*J. Endocr.* (1969), **44**, 63–68
*With* 1 *plate*
*Printed in Great Britain*



# THE EFFECT OF OESTRADIOL-17$\beta$ ON ENZYMES CONCERNED WITH METABOLISM OF CARBOHYDRATE IN HUMAN ENDOMETRIUM *IN VITRO*

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(*Received* 1 *August* 1968)

SUMMARY

Human endometrium in the regenerative phase was maintained for 5 hr. *in vitro* with oestradiol-17$\beta$ alone or together with actinomycin D. Qualitative and quantitative histochemistry of the tissue showed that the activities of glucose-6-phosphate, 6-phosphogluconate, and lactate dehydrogenases were increased by oestradiol, and that actinomycin suppressed the hormonal effect. The activities of succinate and iso-citrate dehydrogenases were unaffected by oestradiol. An attempt is made to correlate the metabolic roles of the enzymes affected by oestradiol. The suppression, by actinomycin, of the oestradiol effect suggests that the increased enzyme activity is due to the formation of new enzyme protein.

## INTRODUCTION

A previous communication (Wilson, 1967) described the stimulation *in vitro* of malate dehydrogenase activity in human endometrium by oestradiol-17$\beta$. The activities of several enzymes involved in carbohydrate metabolism are known to vary in the endometrium during the normal menstrual cycle (McKay, Hertig, Bardawil & Velardo, 1956; Hughes, Jacobs, Rubulis & Husney, 1963; Boutselis, De Neef, Ullery & Overdo, 1963), and it is probable that the alterations in enzyme activity are related to the levels of sex steroid hormones.

The action of oestrogens on endometrium in the regenerative phase is not likely to be influenced by the very low levels of circulating progesterone found at this stage of the cycle. For this reason the action of oestradiol-17$\beta$ on human endometrium in the regenerative phase has been studied. This paper describes the effects of oestradiol-17$\beta$ on the activities *in vitro* of succinate, iso-citrate, glucose-6-phosphate, 6-phosphogluconate, and lactate dehydrogenases in normal human endometrium. In addition the effect of actinomycin D on the action of the hormone is described.

## METHODS AND MATERIALS

Endometrium was obtained by curettage from ten women with normal menstrual cycles who were undergoing minor surgical operations on the uterine cervix for the

correction of a variety of benign conditions. In these circumstances uterine curettage is usual. All patients were parous and between the ages of 25 and 38. Four specimens were obtained on day 7, one on day 10, three on day 12, and two on day 13 of the cycle. In each case histological examination of the specimens showed normal regenerative phase tissue. The in-vitro technique and the quantitative histochemical method have been previously described (Wilson, 1967). Four pieces of tissue were obtained from each specimen of endometrium and one piece of tissue was immediately frozen; the others were used for the experiments *in vitro*. One culture was used as a control. Oestradiol-17$\beta$ (Koch–Light laboratories) was added to the second culture at a concentration of 10 $\mu$g./l. ($3{\cdot}7 \times 10^{-8}$ M). The third culture contained actinomycin D (Merck, Sharpe and Dohme) at a concentration of 600 $\mu$g./l. ($4{\cdot}4 \times 10^{-7}$ M) together with oestradiol at the above concentration. After 5 hr. *in vitro* the tissue was frozen and stored in a cryostat as previously described. One piece of tissue treated with oestradiol and actinomycin was accidentally impregnated with ice and these results have not been included. Sections of the tissue, 10 $\mu$ thick, were prepared in the cryostat with the knife cooled to $-60°$ and with the chamber temperature maintained between $-25°$ and $-35°$. The sections were mounted on warm slides and covered with the histochemical medium without being allowed to dry. Thirty sections were cut from each piece of tissue, six sections being stained for each of the five enzymes. One section was permanently mounted. The areas of the remaining five sections were measured and the formazan was extracted and estimated spectrophotometrically at 550 m$\mu$ in a Unicam SP 500 spectrophotometer. Results were expressed as $\mu$g. formazan/cm.$^2$ of section, and the mean value for the five sections was calculated.

The methods used to demonstrate enzyme activity were basically those of Pearse (1960) but 0·05 M-glycylglycine buffer was used throughout at pH 7·5, except for the 6-phosphogluconate dehydrogenase reaction when the pH was maintained at 8·0. Altmann & Chayen (1966) have found this to be the optimal pH for 6-phosphogluconate dehydrogenase. Nitro-blue tetrazolium, being insoluble, was used as electron acceptor for the preparation of permanently mounted sections and neotetrazolium was used as electron acceptor in the quantitative methods. Only seven specimens were stained for iso-citrate dehydrogenase.

## RESULTS

Examination of the histochemical preparations suggested that the activities of glucose-6-phosphate, 6-phosphogluconate, and lactate dehydrogenases were increased in tissue treated with oestradiol. The enzyme activity in the control tissues was seen as a mixed particulate and diffuse deposit of formazan in the glandular epithelium of the endometrium with a similar, but much weaker, staining reaction in the stromal cells. Blood vessels in the endometrium showed a diffuse stain in the lactate dehydrogenase reaction. The in-vitro control tissue resembled the fresh tissue in every specimen and for all enzymes studied.

The tissue treated with oestradiol showed an increase in the particulate deposit of formazan in the gland cells. Enzyme activity in the stromal cells also seemed to be increased by oestradiol but the effect was less striking than that seen in the gland cells and the blood vessels seemed unaffected. The histochemical appearance of the

oestradiol-treated tissue was similar to that of normal endometrium in the secretory phase on about the 20th day of the cycle (Wilson, 1968) (Pl., figs. 1, 2). The tissue treated with both oestradiol and actinomycin showed no increase in enzyme activity and the histochemical appearance was similar to that of the control tissues.

The results of formazan elution and determinations are shown in Tables 1–3 and substantiate the impressions gained from the microscopic appearances.

Table 1. *Influence of oestradiol-17β on glucose-6-phosphate dehydrogenase activity (μg. formazan/cm.² section) in human endometrium* in vitro (*means* ± S.D.)

| Day of menstrual cycle | Fresh tissue | Cultured tissue | | |
|---|---|---|---|---|
| | | Control | Oestradiol | Oestradiol + actinomycin D |
| 7 | 1·55 ± 0·11 | 1·94 ± 0·20 | 4·15 ± 0·25*** | 1·41 ± 0·23*** |
| 7 | 1·48 ± 0·10 | 1·67 ± 0·24 | 4·32 ± 0·10*** | 1·46 ± 0·12*** |
| 7 | 1·99 ± 0·19 | 1·79 ± 0·26 | 4·05 ± 0·35*** | 1·92 ± 0·15*** |
| 7 | 1·41 ± 0·12 | 1·37 ± 0·08 | 3·09 ± 0·08*** | 1·36 ± 0·14*** |
| 10 | 1·96 ± 0·09 | 2·18 ± 0·19 | 4·22 ± 0·17*** | 2·01 ± 0·19*** |
| 12 | 2·54 ± 0·23 | 2·71 ± 0·16 | 3·42 ± 0·32* | 2·44 ± 0·14*** |
| 12 | 1·42 ± 0·13 | 1·25 ± 0·10 | 2·34 ± 0·07*** | — |
| 12 | 1·61 ± 0·09 | 1·79 ± 0·11 | 3·59 ± 0·13*** | 2·08 ± 0·12*** |
| 13 | 1·50 ± 0·05 | 1·42 ± 0·02 | 3·82 ± 0·15*** | 1·13 ± 0·09*** |
| 13 | 2·30 ± 0·05 | 2·68 ± 0·06 | 4·62 ± 0·23*** | 2·07 ± 0·20*** |

$n = 5$. Differences ($t$-test) between oestradiol-treated and cultured control tissue and between actinomycin plus oestradiol-treated and oestradiol-treated tissue are indicated as follows: *** $P < 0·001$; * $P < 0·05$.

Table 2. *Influence of oestradiol-17β on 6-phosphogluconate dehydrogenase activity (μg. formazan/cm.² section) in human endometrium* in vitro (*means* ± S.D.)

| Day of menstrual cycle | Fresh tissue | Cultured tissue | | |
|---|---|---|---|---|
| | | Control | Oestradiol | Oestradiol + actinomycin D |
| 7 | 0·99 ± 0·09 | 1·26 ± 0·20 | 2·53 ± 0·14*** | 1·09 ± 0·12*** |
| 7 | 1·51 ± 0·14 | 1·79 ± 0·21 | 3·71 ± 0·34*** | 1·79 ± 0·17*** |
| 7 | 1·04 ± 0·10 | 1·03 ± 0·22 | 4·56 ± 0·11*** | 1·03 ± 0·13*** |
| 7 | 1·24 ± 0·08 | 1·35 ± 0·11 | 2·72 ± 0·21*** | 1·30 ± 0·14*** |
| 10 | 1·71 ± 0·23 | 1·80 ± 0·22 | 3·26 ± 0·13*** | 1·88 ± 0·20*** |
| 12 | 2·12 ± 0·04 | 1·97 ± 0·37 | 3·43 ± 0·20*** | 2·22 ± 0·29*** |
| 12 | 0·72 ± 0·10 | 0·95 ± 0·08 | 1·78 ± 0·13*** | — |
| 12 | 1·18 ± 0·12 | 1·35 ± 0·12 | 2·23 ± 0·09*** | 1·32 ± 0·09*** |
| 13 | 1·25 ± 0·07 | 1·16 ± 0·08 | 3·11 ± 0·10*** | 1·10 ± 0·12*** |
| 13 | 2·22 ± 0·05 | 2·39 ± 0·17 | 4·35 ± 0·17*** | 1·86 ± 0·15*** |

$n = 5$. Differences between oestradiol-treated and cultured control tissue and between actinomycin plus oestradiol-treated and oestradiol-treated tissue are indicated as follows: *** $P < 0·001$.

Histochemically. succinate dehydrogenase activity appeared to be unaffected by either oestradiol alone or in combination with actinomycin. The reaction was weak and was seen as a purely particulate deposit of formazan in the gland cells with only occasional stromal cells stained, and no staining of the blood vessels. The results of the quantitative experiments (Table 4) largely confirmed the microscopic findings. However, two specimens showed some change. In one, enzyme activity was increased

Table 3. *Influence of oestradiol-17β on lactate dehydrogenase activity (μg. formazan/cm.² section) in human endometrium* in vitro (*means* ± S.D.)

| Day of menstrual cycle | Fresh tissue | Cultured tissue | | |
|---|---|---|---|---|
| | | Control | Oestradiol | Oestradiol + actinomycin D |
| 7 | 1·47 ± 0·08 | 1·59 ± 0·27 | 5·06 ± 0·11*** | 1·77 ± 0·41*** |
| 7 | 1·66 ± 0·15 | 1·29 ± 0·09 | 3·26 ± 0·19*** | 1·50 ± 0·12*** |
| 7 | 2·31 ± 0·12 | 2·26 ± 0·17 | 5·64 ± 0·17*** | 2·53 ± 0·26*** |
| 7 | 1·33 ± 0·16 | 1·35 ± 0·10 | 3·34 ± 0·17*** | 1·49 ± 0·06*** |
| 10 | 2·53 ± 0·11 | 2·23 ± 0·17 | 5·48 ± 0·20*** | 2·61 ± 0·08*** |
| 12 | 1·99 ± 0·10 | 2·08 ± 0·14 | 4·31 ± 0·10*** | 2·32 ± 0·23*** |
| 12 | 2·07 ± 0·12 | 1·85 ± 0·12 | 4·03 ± 0·15*** | — |
| 12 | 2·13 ± 0·12 | 2·20 ± 0·09 | 4·13 ± 0·09*** | 2·67 ± 0·17*** |
| 13 | 0·73 ± 0·02 | 0·62 ± 0·05 | 1·35 ± 0·10*** | 0·47 ± 0·08*** |
| 13 | 2·09 ± 0·07 | 2·20 ± 0·12 | 4·61 ± 0·21*** | 1·88 ± 0·19*** |

$n = 5$. Differences between oestradiol-treated and cultured control tissue and between actinomycin plus oestradiol-treated and oestradiol-treated tissue are indicated as follows: *** $P < 0·001$.

Table 4. *Influence of oestradiol-17β on succinate dehydrogenase activity (μg. formazan/cm.² section) in human endometrium* in vitro (*means* ± S.D.)

| Day of menstrual cycle | Fresh tissue | Cultured tissue | | |
|---|---|---|---|---|
| | | Control | Oestradiol | Oestradiol + actinomycin D |
| 7 | 0·60 ± 0·07 | 0·71 ± 0·21 | 0·43 ± 0·09 | 0·71 ± 0·24 |
| 7 | 0·82 ± 0·10 | 1·03 ± 0·25 | 1·09 ± 0·16 | 2·07 ± 0·16*** |
| 7 | 0·76 ± 0·19 | 0·78 ± 0·12 | 0·85 ± 0·12 | 0·89 ± 0·17 |
| 7 | 0·85 ± 0·06 | 0·95 ± 0·09 | 0·89 ± 0·08 | 0·86 ± 0·14 |
| 10 | 1·23 ± 0·39 | 1·45 ± 0·21 | 1·25 ± 0·13 | 1·34 ± 0·18 |
| 12 | 1·75 ± 0·18 | 1·98 ± 0·18 | 2·53 ± 0·11*** | 2·81 ± 0·29** |
| 12 | 0·70 ± 0·09 | 0·49 ± 0·16 | 0·46 ± 0·11 | — |
| 12 | 0·59 ± 0·05 | 0·81 ± 0·14 | 0·75 ± 0·14 | 0·54 ± 0·14 |
| 13 | 0·39 ± 0·04 | 0·40 ± 0·07 | 0·32 ± 0·11 | 0·35 ± 0·11 |
| 13 | 1·59 ± 0·05 | 1·48 ± 0·16 | 1·39 ± 0·11 | 1·49 ± 0·20 |

$n = 5$. Differences between oestradiol-treated and cultured control tissue and between actinomycin plus oestradiol-treated and oestradiol-treated tissue are indicated as follows: *** $P < 0·001$; ** $P < 0·01$.

Table 5. *Influence of oestradiol-17β on iso-citrate dehydrogenase activity (μg. formazan/cm.² section) in human endometrium* in vitro (*means* ± S.D.)

| Day of menstrual cycle | Fresh tissue | Cultured tissue | | |
|---|---|---|---|---|
| | | Control | Oestradiol | Oestradiol + actinomycin D |
| 7 | 1·32 ± 0·07 | 1·35 ± 0·22 | 1·14 ± 0·15 | 1·57 ± 0·19 |
| 7 | 1·33 ± 0·17 | 1·19 ± 0·18 | 1·01 ± 0·11 | 1·17 ± 0·16 |
| 7 | 0·85 ± 0·06 | 0·95 ± 0·09 | 0·89 ± 0·08 | 0·86 ± 0·15 |
| 10 | 2·58 ± 0·16 | 2·87 ± 0·22 | 2·53 ± 0·11 | 2·64 ± 0·27 |
| 12 | 1·26 ± 0·02 | 1·38 ± 0·19 | 1·13 ± 0·08 | — |
| 12 | 1·70 ± 0·05 | 1·68 ± 0·10 | 1·48 ± 0·11 | 1·53 ± 0·21 |
| 13 | 1·73 ± 0·08 | 1·88 ± 0·11 | 1·84 ± 0·15 | 1·78 ± 0·12 |

$n = 5$. No significant differences between oestradiol-treated and cultured control tissue and between actinomycin plus oestradiol-treated and oestradiol-treated tissue exist.

in the tissue treated with oestradiol and actinomycin, in the other a similar finding was recorded and the tissue exposed to oestradiol alone also showed increased enzyme activity.

Iso-citrate dehydrogenase activity was also unaffected by oestradiol or by oestradiol and actinomycin together. The activity of this enzyme is histochemically demonstrated as a mixed diffuse and particulate deposit of formazan in the gland and stromal cells of the endometrium with an intense diffuse staining of the walls of the blood vessels. There were no apparent differences in the intensity of staining of any of these structures under the different experimental conditions. The quantitative results shown in Table 5 confirm the microscopic findings.

## DISCUSSION

Although the conclusions in this study are based mainly on the quantitative results set out in the tables, descriptive histochemistry was felt to be important because it was not known which cell types in the endometrium would be affected by oestradiol. The cells of the glandular epithelium showed the greatest increase in enzyme activity, but the stromal cells also showed evidence of being influenced by the hormone. Blood vessels were seemingly unaffected. Although the quantitative techniques do not provide a high degree of accuracy the results are sufficiently clear-cut and consistent to draw several conclusions. Glucose-6-phosphate and 6-phosphogluconate dehydrogenases as representative enzymes of the pentose phosphate pathway are known to have considerable activity in the human endometrium (Hughes *et al.* 1963; Cohen, Bitensky, Chayen, Cunningham & Russell, 1964). The present findings suggest that these enzymes are under the control of oestrogens. Hilf, Michel & Bell (1966) showed that glucose-6-phosphate dehydrogenase and malate dehydrogenase were increased in rat mammary gland after the administration of oestradiol, but that iso-citrate dehydrogenase activity was, if anything decreased. The results presented here, and those for malate dehydrogenase which have been previously reported (Wilson, 1967), are in remarkable agreement with the findings of these workers. The reason why malate dehydrogenase alone of the citric acid cycle enzymes is stimulated by oestrogen in hormone-sensitive tissue is far from clear. One possibility is that it is the decarboxylating function of malate dehydrogenase which is stimulated by oestrogen. This would provide a means of utilizing the NADPH generated by the pentose phosphate pathway for the conversion of pyruvate to phosphoenol pyruvate (Hollmann, 1964), the phosphoenol pyruvate formed as a result of this reaction could then be used for the resynthesis of glucose and the ultimate synthesis of glycogen for secretion by the endometrium. Similarly, the stimulation of lactate dehydrogenase may be related to the increase in NADPH resulting from increased activity in the pentose phosphate pathway. NADPH does not take part to any extent in oxidative phosphorylation and is therefore comparatively useless as an energy source. However, lactate dehydrogenase can utilize NADPH as coenzyme for the conversion of pyruvate to lactate. The reverse change—from lactate to pyruvate—results in the reduction of NAD and the net effect is the transfer of hydrogen from NADPH to NADH (Holzer & Schneider, 1958). The NADH so formed is then free to take part in oxidative phosphorylation. It is suggested that

this provides an explanation for the stimulation of lactate dehydrogenase activity by oestradiol.

Malate dehydrogenase and lactate dehydrogenase have been shown to reach a peak level of activity in human endometrium on about day 20 of the menstrual cycle (Hughes *et al.* 1963) and it is interesting to note that these enzymes are under the influence of oestrogen. It would seem, therefore, that some at least of the functions of the endometrium during the secretory phase are controlled or directed by oestrogen alone and that progesterone may play a lesser part at this early period in the secretory phase than has previously been thought. Further support for this concept comes from the finding in this study that the intracellular distribution of enzyme activity after exposure of the tissue to oestrogen is very similar to that seen on about day 20 in normal endometrium in the secretory phase.

The suppression of the stimulation of enzyme activity by actinomycin D is in accordance with current theories of the mechanism of action of oestradiol in other species (Karlson & Sekeris, 1966). The results with actinomycin suggest that DNA-dependent RNA synthesis is essential for the increase in enzyme activity, and therefore that the increased activity is due to the formation of new enzyme protein. The accumulation of [$^3$H]oestradiol in the nuclear fraction of human endometrial cells (Brush, Taylor & King, 1967) supports this hypothesis.

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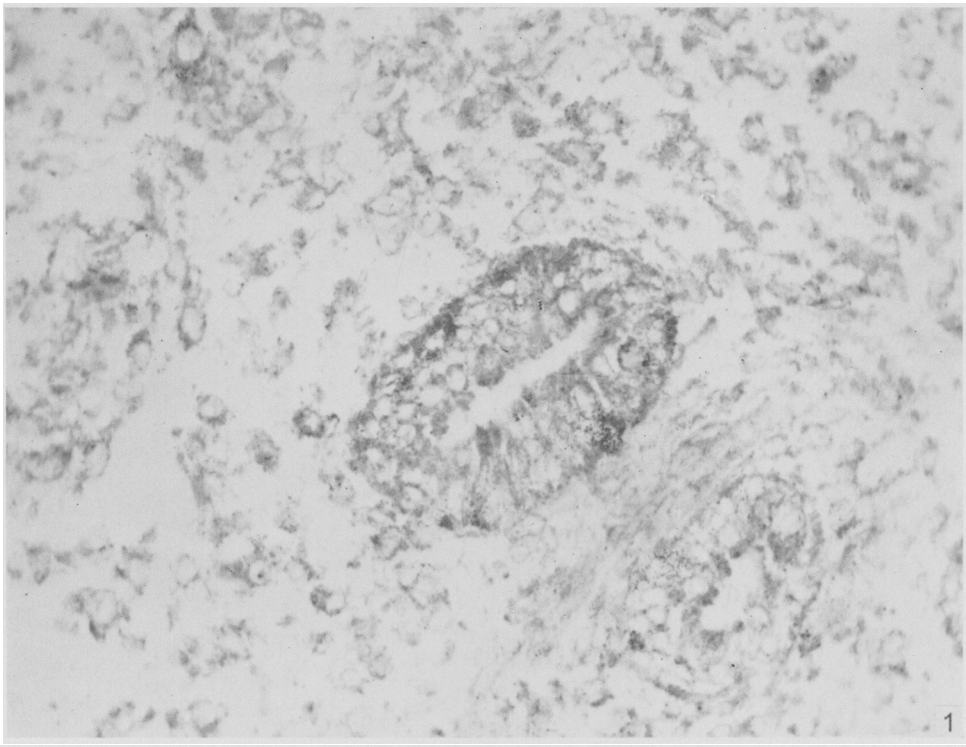
## DESCRIPTION OF PLATE

Fig. 1. Regenerative endometrium from a woman on day 10 of the menstrual cycle. Fresh tissue stained for lactate dehydrogenase. Nitro-blue tetrazolium reduction. Moderate enzyme activity in the cells of the glands and stroma. ( × 400.)

Fig. 2. The same endometrium, maintained for 5 hr. *in vitro* with 10 $\mu$g. oestradiol-17$\beta$/l. Trowell T 8 tissue culture medium (Difco). Stained for lactate dehydrogenase. Nitro-blue tetrazolium reduction. Increased enzyme activity in the gland cells. ( × 400.)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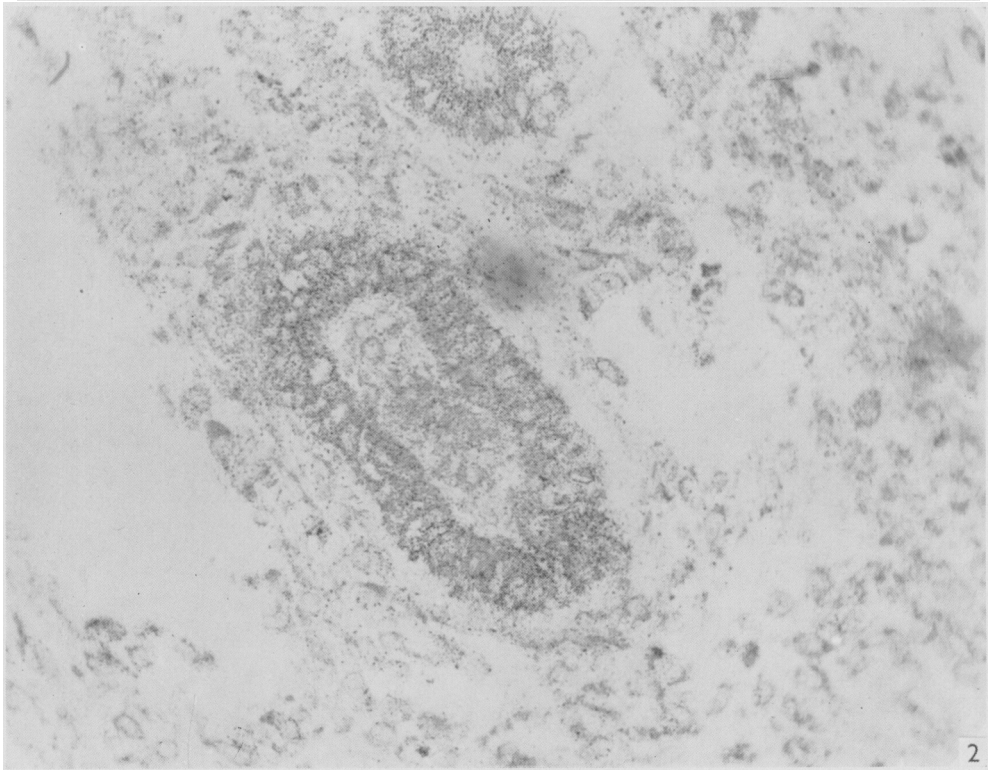
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