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THE PHARMACOLOGICAL ACTIONS OF DIETHYL-STILBOESTROL AND OTHER OESTROGENIC AND NON-OESTROGENIC SUBSTANCES

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It is well known that oestrogens have a marked effect on certain pharmacological properties of the uterine smooth muscle. Thus, the spontaneous rhythmic contractions and the response of the uterine muscle to the oxytocic hormone of the posterior pituitary lobe are both increased in animals treated with oestrin [Reynolds, 1937; Robson, 1934]. Recently Dodds, Goldberg, Lawson & Robinson [1938] have described a synthetic substance diethyl-stilboestrol which has a very high oestrogenic activity, and since the sodium salt of this substance can easily be formed and is water soluble, it was decided to investigate its effects on the uterine muscle. The unexpected and potent inhibitory actions obtained in the preliminary experiments led to a more extensive investigation of the pharmacological activities of this substance and especially of its actions on various types of smooth muscle in the body.

Experiments on the action of stilboestrol on smooth muscle in vitro

The effects of the drug were determined on the uterus and small intestine of the rabbit and the guinea-pig; on the uterus of the rat; on the small intestine of the frog; and on the rabbit's aorta (spiral preparation). All the mammalian smooth muscle preparations were suspended in 100 c.c. baths of oxygenated Ringer-Locke solution at 37·5° C. and records taken on a smoked drum. The small intestine of the frog was suspended in a 10 c.c. bath of oxygenated frog's Ringer at room temperature.

Experiments were performed with three different samples of diethyl-stilboestrol and the effects obtained when the samples were tested on the same preparations of smooth muscle proved to be identical within the limits of experimental error. We are indebted to Dr Carr of B.D.H. Ltd. and Dr Neumann of Schering Ltd. for supplies of the drug.

Fresh solutions of stilboestrol were made up for each experiment: 5 mg. were dissolved in 3 c.c. of warm *N*/10 NaOH, diluted with Ringer-Locke, and sufficient of the dilution added to the bath to make up the required concentration. Control experiments showed that none of the effects ascribed to stilboestrol were due to changes in *p*H. When the stilboestrol was first diluted with Ringer-Locke a clear solution was obtained, but this usually became cloudy in some 20 min., forming a colloidal suspension. There was no demonstrable difference between the activity of the clear solution and of the colloidal suspension.

It was found that stilboestrol produced an inhibitory effect on all types of smooth muscle tested. This inhibitory effect was exerted both on the spontaneous rhythmic contractions, and on the tone of the muscle. Following the addition of the drug to the bath, the spontaneous contractions decreased or ceased altogether, and the tone was diminished. With large doses these effects came on very quickly and the spontaneous contractions ceased altogether, whilst with smaller concentrations the effects came on more gradually, and the inhibition of movement was only partial. The concentrations of the drug needed to inhibit the different types of smooth muscle investigated varied somewhat, though most preparations showed very definite inhibition with concentrations of 1/1,000,000. Such an effect is illustrated in Fig. 1, which shows the action of this concentration of stilboestrol on the small intestine of the rabbit. The threshold doses for the various tissues are shown in Table I, and it is noteworthy that the rat's uterus is the most sensitive, showing some inhibition with concentrations of 1/10,000,000. A further feature of the action of stilboestrol was its effect on the response of smooth muscle to drugs. The oestrogen decreased or completely abolished the response of smooth muscle to drugs which usually caused contraction. The degree of inhibition depended on the doses used. Such an effect is illustrated in Fig. 1, which shows that the action of a dose of 0·5 μg. of acetylcholine on the rabbit's small intestine was totally abolished in the presence of a concentration of stilboestrol of 1/1,000,000. The whole series of experi-

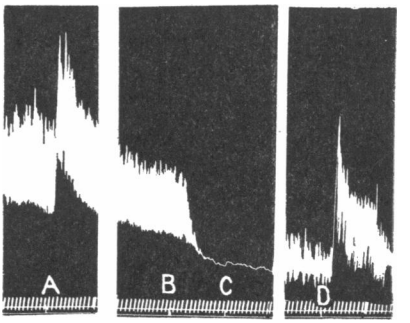

Fig. 1. Rabbit's small intestine *in vitro*. Showing the effect of stilboestrol on the rhythmic contractions and response to acetylcholine. A, C, D, 0·5 μg. acetylcholine added to bath. B, Stilboestrol 1 in 10^6 added to bath. Time intervals, 1 min. Intervals, A–B, 10 min.; C–D, 20 min.

TABLE I. Concentration of stilboestrol (negative logarithm) which inhibits *in vitro*

Tissue	Rhythmic contractions and tone	Response to acetylcholine	Response to adrenaline	Response to pituitary (posterior lobe) extract	Response to histamine	Response to $BaCl_2$
Small intestine of rabbit	6·4	6·4	—	>5·7	>6	6·3
Uterus of rabbit	6	>5·7	>5·7	6	—	—
Uterus of rat	7	6·7	—	6·7	—	>6
Small intestine of guinea-pig	6	6	—	—	—	6
Uterus of guinea-pig	6	—	—	6	6	—
Frog's small intestine	6·3	6	—	—	—	—

ments is summarized in Table I, which gives all the types of smooth muscle investigated. This table shows that the motor response to all the drugs tested was abolished or diminished by the concentrations of stilboestrol sufficient to produce a marked diminution in the spontaneous activity. The doses of the drugs used to produce a motor effect were so adjusted for each tissue as not greatly to exceed the amounts necessary to produce the threshold response.

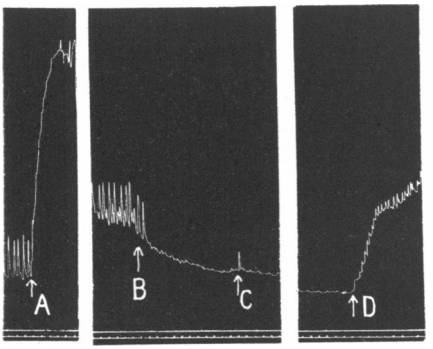

Fig. 2. Guinea-pig's uterus *in vitro*. Showing the effect of stilboestrol on the rhythmic contractions and response to pituitary (posterior lobe) extract. A, C, D, 0·5 unit pituitary (posterior lobe) extract added to bath. B, Stilboestrol 1 in 10^5 added to bath. Solution changed between A and B, C and D. Time intervals, 1 min. Intervals, A–B, 10 min.; C–D, 40 min.

The effects of stilboestrol on the spontaneous contractions, on the tone, and on the responses to motor drugs are slowly reversible on changing the solution. In some experiments the muscle returned almost to its normal condition within half an hour of changing the solution, but in other experiments the action of stilboestrol was more persistent. This was especially the case when high concentrations of stilboestrol had been added to the bath. This return to normal is illustrated in Figs. 1 and 2.

The effect of stilboestrol on the reaction of smooth muscle to drugs which usually produce an inhibitory effect was also investigated. The results are complicated by the fact that stilboestrol itself produces inhibition, and that therefore only small concentrations of the drug could be used. The results suggest, however, that stilboestrol does not interfere with the action of drugs producing inhibition.

The effect of stilboestrol on the response of smooth muscle to direct electrical stimulation was also investigated, the small intestine of the

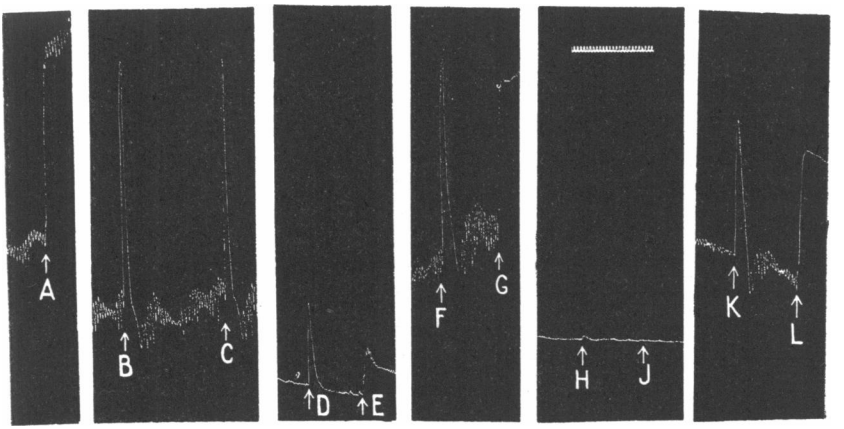

Fig. 3. Rabbit's small intestine *in vitro*. Showing the effect of stilboestrol on the response to direct electrical stimulation and to acetylcholine. B, C, D, F, H, K, Direct electrical stimulation. A, E, G, J, L, 0·5 μg. acetylcholine added to bath. Between C and D, Stilboestrol 1 in $10^{5\cdot7}$ added to bath. Between G and H, Stilboestrol 1 in $10^{5\cdot3}$ added to bath. Solution changes between A and B, E and F, G and H (previous to addition of stilboestrol), and J and K. Time intervals, 5 sec.

rabbit being used. The method of stimulation was similar to that described by Prasad [1935]. Two lamps in series acted as a potential divider, giving 40–50 V. A.C., which was led to the muscle through a variable resistance. It was found that stilboestrol inhibited the response of smooth muscle to electrical stimulation, though this effect appeared to be rather less than the effect on the response of the same muscle to acetylcholine. Fig. 3 illustrates this inhibition.

The effect of stilboestrol on the smooth muscle of blood vessels was also investigated by perfusion of the rabbit's ear, the method used being that recently described by Gaddum & Kwiatkowski [1938]. This experiment showed that the addition of stilboestrol (1/10,000) was followed by an increase in the perfusion rate due to vaso-dilatation of the blood vessels.

Experiments on cardiac muscle. The action of stilboestrol was investigated on the cardiac muscle of the rabbit and of the frog. The frog's isolated auricle strip was suspended in a 10 c.c. bath of oxygenated frog's Ringer at room temperature, and the contractions recorded on a smoked drum with a light balanced lever. In the case of the rabbit the isolated auricle preparation described by Chang [1937] was used, the temperature of the bath being maintained at 27° C. No demonstrable effects on the rate or amplitude of the spontaneous contractions of the auricle preparations were produced by concentrations of stilboestrol up to 1/50,000, i.e. with concentrations some twenty times greater than those usually effective for smooth muscle.

Actions of allied substances in vitro. The question arose whether the inhibitory action of stilboestrol on smooth muscle was related to its oestrogenic activity, and in order to investigate this question, experiments were performed to determine the effects on the isolated rabbit's intestine of a number of other substances, namely: of oestradiol, progesterone, testosterone; of phenol, triphenyl ethylene and triphenyl chlorethylene; of triphenyl vinyl alcohol, α-phenyl cinnamic acid, and acetyl α-phenyl cinnamic acid; and of benzpyrene. We are indebted to Dr Macbeth of Organon Ltd. for supplies of oestradiol and progesterone and to Dr Miescher of Ciba Ltd. for the supply of testosterone. The substances were in all cases dissolved in absolute alcohol, and sufficient of this solution (0·1–0·3 c.c.) added to the bath (100 c.c.) to produce the desired concentration; a colloidol suspension was in all cases formed. The possibility that alcohol produced any of the observed effects was always excluded. In each experiment the effects of the substance under investigation were compared with those of stilboestrol. It was found that oestradiol caused, in a concentration of 1/1,000,000, a slight inhibition of the spontaneous rhythmic activity of the isolated intestine, and a slight diminution of the motor response to acetylcholine: in concentrations of 1/200,000, however, it caused an inhibition of spontaneous rhythmic activity and of the motor response to acetylcholine comparable to that produced by a concentration of stilboestrol of 1/1,000,000. These results are illustrated in Fig. 4.

Progesterone produced an effect on the spontaneous contractions and response to acetylcholine similar to oestradiol, while testosterone had little or no effect on either in concentrations of 1/200,000.

Phenol, when added in a concentration of 1/500,000, caused a slight augmentation of the spontaneous rhythmic movements of the gut and in concentrations of 1/100,000 markedly augmented the contractions.

Triphenyl ethylene and triphenyl chlor-ethylene in concentrations of 1/1,000,000 had no demonstrable effect on the spontaneous rhythmic movements of the gut; in concentrations of 1/200,000, however, there was a slight motor effect: but in neither case was there any effect on the extent of the motor response to acetylcholine.

Triphenyl vinyl alcohol, α-phenyl cinnamic acid and acetyl α-phenyl cinnamic acid all had no effect on the spontaneous movements or tone of the isolated gut in concentrations of 1/1,000,000.

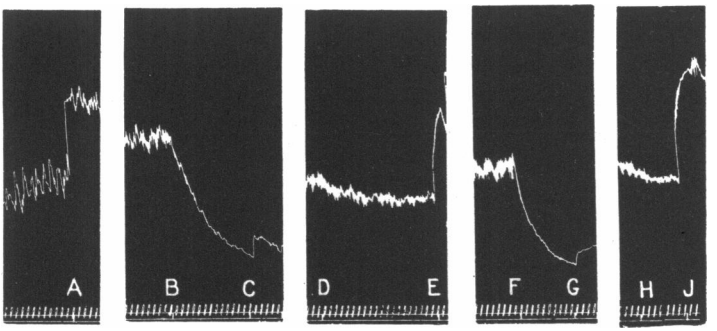

Fig. 4. Rabbit's small intestine *in vitro*. Comparing the effects of oestradiol and stilboestrol. A, C, E, G, J, 2 μg. acetylcholine added to bath. B, Stilboestrol 1 in 10^6 added to bath. D, Oestradiol 1 in 10^6 (in 0·06 c.c. alcohol) added to bath. F, Oestradiol 1 in $10^{5\cdot3}$ (in 0·3 c.c. alcohol) added to bath. H, 0·3 c.c. alcohol added to bath. Solution changed between A and B, C and D, E and F, and G and H. Time intervals, 1 min.

The carcinogenic substance benzpyrene when added to the bath in concentrations of 1/200,000 had no effect on either the spontaneous rhythmic activity and tone or on the motor response to acetylcholine.

Experiments in vivo

In view of the results obtained with stilboestrol *in vitro* it seemed desirable to determine whether similar results could be produced on smooth muscle in the intact animal by the administration of the drug intravenously.

Methods. Cats were investigated under chloralose anaesthesia and rabbits under nembutal anaesthesia, except for the investigation of the uterine movements, when ether anaesthesia was used. The blood pressure was recorded with the vagi cut. The intestinal contractions were recorded by means of a Cushny myograph similar to that previously used for recording the longitudinal contractions of the uterus [Robson & Schild,

1938], while the contractions of the rabbit's uterus were recorded by means of a cannula introduced through the cervix [Robson, 1935].

The intravenous injection of stilboestrol causes a temporary fall in blood pressure. Even when the doses of the drug are large (up to 10 mg.) this fall is not great, and only lasts for a few minutes. The effect is illustrated in Fig. 5. The effects on the movements of the small intestine and of the uterus of a similarly large dose of stilboestrol are also small and of short duration. Furthermore, no effect on the response of these smooth muscle systems to drugs could be observed. Thus the vasomotor reaction to various doses of adrenaline was the same before and after the administration of stilboestrol and the response of the uterus to pituitary (posterior lobe) extract also remained unaffected by large doses of stilboestrol.

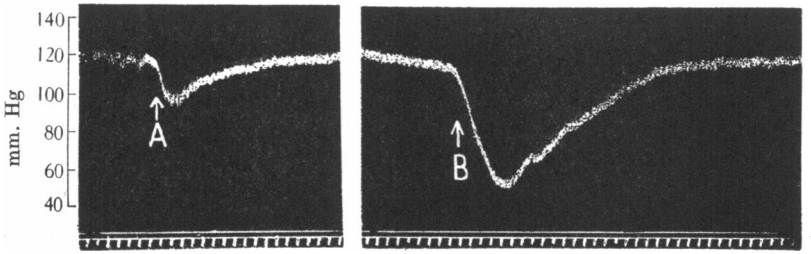

Fig. 5. Cat's carotid blood pressure. A, 1 mg. stilboestrol injected intravenously. B, 5 mg. stilboestrol injected intravenously. Time intervals, 5 sec.

Moreover, the contractions of the nictitating membrane of the cat produced by various doses of adrenaline were not affected by the intravenous injection of the same large doses of stilboestrol. In all these experiments an interval of a few minutes was allowed to elapse after the intravenous injection of stilboestrol before the various doses of the other drugs were given.

These results suggested that when stilboestrol is injected intravenously it is in some way very rapidly inactivated or removed from the circulation, so that the injection of even large doses produces only small and temporary effects on the activity of smooth muscle under the experimental conditions described. In a further experiment, therefore, the drug was injected intravenously at the constant rate of 1 mg./min. into a cat weighing 3·5 kg. It was found that this had no effect on the blood pressure, nor was the response to adrenaline of the vascular system or of the nictitating membrane decreased during the period of the infusion. It therefore seemed desirable to produce a high local concentration of the drug by introducing it into the circulation at a point as near as possible to the organ under investigation.

Experiments on perfusion of intact organs

Method. The experiments were performed on cats under chloralose anaesthesia. A loop of the small intestine was perfused through a branch

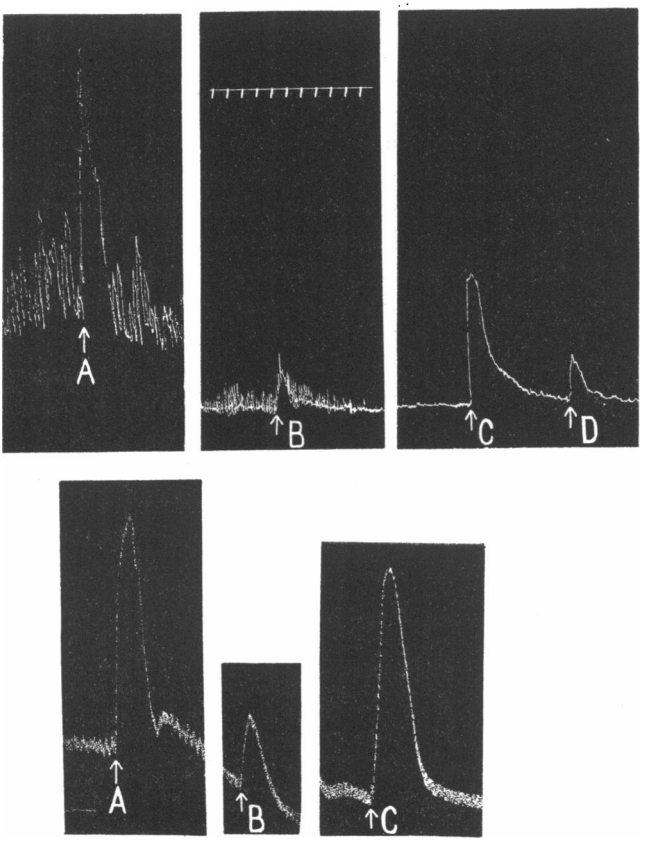

Fig. 6. Showing the effects of stilboestrol on the perfused intestinal loop and on the perfusion pressure (cat). Upper tracings, contractions of perfused loop. A, acetylcholine 10μg. injected into perfusion cannula. Between A and B, 20 μg. of stilboestrol were added to the perfusion reservoir. B, 50 μg. acetylcholine injected into perfusion cannula. C, 50 μg. acetylcholine injected into perfusion cannula. D, 10 μg. acetylcholine injected into perfusion cannula. Lower tracings, perfusion pressure. A, B, C, 1 μg. adrenaline injected into perfusion cannula. Between A and B, 20 mg. of stilboestrol were added to the perfusion reservoir. Time intervals, 30 sec.

of the mesenteric artery by means of a perfusion pump similar to that previously used for the perfusion of the uterus [Robson & Schild, 1938]. The blood for the reservoir was taken from the carotid artery, and the

rate of blood flow from the carotid into the reservoir was automatically controlled by the following device. The carotid cannula, the reservoir, and the pressure tubing connecting the two were made into a closed system filled with blood, with an air pocket in the reservoir, and as the blood was emptied by the pump from the reservoir it was automatically filled again from the carotid. The perfusion pressure was recorded on the kymograph as well as the contractions of the perfused loop. The blood from the perfused loop returned into the general circulation. Stilboestrol was added to the reservoir in the usual solution in alkaline Ringer-Locke. Control experiments showed that the results were not due to the small amount of alkali introduced. Other drugs were directly injected by means of a syringe into the cannula in the mesenteric artery. Clotting was prevented by means of injections of heparin. That the loop was completely perfused was proved at the end of the experiment by the addition of methylene blue to the perfusion fluid.

Results. The addition of 10–20 mg. of stilboestrol to the perfusion reservoir led to a prolonged fall of the perfusion pressure and to a depression of the contractions of the perfused intestinal loop. The vasomotor response obtained when adrenaline was injected into the perfusion cannula and the contractions of the intestinal loop brought about by the injection of acetylcholine were both decreased in the presence of stilboestrol. These effects are illustrated in Fig. 6.

Discussion

The experiments show that diethyl-stilboestrol produces a marked inhibitory effect on smooth muscle, affecting not only the spontaneous activity and tone but also its motor response to a variety of drugs and to direct electrical stimulation. Since these effects are produced by comparatively high dilutions of the drug and since they are entirely reversible it would appear that they are not due to a toxic action which results in injury to the tissue. The fact that the response of the muscle to barium chloride and to direct electrical stimulation are both inhibited suggests that the action of the drug is exerted directly on the muscle, since it is generally accepted that barium chloride and electrical stimulation act directly on the muscle. The results, indeed, suggest that diethyl-stilboestrol produces a reversible inhibitory effect on the contractile mechanism of smooth muscle, though the nature of this effect requires further investigation. Why the contractions of cardiac muscle remain unaffected by much larger concentrations of the drug can at present only be a subject of speculation, but these results show that the drug does not merely

exert a general depressant action such as is produced by alcohol and anaesthetics.

The results show that the inhibitory action on smooth muscle produced by certain compounds is not necessarily correlated with the oestrogenic activity of these compounds. Thus, although oestradiol has an oestrogenic activity equal to, if not greater than, stilboestrol, its inhibitory effect on smooth muscle is much less; further, progesterone, which has no oestrogenic activity, exerts a definite inhibitory action on smooth muscle equal to that of oestradiol. Moreover, the investigation of action of smooth muscle has revealed definite differences between the pharmacological action of certain types of oestrogens: thus the synthetic oestrogen stilboestrol has a much more potent action than a natural oestrogen oestradiol, while two other synthetic compounds, triphenyl ethylene and triphenyl chlor-ethylene, not only do not inhibit smooth muscle but even possess a slight motor effect.

Another interesting feature of the actions of stilboestrol is the wide difference between the potency of its effects on smooth muscle *in vitro* and *in vivo*. Thus while a concentration of the drug of 1/1,000,000 added to the bath produces a marked and prolonged effect, the intravenous injection of 5 mg. giving an approximate blood concentration of 1 in 20,000 produces only a small and transitory effect or no effect at all. There are three possible explanations for this discrepancy in the apparent effective concentrations, namely: (1) that the drug is rapidly broken down in the blood and tissues, though this appears unlikely in view of the known duration of the oestrogenic effect of stilboestrol, (2) that the drug is removed from the circulation or inactivated by a definite organ or organs (e.g. the liver), and (3) that the drug is in some way rapidly rendered inactive in the blood. The fact that in the experiments on the perfusion of an intestinal loop concentrations of stilboestrol of the order of 1/3000 produced effects less than are produced by the concentration of the drug *in vitro* of 1/1,000,000 offers strong support to the view that, in so far as its pharmacological activity on smooth muscle is concerned, the drug is rapidly inactivated in the blood stream.

The question arises whether the pharmacological actions of the drug are of any significance in its clinical use. Since the action in the intact animal is so much smaller than that on the isolated organs it seems unlikely that, in the doses at present used, any appreciable pharmacological effect could be produced on smooth muscle. There is thus not much likelihood that the drug can be used as an antispasmodic, nor is it likely that any toxic effects are to be expected either through an action on the

heart or on smooth muscle. Clinical observation has shown that oral administration of stilboestrol occasionally produces nausea and vomiting. Our experiments show that the drug is a powerful inhibitor of plain muscle, but we cannot suggest any connexion between this fact and the side effects mentioned above.

SUMMARY

1. Rabbit's, guinea-pig's and frog's small intestine and rabbit's, guinea-pig's and rat's uteri were suspended in oxygenated physiological solution; the addition of diethyl-stilboestrol in concentrations of $1/10^6$–$1/10^7$ produced:

(*a*) Inhibition of the spontaneous rhythmic contractions and of the tone.

(*b*) Inhibition of the motor responses of the muscles to adrenaline acetylcholine, histamine, pituitary (posterior lobe) extract, $BaCl_2$, and direct stimulation.

All these effects were slowly reversible.

2. Oestradiol and progesterone in concentrations of 1/200,000 produced an effect on the rabbit's small intestine *in vitro* similar to that produced by a concentration of stilboestrol of 1/1,000,000. Triphenyl ethylene and triphenyl chlor-ethylene in concentrations of 1/200,000 produced a slight motor effect but did not affect the reaction to acetylcholine.

3. No effects were produced on the rabbit's and frog's auricle *in vitro* by concentrations of stilboestrol of 1/50,000.

4. The intravenous injection of 10–20 mg. of stilboestrol produced only a temporary and small effects on the blood pressure and on the intestinal and uterine movements recorded *in situ*.

5. When an intestinal loop was perfused with the animal's own blood, addition of 10–20 mg. of the drug caused a prolonged fall in the perfusion pressure, in the movements of the perfused loop, in the response of the perfused vessels to adrenaline and in the response of the perfused loop to acetylcholine.

It is with pleasure that we acknowledge our gratitude to Prof. A. J. Clark for his advice and interest, and to the Medical Research Council for defraying the expenses of this investigation.

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