out in the following way. After the addition of hydrochloric acid to a reaction of *p*H 3–4, 10 volumes of ether were added and the whole shaken for half an hour. The separated ether phase was afterwards taken down to dryness and the residue laked with water. On drying this watery solution again, it was found that amounts less than 0·05 mg. were sufficient to cause distinct actions on the test preparations, i.e. on the rabbit's blood pressure or on the isolated gut of the rabbit.

In a series of experiments the partition coefficient between ether and water was determined at room temperature. The value of this coefficient in a number of biological tests was 0·07–0·10. This means that, in order to obtain a fairly complete extraction of the prostaglandin from a watery solution, it will be necessary to treat the solution several times with, say, 10 volumes of ether.

(3) *Solubility.*

Goldblatt has reported that the active substance is soluble in alcohol and acetone. Euler found that it was also, to a certain extent, soluble in ether and chloroform, as stated above. The prostaglandin is also soluble in concentrated acetic acid. The solubility in petroleum ether seems to be small. From the above it is clear that the solubility differs widely from that of the substance P, which can be precipitated by ether from an alcoholic solution.

(4) *Precipitation and adsorption.*

Hitherto all efforts to precipitate the prostaglandin by means of metal salts, strychnine or brucine have been without result. Phosphotungstic or reinecke acid did not precipitate appreciable quantities of the active substance from concentrated solutions. It appeared also from the experiments that the active substance was not readily adsorbed on precipitations. Special adsorption experiments with fuller's earth have corroborated this finding, in that 1 g. of fuller's earth in 15 c.c. of a solution

of prostaglandin, shaken intermittently for 2 hours, removes less than 20 p.c. of the activity of the solution. Treatment with fuller's earth, therefore, affords a useful method for the purification of solutions.

(5) *Stability.*

The stability was determined at varying *p*H at 100° C. and after 20 min. heating. The activity which remained after such treatment was determined on the rabbit's blood pressure, as well as on the rabbit's isolated gut. To acidify hydrochloric acid was used, and this was neutralized with sodium hydroxide and vice versa. The activity left after treatment is shown in Table I.

TABLE I

*p*H	Activity left after treatment at 100° C. for 20 min. p.c. of original
0	0
1–7	100
8·5	75
9·5	50
14	0

From the table it is evident that the stability is rather high, even as far on the acid side as corresponds to *p*H 1. On the alkaline side, however, the activity shows a rapid decrease.

(6) *Dialysis, electrodialysis, cataphoresis.*

The prostaglandin dialyses readily through cellophane, parchment or collodion. On electrodialysing active solutions, only traces of the activity could be recovered on examining the anode and the cathode fluid, though the middle chamber was deprived of most of its original activity. The type of electrodialysis apparatus used was a small specimen of the Ostwald type, both electrodes being continuously washed by a small current of distilled water. From these experiments it was clear that the active substance must have been destroyed, and since it will pass out at the anode, the acidity of the solution alone cannot be the cause of its destruction. It could be shown, however, that small amounts of free bromine rapidly destroyed the activity, and since free chlorine is regularly liberated at the anode it seems very likely that this is the cause of the failure of the active substance to appear in the anode solution.

In the cataphoresis experiments the Theorell technique [1934] was used, permitting an exact determination of the migration velocity at the same time. It was found that at an approximately neutral reaction

(pH = 6·54) the active substance went to the anode at a rate of 5·4 × 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}, whereas the fluid on the cathode side was inactive. After 4 hours' cataphoresis with 6 mA. at 20° C. of a purified solution of prostaglandin, the activities in the different segments (see Fig. 1) were determined on the rabbit's blood pressure and on the rabbit's isolated intestine. The activity of the contents in the numbered segments is given as a percentage of the original activity of the solution in Table II below.

TABLE II

Segment	−1 to −3	−4	−5	−6	4+ to +6	+3	+2	+1
Activity	0	30	60	85	100	100	30	15
	Cathode→				←Anode			

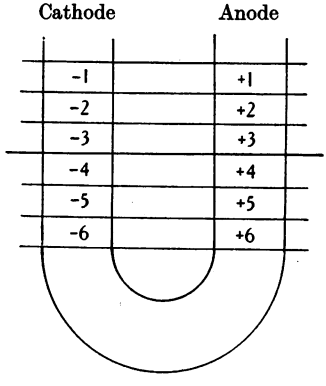

Fig. 1.

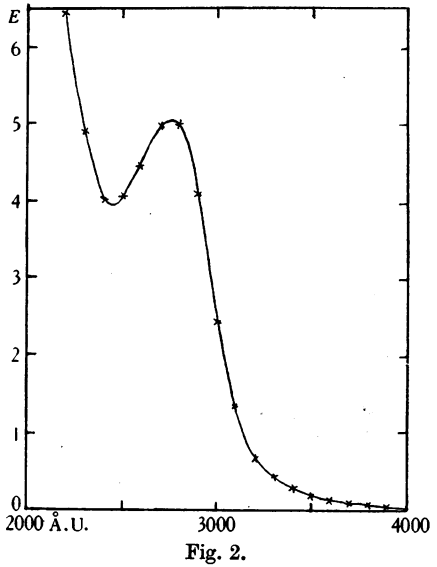

Fig. 2.

Fig. 1. Cataphoresis (Theorell).

Fig. 2. Absorption spectrum in ultra-violet of a purified solution of prostaglandin. Ordinate: $E = \frac{1}{c_1} \cdot \frac{1}{d} \cdot \log \frac{I_0}{I}$, c_1 meaning mg. purified substance per c.c. Abscissa: wave-length in Ångström units.

The prostaglandin thus has acid properties, which affords an explanation of the observation that only from acid watery solutions would ether and chloroform extract the active substance, whereas it was left behind in alkaline solutions. Furthermore, this property is a proof that the active substance cannot be mistaken for pharmacodynamically active substances already known in the body, since probably adenylic acid alone would come into question among these.

The absorption spectrum in ultra-violet of a purified solution of prostaglandin was also determined, Dr H. Theorell kindly doing this for me. The solution contained 2·25 mg. per c.c. and was prepared in the following way. The primary alcoholic extract was evaporated to dryness, the residue laked with a small quantity of absolute alcohol and the clear yellowish solution precipitated with 5 volumes of dry ether. The clear filtrate was dried and the residue taken up in water. This solution was slightly cloudy owing to the presence of lipoids, and was cleared with fuller's earth. The clear watery solution was again dried and submitted to the same treatment as outlined above. The final watery solution from the alcohol-ether filtrate residue was completely clear and colourless. It was active on the rabbit's blood pressure in an amount corresponding to 0·03 mg. The absorption curve is shown in Fig. 2. From the curve it is evident that the purified solution has a marked absorption band in the vicinity of 2750 Å. Whether this band is due to the active principle or to persisting impurities cannot be decided yet, though the high activity of the solution may be taken as evidence for the former assumption.

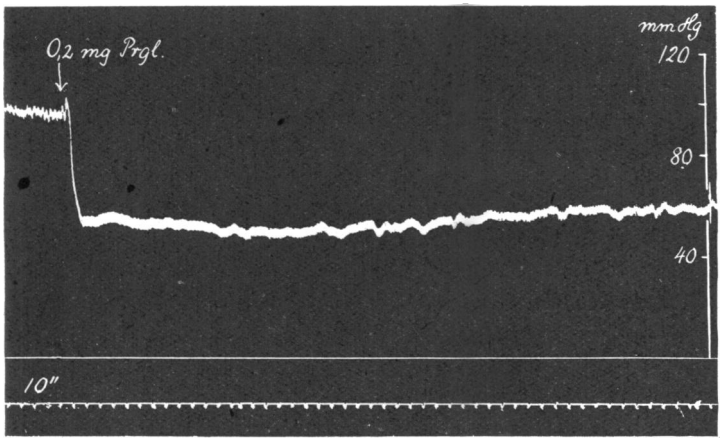

Fig. 3. Rabbit's blood pressure, urethane, atropine. 0·1 c.c. of a solution containing 2 mg. purified prostaglandin per c.c.

(7) *Action on the blood pressure.*

After intravenous injections of human seminal fluid or corresponding amounts of purified solutions of prostaglandin, a prolonged lowering of the blood pressure was observed in all animals tested (rabbit, cat, dog) (Fig. 3). After atropine the effect persisted. Of special interest is the long duration of the depressor action; in some cases as much even as

half an hour elapsed before the blood pressure had reached the original level. In this respect the known depressor substances, acetylcholine, choline, adenosine, histamine or substance P all produce an effect of fairly short duration; kallikrein alone has a prolonged action. This substance however shows no stimulating action on the rabbit's isolated gut and differs also in several other respects from prostaglandin.

(8) *Action on the heart.*

In the blood-pressure experiments, no definite influence was observed on the frequency of the heart or on the blood-pressure amplitude, as recorded with a membrane manometer. In some experiments a cardiometer was placed on the heart *in situ* but no definite changes occurred on

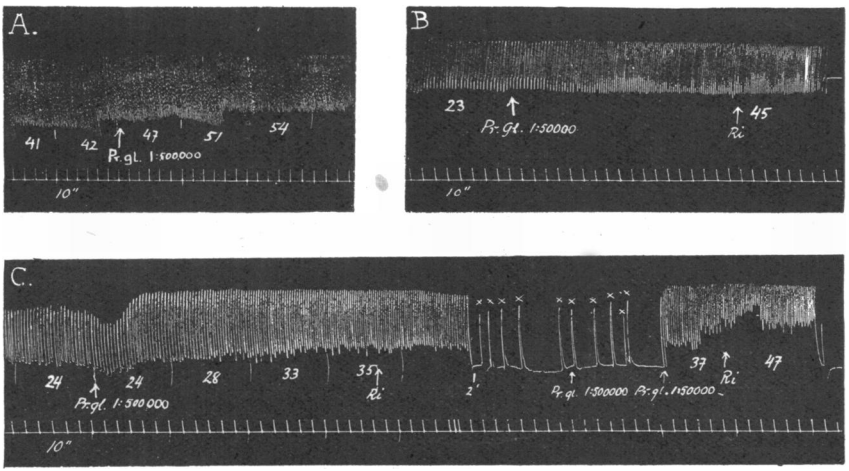

Fig. 4. Frog's isolated heart (Straub). *Prgl.* same preparation as in Fig. 2. Concentrations are shown in the figure. *x* denotes mechanical stimulation. *Ri*, frog's Ringer.

the injection of prostaglandin in amounts which lowered the blood pressure to half its normal value. The addition of corresponding amounts of prostaglandin to fluid perfused through the rabbit's isolated heart by Langendorff's technique did not cause any certain effect either on the frequency or on the amplitude of the contractions. These experiments make it highly improbable that the depressor action is of cardiac origin.

The effect of prostaglandin on the frog's heart was investigated with the Straub preparation. By means of a special arrangement, the heart could be set in communication with four different solutions, the change from one to another being made without loss of time. Frog's Ringer was

used at a pressure of about 5 cm. of water. For these experiments highly purified preparations of prostaglandin were used, of which even 20γ would cause a drop of about 20 p.c. in the rabbit's blood pressure. On changing the Ringer for prostaglandin-Ringer (P.R.) 1 : 500,000, an increase occurred in the heart rate from 24 to 35 as shown in Fig. 4. After adding P.R. 1 : 50,000 the effect was considerably accentuated, the frequency rising from 23 to 45 per min. At the same time a definite change in the type of the contractions could be observed which was characterized by a predominance of the systolic phase and an incomplete diastolic relaxation. In some respects the effect was similar to that caused by an excess of calcium. In one case a heart which had stopped when ordinary Ringer was used was started on simply changing the Ringer for P.R. 1 : 50,000, without any mechanical or other inducements. In this case the effect on the frequency and on the enforcement of the systolic phase was especially evident (Fig. 4C).

(9) *Effect on the peripheral vessels in the frog.*

In a number of cases purified prostaglandin preparations were tested on the peripheral vessels of the hindlimbs of the frog (Laewen-Trendelenburg's preparation). *Rana temporaria* was used throughout and the pressure was 30 cm. water. After the frequency of the drops had become constant, the frog's Ringer was changed for P.R. 1 : 50,000. The Ringer was improved by adding about 10 p.c. of rabbit's heparine plasma to it, and this decreased the tendency to the development of œdema rather strikingly. Shortly after the change to P.R., vaso-dilatation occurred, showing itself in an increase in the number of drops (Table III).

TABLE III

No. of drops per 2 min.	Perfusion fluid
28	Frog's Ringer with 10 p.c. rabbit's heparine plasma
29	" " " " "
34	Same with prostaglandin 1 : 50,000
37	" " "
37	" " "
38	" " "
36	Frog's Ringer alone as above
34	" "
31	" "
26	" "

Though the vaso-dilating power of prostaglandin seldom exceeded the order of that shown in Table III, it could be demonstrated constantly.

(10) *Action on the isolated gut.*

The intestinal bath used was of the type described by Burn & Dale [1922], the capacity of the bath being 30 c.c. The suspension fluid was Tyrode, to which in most cases glucose was added (0·1 p.c.). The oxygenation was maintained by bubbling a mixture of 5 p.c. CO_2 in O_2 through the solution. The temperature of the thermostat was 38° C.

Prostaglandin caused a contraction of considerable duration on the isolated gut in all animals tested (rabbit, guinea-pig, rat, mouse, squirrel), as is seen in Figs. 5, 6, 9, 11, 12. The effect comprises partly an increase in tone, which sets in fairly quickly, though not so rapidly as in the case of acetylcholine or histamine, and partly an increase in the amplitude of the contractions. On the guinea-pig's intestine the difference in the time

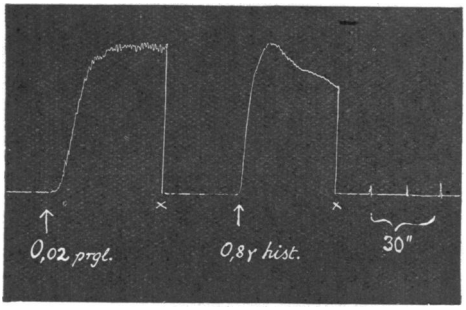

Fig. 5. Guinea-pig's isolated intestine. *Prgl.* purified solution of prostaglandin; *hist.* histamine dihydrochloride.

course between the effect of histamine and that of prostaglandin was clearly evident, as shown in Fig. 5. In no case was the effect on the intestine test preparations from different animals affected distinctly by atropine. After the contraction had reached its maximum, it remained there for a relatively long time, in a way rather similar to that in the case of substance P. With regard to the frequency of the contractions no definite action could be observed.

(11) *Action on the intestine* in vivo.

The stimulating effect of prostaglandin on the gut could also be demonstrated *in vivo*. After subcutaneous injections of 5–10 rabbit doses, one rabbit dose causing a decrease of about 25 p.c. in the blood pressure, in white mice of 20–25 g. weight, a strong effect on the peristalsis of the intestine could be observed in a few minutes, resulting in the passage of semifluid fæces.

(12) *Action on the isolated uterus.*

Prostaglandin was found to stimulate the isolated uterus or strips of uteri of all animals investigated (cow, rabbit, guinea-pig, rat) and also strips of human uteri. The experiments with isolated uteri were performed in the same way as those on the intestine.

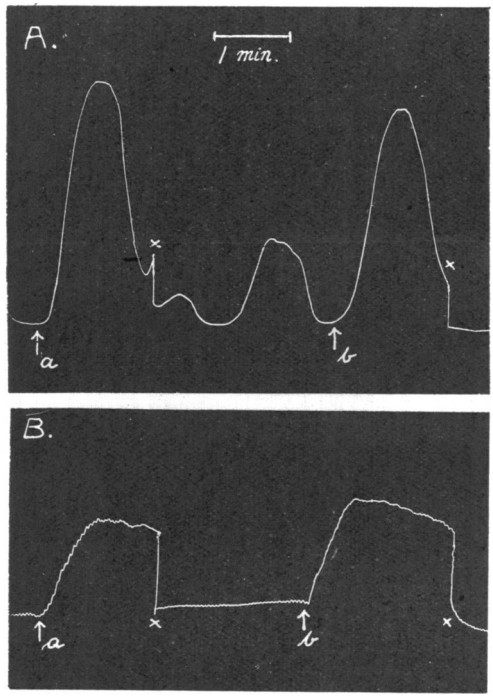

Fig. 6. A, rat's isolated uterus. *a*, 0·22 c.c. secretion from human semina vesicle (autopsy material). *b*, extract of 1·4 g. human prostate (autopsy material). B, rat's isolated intestine. *a* and *b* as in A.

Figs. 6 and 7 show the effect of prostaglandin on uterus preparations of the rat and the rabbit. On the virgin rabbit's or guinea-pig's uterus the effect was small, however, even when rather bigger doses were employed (Fig. 7).

It might be thought that the effect observed on the guinea-pig's uterus was at least partly due to contaminating histamine. That this was not the case could be shown in several ways: (1) the histamine equivalent of the purified solutions used—as determined on the cat's blood pressure —was of a much smaller order; (2) the effect was absent or very weak on the virgin uterus; (3) the effect disappeared after boiling for 20 min. in

normal acid, and this is not the case with histamine. The otherwise disturbing contamination of histamine and choline was chiefly avoided by extraction with ether, which leaves practically all of these substances behind. It could also be shown that, on partial inactivation by means of acids or alkali, the actions on the blood pressure, on the isolated intestine and on the isolated uterus always showed very close agreement. The opinion of Goldblatt [1935] that the active substance in human seminal plasma has no action on the uterus, but that the effect observed is due solely to histamine, is not supported by the present investigation.

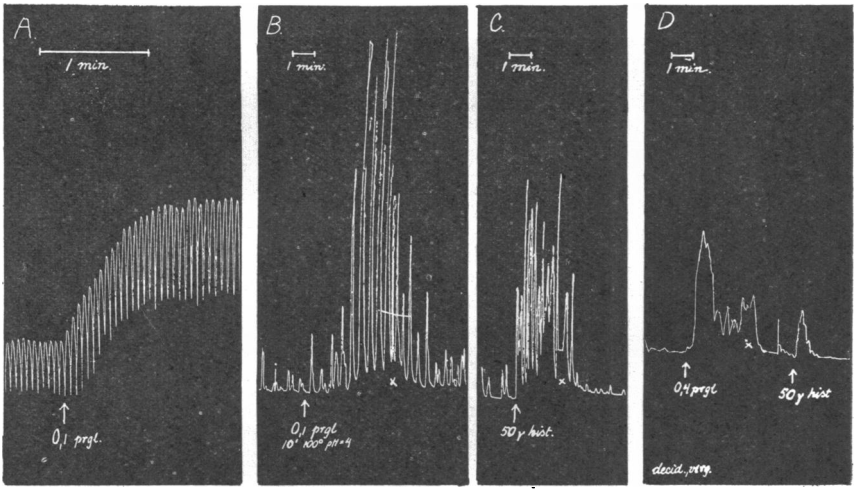

Fig. 7. A, rabbit's isolated intestine, purified solution of prostaglandin. B, rabbit's isolated uterus, same amount of prostaglandin. C, rabbit's isolated uterus, 50γ histamine dihydrochloride. D, rabbit's decidualized uterus (virgin), weak response to big dose of prostaglandin and histamine.

On strips of human uteri a stimulating effect was regularly found, which confirms some of the observations made by Kurzrok and his co-workers [1931, Cockrill *et al.* 1935] and is illustrated in Fig. 8. The last-mentioned authors found in many cases an inhibition, however, but in our experiments this was never the case. Strips of human uteri were tested in five cases. The operation material was kindly placed at my disposal by Prof. A. Westman, Lund. The strips of uteri used in my experiments were in most cases taken from the circular fibres arranged round myomatous nodes. The test pieces were about 4 cm. long and some millimetres thick. In one case a piece consisting of longitudinal fibres was tested, with the same result.

The effect on the human uterus consisted mostly in an increase in the contractions, but in a few cases also a marked increase in the frequency of the contractions could be observed. In the latter case the amplitude of the contractions was not influenced (apparently it was maximal). In one single case only was inhibition observed on a strip of a cow's uterus, such as Kurzrok and co-workers have described for human uteri. The piece on which inhibition was found consisted of longitudinal fibres, whereas another preparation of circular fibres reacted with a contraction. The uterus in question contained a fœtus the size of a hazel nut. In order to try to determine whether the inhibition reaction of the longitudinal

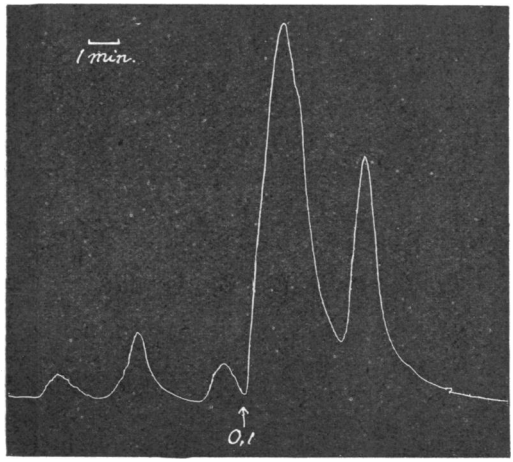

Fig. 8. Longitudinal strip of human uterus, grav., mens. IV. 0·1 c.c. of human seminal fluid.

piece was specific or not, choline and histamine were added to the bath, both substances causing a definite contraction, however. The inhibitory action of prostaglandin in this case was registered repeatedly with the same result, but with other (non-pregnant) preparations a similar reaction was never found, the only answer to prostaglandin being contraction.

(13) *The occurrence of prostaglandin in animals.*

After the demonstration of this new active substance in secretion and extracts from the prostate and seminal vesicles in man, its occurrence in corresponding organs of various animals was searched for. The following organs were investigated and tested for the presence of prostaglandin: prostate of monkey, horse, pig, dog, cat and rabbit; the vesicular gland

of monkey, bull, sheep, pig; the seminal vesicles of guinea-pigs; Cowper's glands of pig and sheep, and also fresh semen from bull and horse (I am indebted to Dr E. Åkerblom for these samples). Of the preparations mentioned, which were tested either in their native condition when this was possible, or in suitably prepared extracts, only the secretion from the vesicular gland of the sheep was found to exert a strong and typical prostaglandin effect on the rabbit's blood pressure and on the isolated gut (Fig. 9). As already stated, the accessory genital glands are relatively rich in adrenaline, but after inactivation of the adrenaline the extracts

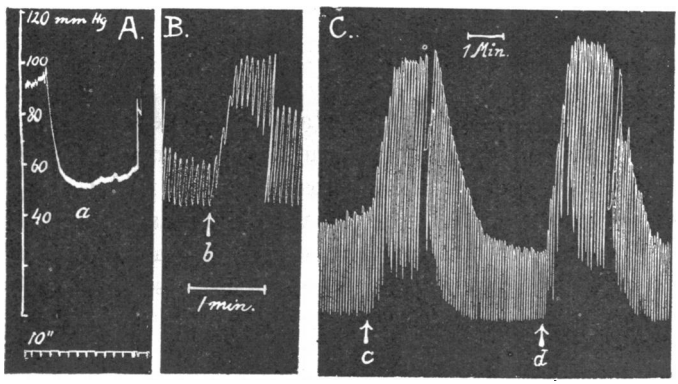

Fig. 9. A, rabbit's blood pressure, urethane, atropine, 0·1 c.c. of secretion from sheep's vesicular gland. B, same amount added to rabbit's isolated intestine. C, *c*, prostaglandin extracted with ether from extract of human seminal fluid; C, *d*, ether extract from 0·1 c.c. of secretion from sheep's vesicular gland. (C, rabbit's isolated intestine.

too showed a prostaglandin effect. The identification of the active substance of the vesicular gland of the sheep with prostaglandin is founded on the following facts: (1) the quantitative relations with regard to the action on the rabbit's blood pressure, the isolated intestine, and on the isolated uterus of the rabbit, agreed with the action of purified prostaglandin prepared from human semen; (2) the active substance is inactivated in the same way by acids and alkali; (3) the active substance from the sheep can be extracted with ether in a similar way as for prostaglandin. These facts are stressed, since it is astonishing that the active substance in question, apart from being found in the secretion of the prostate and seminal vesicles of man, appeared only in the secretion of the vesicular gland of the sheep, and not in the corresponding secretions from the bull, the horse, or the pig. The assumption that the specific substance is produced perhaps only at the time when the animals are on heat is opposed

by the fact that none of the substance was found in the semen of the bull or the horse. In a number of cases the secretion of the vesicular glands of the pig was investigated, where these glands contained more than 600 c.c. fluid, the animals being in a period of sexual activity.

On the other hand, it cannot be doubted that the amount of the active substance in man, as well as in the sheep, varies greatly with fertility age. Thus only small quantities were found in prostates from children or old persons (autopsy material) whereas the same organs or the secretion from men between 20 and 50 were comparatively rich in the same. Similar effects were observed in the sheep, and furthermore the periods of heat showed themselves clearly in the size and the amount of secretion in the vesicular gland. The amount of prostaglandin seemed to follow the amount of secretion.

Whether prostaglandin is totally absent or present only in minute quantities in the secretions of the other animals investigated cannot be decided. In the secretion of the prostate of the rabbit an effect was sometimes observed, but whether this was due to a substance of the type of substance P or prostaglandin is uncertain. At any rate the amount of this substance prevails with a broad margin in the secretions of man and the sheep.

Vesiglandin

(1) *Occurrence and properties.*

With extracts from the vesicular gland of a monkey, *Macacus rhesus*, the observation was made [Euler, 1935*b*] that they caused a marked fall in the blood pressure of the rabbit and other animals. This action, like the action of prostaglandin, was of long duration and was not affected by atropine. The source of the two active substances being similar, it was thought conceivable that they were identical, but the behaviour on the isolated intestine and uterus, as well as inactivation experiments later on, showed that this could not be the case. It thus became clear that the substance from the monkey was different from others and, in order to facilitate reference, it has been called vesiglandin.

Vesiglandin shows a number of properties similar to those of prostaglandin, not only with regard to the effect on the blood pressure but also with regard to solubility and chemistry. It is soluble in water, alcohol, acetone, and is not precipitated by dry ether from an absolute alcoholic solution. It may be extracted by ether from the dry substance or from an acid watery solution. It is dialysable through cellophane. It was not precipitated by phosphotungstic or reinecke acid, nor by silver or lead salts.

On cataphoresis vesiglandin, like prostaglandin, goes to the anode. In electrodialysis experiments no activity was recovered, which is explained by the fact that vesiglandin, like prostaglandin, is rapidly destroyed by the free halogens at the anode.

It might be assumed, however, that, since the two substances have so many similar properties, vesiglandin might be a component of prostaglandin, the former substance having only a depressor action. Against the assumption that prostaglandin is composed of one depressing substance (vesiglandin) and one intestine-uterus-stimulating one, definite evidence is afforded by the fact that the two actions of the prostaglandin are inactivated at the same rate, which is markedly different for vesiglandin, as is shown in Table IV.

TABLE IV. Inactivation of vesiglandin at 100° C. for 20 min. at different reactions

*p*H	Activity left. p.c. of original
0	0
1	10
4	100
7	50
8–8·5	40
9–9·5	20
14	0

From the table it is evident that vesiglandin shows a marked maximum of stability at *p*H 4. When compared with prostaglandin, the stability curve shows definite differences, the stability of prostaglandin being greater, especially at *p*H 1, where none of the activity of the latter substance was destroyed. As practically all the vesiglandin is destroyed at this *p*H under the conditions mentioned, this difference may well be used for discriminating between the two substances. At *p*H 7, too, vesiglandin is definitely less stable than prostaglandin. The biological titration was made on the blood pressure of the atropinized rabbit, and prostaglandin was tested simultaneously in the same way and after the same treatment in order to strengthen the evidence with regard to the difference of the stability of the depressing effect of the substances. The results thus obtained with prostaglandin agreed very well with those previously found.

The material for the preparation of vesiglandin was partly the vesicular glands of monkeys, in extracts of which the new substance was detected, and partly semen from monkeys.[1] Also in extracts from the prostate glands of monkeys (chiefly *Macacus rhesus*) the active substance

[1] On this occasion I wish to thank Prof. Carl G. Hartman, Baltimore, who kindly supplied specimens of monkey's semen, which were taken up in alcohol.

could be demonstrated. In the case of prostaglandin, the secretion of the glands and extracts of them yielded the same active substance, and the same was so for vesiglandin. From this it may be concluded that the activity of the extracts was due to the secretion products of the glands. It follows also that, apart from the vesiglandin, no other pharmacodynamically active substance of importance is present in these glands when tested on the rabbit's blood pressure or the rabbit's gut. The conditions in the monkey are thus rather similar to those in man and in the sheep, with the difference that the active substances show distinct differences biologically as well as chemically.

(2) *Biological actions.*

Of the pharmacodynamical actions of vesiglandin, the effect on the blood pressure of the atropinized rabbit has already been mentioned.

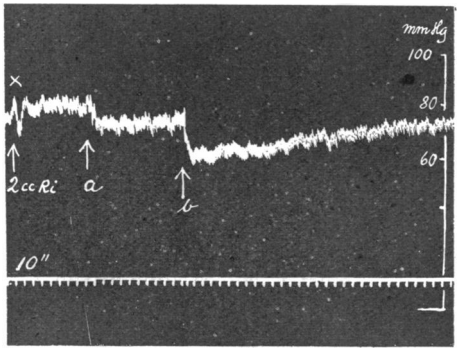

Fig. 10. Rabbit's blood pressure, urethane, atropine. *a*, extract of 4 mg. fresh vesicular gland of *Macacus rhesus*; *b*, extract of 8 mg.

This very much resembles that caused by prostaglandin in that it is of long duration. A more detailed analysis of the effect could not be carried out, as the supply of material was very limited. Fig. 10 shows the effect of vesiglandin on the rabbit's blood pressure after atropine. The amounts of fresh gland which corresponded to the preparations injected intravenously were in this case 4 and 8 mg. respectively. On the injection of an extract corresponding to 20 mg. of the fresh vesicular gland, depression of about 50 p.c. of the normal blood pressure was repeatedly observed.

On pieces of rabbit's isolated gut such amounts as were highly active on the blood pressure mostly caused either a very weak stimulating action or no action at all. Fig. 11 shows the effect of vesiglandin on the isolated

gut of the squirrel (which reacts like the rabbit in this respect) in comparison with that of a dose of prostaglandin having the same depressor effect.

It seemed to be of special interest to compare the effect of vesiglandin with that of prostaglandin on the uterus. Active preparations were

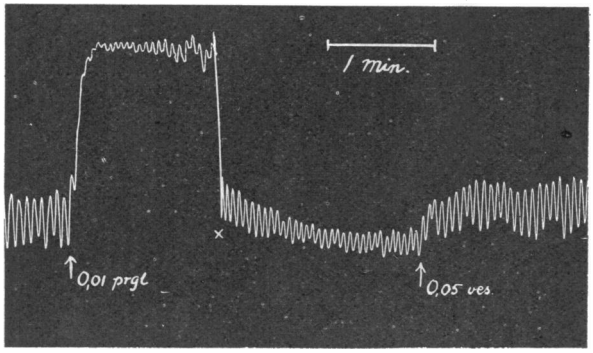

Fig. 11. Squirrel's isolated intestine. Purified solutions of prostaglandin and vesiglandin having equivalent actions on the blood pressure of the atropinized rabbit.

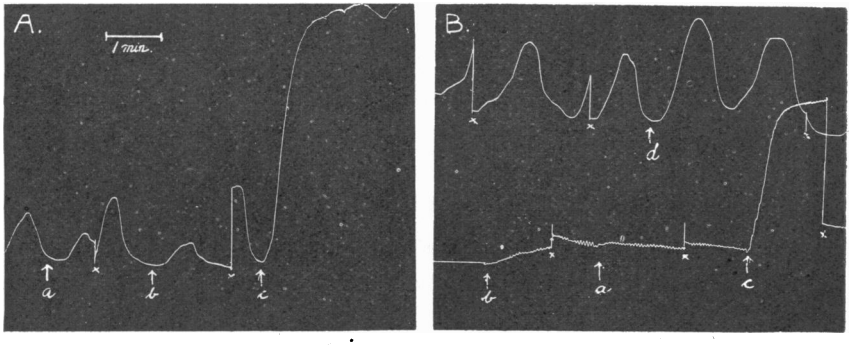

Fig. 12. A, rat's isolated uterus. B, upper tracing, rat's uterus, lower tracing, rat's intestine. *a*, 10γ histamine dihydrochloride; *b*, extract of 13 mg. vesicular gland of *Macacus rhesus*; *c*, depressor equivalent amount of prostaglandin; *d*, same as *c*, treated with NaOH *N*/1 for 5 min. at 100° C.

tested on the isolated uterus of the rabbit, the rat and the guinea-pig. On the rabbit's isolated uterus a stimulating action could be observed in some cases, but this was only about one-tenth of that of prostaglandin, comparing doses with the same depressor action. The action on the uterus agreed well with that on the intestine in these cases, and it is conceivable

that the preparations of vesiglandin contain a small amount of prostaglandin, a suggestion which does not seem unlikely in view of the fact that both substances show certain resemblances in biological and chemical respects and also with regard to their origin. Fig. 12 shows the effect of equivalent depressor amounts of vesiglandin and prostaglandin on the isolated intestine and uterus of the rat. On the guinea-pig's uterus only an indistinct effect was observed.

From these observations it follows that, though vesiglandin has effects on the blood pressure similar to those of prostaglandin, its action on certain plain muscle organs is either small or absent.

TABLE V. A schematic survey of some properties of certain biological "depressor substances"

		Solubility					(Rabbit) Biological actions			
						Maximal		Isolated		Influence
Substance	Dialysability	Water	Alcohol	Acetone	Ether	stability at 100°, *p*H	Blood pressure	Jejun.	Uterus	of atropine
Kallikrein	−	+	−	−	−	*	−†	0	0	0
Adenosine	+	+	−	−	−	7–14	−	−	+	0
Acetylcholine	+	+	+	(−)	−	4	−	+	+	+
Histamine	+	+	+	(−)	−	0–7	(+)	0	0	0
Substance P	+	+	+	(+)	−	1–7	−	+	+	0
Prostaglandin	+	+	+	+	+	1–7	−†	+†	+	0
Vesiglandin	+	+	+	+	+	4	−†	0	0	0

* Unstable at 100°. † Prolonged action.

In Table V some properties of a number of biologically occurring "depressor substances" are summarized in order to show some characteristic differences with regard to solubility, stability and biological actions.

DISCUSSION

The occurrence of two substances, here called prostaglandin and vesiglandin, which were unknown up to a few years ago, and which exert remarkable biological actions in small amounts, in the secretion of accessory genital glands of man and certain animals, must be considered to point to a physiological function of these substances. From the experience available at present, it seems that the occurrence in these organs is specific, as it has not been possible to demonstrate a similar action in reasonable quantities of other organs. From the location of the active substances in the organism, the possible significance for the sexual activity as a whole or in certain details suggests itself. The chief actions observed, vaso-dilatation and strong contraction of plain muscle, are both intimately connected with the sexual functions. In spite of the fact that a physiological function of the substances is merely hypothetical as yet, it

may be suggested, on account of the presence of the substances in the secretion of certain genital glands, that they act as a stimulus for the emptying of these glands when the substances have accumulated sufficiently. It is perhaps also conceivable that after being produced in the gland the substances are absorbed into the blood, and act from there in some way or other. In both cases the substance would act as a sort of automatic regulator for the emptying of the glands. The testing of these possible physiological functions is rather difficult, however, especially in view of the important psychical factors which to a great extent regulate the sexual functions. In favour of the idea of a chemical stimulation by the secretion products it might be mentioned that the emptying of these glands is known to decrease greatly for some time their tendency to contract until the new-formed secretion products have accumulated sufficiently again. It is, however, difficult at present to see why such a substance should occur only in certain animals and in man.

Summary

In secretion and extracts from the prostate and seminal vesicles of man and the vesicular gland of the sheep a pharmacodynamically highly active substance, prostaglandin, has been demonstrated and described in respect of certain biological and chemical properties.

The substance which is soluble in water, alcohol, acetone, and, under certain conditions, in ether and chloroform, is very stable at *p*H 1–7 but is readily destroyed in normal acid and alkali, and also by free halogens.

In cataphoresis experiments prostaglandin goes to the anode with a migration velocity of $5{\cdot}4 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ volt^{-1} at *p*H 6·54.

It dilates the vessels of the hindlimbs of the frog and lowers the blood pressure of the rabbit, the cat and the dog.

It does not greatly affect the mammalian heart, but causes an increased frequency and predominance of the systolic phase of the frog's isolated heart.

The activity of the intestine of various animals is increased *in vitro* and *in vivo*.

The activity of the isolated uterus of various animals is enhanced, and this is also true for strips of human uterus.

The biological actions are not abolished by atropine.

Prostaglandin could not be traced in ejaculates from the horse or the bull, nor in secretions from the pig.

In semen or extracts from the vesicular gland or the prostate of the monkey (different kinds) another highly active substance was demonstrated (vesiglandin).

Like prostaglandin, vesiglandin is acid in character and shows similar properties with regard to solubility. It is, however, less stable in acids and alkali than prostaglandin.

Vesiglandin causes a lowering of the blood pressure of the atropinized rabbit, but shows no or only a weak effect on the isolated intestine of the rabbit or guinea-pig.

The possible function of these substances in the regulation of the expulsion of secretions from the accessory genital glands is briefly discussed.

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