THE ASSOCIATION OF CERTAIN OVARIAN CELLS WITH ENDOMETRIAL CANCER*

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Much attention has been paid in recent years to a possible relation between ovarian function and the development of adenocarcinoma of the endometrium. Marital status and previous pregnancies have been studied by Healy and Brown (1939), and delayed menopause followed by the development of endometrial carcinoma by Crossen and Hobbs (1935), Randall (1945), Masson and Gregg (1940), and Taylor and Millen (1938). A great deal has been written on a possible connexion between endometrial hyperplasia and the development of carcinoma (Novak and Yui, 1936; Jones and Brewer, 1941; Howard Taylor, 1932). The high incidence of corpus carcinoma with granulosa- and theca-cell tumours has been studied by Schröder (1922), Dockerty (1940), Hodgson, Dockerty, and Mussen (1945), Banner and Dockerty (1945), and Hertig (1945). Recently a paper by Woll, Hertig, Van S. Smith, and Johnson (1948) has drawn attention to the presence of cortical granulomata and thecomatosis in association with adenocarcinoma of the endometrium.

Case 1

On June 5, 1947, a married woman aged 81, with three children, was operated upon. A large combined Brenner tumour and pseudomucinous cystadenoma of the left ovary, together with a small cystic right ovary and the uterus, were removed (W.S.). An early adenocarcinoma of the endometrium was found in the uterus. The large tumour was a typical combined Brenner tumour and pseudomucinous cystadenoma. The case was examined with some care histologically, because it was hoped to investigate the cause of uterine bleeding with Brenner tumours. In the right ovary was a small Brenner tumour together with a cystoma, but the mesovarium contained unusual cells. The cells were localized to the part of the mesovarium immediately adjacent to the cortex of the ovary, approximately at the level of the white line. The cells were large and polyhedral and the protoplasm stained deeply with eosin. The nuclei were large and spherical (Fig. 1) and the cells were superficially similar to the paralutein cells of a mature corpus luteum.

The appearance of the cells suggested that they were in a state of activity, and from their resemblance to paralutein cells it seemed reasonable to believe that they were secreting a hormone-like substance. The rete ovarii also showed some activity. The patient was aged 81, so there was no question that the cells were derived from follicles. Moreover, the location of

the cells was quite different from that of Graafian follicles. At the time it was believed that the cells were probably oestrogenic, and it was thought that they might be responsible for

FIG. 1.—Cells under high magnification (Case 1). Although mainly polyhedral, their shape varies. In the lower part of the photograph the cells seem to be fused together. The characteristic rounded nuclei of the cells can be seen. These cells were found near the hilum at the level of the white line.

the myohyperplasia found in the uterus and might explain the post-menopausal haemorrhage encountered with Brenner tumours.

Case 2

On Sept. 24, 1947, a nulliparous married woman, aged 40, was operated upon (W.S.) for what was believed to be metropathia haemorrhagica. There was a history of amenorrhoea followed by continuous bleeding. The uterus was found to be slightly enlarged, and the right ovary was palpably about twice the normal size. A subtotal hysterectomy and a right salpingo-oophorectomy were performed. At operation the right ovary was found to be symmetrically enlarged and not to con-

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FIG. 2.—Cell clusters, Case 2. The large deeply staining cells which surround the blood vessel in the middle of the photomicrograph are the cells which we describe. The cells seem to be spreading out from the wall of the blood vessel. The light area represents the medulla of the ovary, while, to the left and above, the deeply staining cells form part of the cortex. The cells shown to the left are better differentiated than those which surround the vessel in the centre.

tain a cyst. The endometrium was not polypoidal and was only slightly irregular to the naked eye. The specimen was examined histologically, when a very early adenocarcinoma of the endometrium was found. The tumour was only a few millimetres in diameter.

Comment on Cases 1 and 2

For various reasons it was felt that these two cases should be investigated in detail and reported in the literature.

It was not until March, 1949, that we were able to start work on these and other cases. Case 2 was clearly of an unusual type; consequently the ovary which had been removed was examined in great detail. It contained a small number of primordial follicles, a normal number of interstitial cells, but no corpus luteum and no active Graafian follicle. To our surprise we found deeply staining polyhedral cells in the ovarian medulla which were morphologically identical with those found in Case 1. The identification of the cells was much more difficult than in the first case, for the patient was aged 40 and it was essen-

FIG. 4.—Case 2, illustrating the close relation of the cells to the endothelium of the blood vessels. The syncytial-like appearance of the cells is well shown. Above lies the lumen of the vessel with red blood corpuscles.

tial to prove that the cell clusters were independent of the follicle system. We have had some experience of ovarian histology and in the past have investigated the morphology of Graafian follicles (Shaw, 1927a), atretic follicles (Shaw, 1925), corpora lutea, and interstitial cells (Shaw, 1926). We concluded that the cells were independent of the follicular system and were not derived from either granulosa or theca interna cells. The analysis was rigorous and critical. No atretic body was found in the vicinity of the cells. The cells were localized to the medulla and the cell clusters were found in separate parts of the ovary without any communication between the clusters. The cells were mainly polyhedral, with rounded nuclei, but sometimes they were fusiform and sometimes stellate. In other areas they were syncytial-like, with the protoplasm of adjacent cells fusing together. The unusual forms of cells found in chocolate cysts have been investigated previously (Shaw, 1927b; Shaw, 1933), and the cell clusters identified in this specimen were different from any of the types of cell found with chocolate cysts of the ovaries. In this case the cells were not located to the subcortical area of the mesovarium but had spread into the medulla. (Photomicrographs of the cells are shown in Figs. 2-5.)

FIG. 3.—Cells from Case 2, under higher magnification than Fig. 1. The same rounded nuclei and the same peculiarities of the cell outline can be seen.

FIG. 5.—Another illustration showing the close relation of the cells to the endothelium. The lumen of the vessel lies in the upper part of the photograph.

As a result of the investigation of these two cases it seemed possible that the cell clusters might have some relation to the development of an adenocarcinoma of the endometrium.

Case 3

A married woman, aged 57, had been treated in 1940 by one of us for menopausal haemorrhage by means of an intra-uterine application of 50 mg. of radium for 48 hours. The patient was nulliparous. She was admitted to hospital on March 10, 1949, complaining of vaginal haemorrhage of 14 days' duration. A b.opsy combined with curettage of the cervical canal was carried out next day, and a squamous-celled carcinoma was demonstrated in the specimen. On March 25 a Schauta operation was performed (W.S.) and both ovaries were removed. The uterus contained an early endocervical carcinoma. The ovaries were shrunken.

In view of the findings in the first two cases, both ovaries were examined in great detail. The same cell clusters found in the first two cases were demonstrated in the vicinity of the white line in one ovary. The cells had the typical appearance of those found in the other cases. The cell clusters were also found in the second ovary. Some were localized to the vicinity of the white line, while others were identified in the medulla in the neighbourhood of strands of hyaline tissue.

Case 4

A single woman, aged 51, was admitted to hospital on May 18, 1949, complaining of lower abdominal pain and irregular post-menopausal haemorrhage. Curettage was performed by the house-surgeon, and the pathologist reported that the material removed suggested that the patient had an endocervical carcinoma. Because of this report a Wertheim operation was performed on May 27 (W.S.). The growth was found to be an adenocarcinoma of the endometrium, and it was clear that the material removed at the curettage was the lower pole of a polypoidal projection. The ovaries were examined in detail. They were more vascular than normal. They contained no Graafian follicles and no recently atretic follicles. Again, the cell clusters were found in the hilum of one ovary in the mesovarium at the level of the white line. The cells have been found, up to the present, in only one ovary. In this case the cell clusters were less numerous than in the first three cases. On the other hand, the carcinoma was more advanced.

Examination of Controls

At this stage of the investigation it became obvious that controls must be examined, although one of us has spent much time upon the histology of the normal ovary and could not recall seeing such cells previously. It was realized, however, that much of this previous work had dealt with the follicular system. One of us has a large collection of slides of ovaries obtained from the Williamson laboratory. They have been regarded in the past as being extremely valuable, and were removed from the laboratory before the war. The slides are numbered in accordance with the registration system of the laboratory.

One evening some 200 of these slides were examined, and in only one were the cell clusters discovered. The next day it was possible to identify the slide. The specimen had been obtained in 1932 by a Wertheim operation (W.S.) performed upon a multipara of 55. In this case the carcinoma was endocervical in type and a pyometra had developed in the cavity of the uterus above the level of the carcinoma. Although the report given from the laboratory stated that the ovaries were normal, one of us had a vague feeling that he had seen the cells as long ago as 1932 and had been puzzled by them but had not investigated them further. After the identification of the cell clusters in this fifth case of carcinoma of the uterus, particularly as the identification had been made without any possibility of bias, we became convinced that the cell clusters had

some association with adenocarcinoma of the endometrium and cervical canal.

The next step was to examine the collection of slides in the Williamson laboratory. One of us had been in charge of the laboratory when it was started, and had insisted that every slide and every paraffin block should be stored. Unfortunately, the collection was badly damaged by bombing, and only with the greatest difficulty have old slides been found and identified. Moreover, it seems almost impossible to sort out the paraffin blocks, with the result that the investigation of the specimens is imperfect and incomplete. We have collected together 58 specimens of ovaries from cases of carcinoma of the uterus. We have thought it best to group the cases into those of adenocarcinoma of the endometrium, endocervical carcinoma, and squamous-celled carcinoma of the vaginal portion of the cervix. The results are as follows:

Adenocarcinoma of the endometrium.—Total specimens, 48 (cell clusters found, 24; no cells found, 24).

Endocervical carcinoma.—Total specimens, 9 (cell clusters found, 6; no cells found, 3).

Squamous-celled carcinoma of the portio.—Total specimens, 1 (cell clusters found, 0; no cells found, 1).

The interpretation of these figures is important. The four cases completely investigated were all positive. In the third of these four cases many sections were made before the cells could be identified. In all cases, apart from these four, only one slide of the ovaries has been available. Consequently the negative findings are not regarded as conclusive. We believe that it may be necessary to make a large number of sections from an ovary before the cells can be identified. The small number of cases of squamous-celled carcinoma of the portio is explained because carcinoma of the cervix has usually been treated with radium at St. Bartholomew's Hospital. We are not prepared to draw any conclusions about the incidence of cell clusters in squamous-celled carcinoma of the portio from the material we have at our disposal.

We must stress the difficulty of demonstrating the cells. If they are aggregated in the mesovarium, at the level of the white line, in close proximity to the edge of the cortex, they can be recognized without much difficulty. If, however, they are scattered through the medulla it may be necessary to examine the whole ovary fairly extensively before they can be demonstrated. The size of the cells and their deep staining with eosin help in their recognition. In no case have we found a large number of cells, and we do not regard their detection as being particularly simple.

We have not been able to trace any reference to the cells in the literature. It seems highly improbable that they have not been recognized before and we believe that some previous description will be found.

Significance of the Cells

The number of cases examined is small and we are unlikely to be able to collect a large number of specimens in the immediate future. We have therefore thought it advisable to publish these results so that a wide search can be made in other laboratories. We are convinced that the cells are found in association with carcinoma of the endometrium and cervical canal. It may be that time will show that our work on control specimens has been incomplete, and that similar cells will be found in ovaries not associated with carcinoma of the uterus, but that is not our belief at the present time. Furthermore, at this early stage of the investigation any views that we may put forward are conjectural, hypothetical, and perhaps unsound.

In our view the cells are carcinogenic and lead to the development either of an adenocarcinoma of the endometrium or of an endocervical carcinoma of the cervix. At the moment we have insufficient evidence to show that there is a relation between the cell clusters and the development of squamous-celled carcinoma of the portio. Against this view is the possibility that the cell clusters appear as the result of the stimulation of the ovaries by the carcinoma, and it may be that both the cell clusters and the carcinoma are induced coincidentally by some third factor. The dominance of the uterus by the ovaries has always been the main theme of our previous work on the ovaries. Indeed, the cells of the cell clusters are so similar in type to granulosa-lutein and theca-lutein cells that we feel that the cell clusters are the cause and the uterine cancer the effect.

We are of course familiar with the literature on the development of adenocarcinoma of the endometrium in the absence of both ovaries; three cases were recorded by G. Van S. Smith (1941). We are also acquainted with the large number of cases of stump carcinomata arising after subtotal hysterectomy. Nevertheless, only very few cases of adenocarcinoma of the endometrium arising after the removal of both ovaries are on record. It is possible that cell clusters may be found in the mesovarium further along the ovario-pelvic fold, and that such cells left behind at operation initiated the adenocarcinoma in the cases described. The stump-carcinoma cases arising after the removal of both ovaries cannot be explained by the theory we have put forward. Although it is well known that in many of the cases the carcinoma was already present before the hysterectomy was performed, we must accept the view that a stump carcinoma may develop in the absence of both ovaries. It may be that these cases are mainly squamous growths of the portio.

The frequent localization of the cell clusters to the specific zone in the vicinity of the white line seems to us to be of the utmost significance. We believe that this area should be regarded as an organizing centre for activating cells which are carcinogenic to the endometrium of the body of the uterus and of the cervical canal.

Origin of Cells

We believe that the cells described above are not found either in the normal ovary or in the hilum of the ovary of patients who are not suffering from carcinoma of the uterus. An attempt has been made with the material at our disposal to trace the origin of the cells. Case 2 was of much interest, because the cell clusters seemed to be spreading not only into the medulla of the ovary but circumferentially between the medulla and the cortex. The variation in shape and the syncytial-like arrangement of the cells imply that they were actively dividing. They were invariably found in the vicinity of blood vessels, and we believe that we have traced their origin from the endothelium of small capillaries and of lymphatics. Neither of us is a trained cytologist, so that this opinion may be of little value.

If, however, our main thesis is correct, that the cell clusters are carcinogenic to the endometrium of the body of the uterus and the cervical canal, some attempt must be made to explain why the cell clusters appear. The obvious interpretation is that a hypothetical hormone causes the cells to develop from the endothelium of the blood vessels or lymphatic spaces. This implies that the cause of carcinoma of the endometrium and cervical canal is not a local phenomenon. We realize that such a possibility may alter previous views on the development of carcinoma.

Other lines of investigation present themselves immediately. We feel that wherever possible ovaries from cases of carcinoma arising elsewhere in the body should be examined histologically. This is particularly so in the case of carcinoma of the breast, for there is evidence that malignant disease of the breast sometimes retrogresses after the removal of both ovaries. A similar investigation might be made of the testis and epididymis in cases of carcinoma of the prostate.

Case 3 is of some importance gynaecologically. The patient had been treated previously with radium and an artificial menopause had been created. Although the follicular system in the ovaries had retrogressed completely, the cell clusters were found not only near the white line, but also in the medulla. The findings suggest that the cells appear after the creation of an artificial menopause, and that many of the puzzling features of the development of carcinoma of the endometrium after radiotherapy can be explained. Again, in the radium treatment of carcinoma of the uterus it seems that the cells must first be destroyed by radium, otherwise a recurrence of carcinoma is to be expected. It seems to us important to determine whether the cell clusters disappear after the application of radium to the cervix. If the cell clusters are not destroyed, and our thesis that they are carcinogenic is correct, the procedure in such cases should be to remove the ovaries before radium treatment is instituted.

Conclusions

Unusual cells have been demonstrated in the mesovarium and medulla of the ovaries of cases of adenocarcinoma of the endometrium and of endocervical carcinoma of the uterus. So far as we know, the cells have not been described before. If they have been described, little attention has been paid to them. The cells do not seem to be found in normal ovaries, and their presence seems to be restricted to cases of carcinoma of the uterus. They are usually found in the mesovarium, close to the termination of the cortex of the ovary, but they may also be found in the ovarian medulla and, very rarely, in the cortex. Although they may possibly appear as the result of some reaction from the uterine carcinoma or may be stimulated to appear by a hypothetical factor which also initiates the carcinoma of the uterus, we incline to the view that they are carcinogenic. Our main difficulty has been to obtain material.

In only four cases have the ovaries been investigated in great detail. In 54 other cases of carcinoma of the body of the uterus and carcinoma of the cervix, only one slide of one ovary has been available. Of these 54 cases the cells have been found in 26. We emphasize the difficulty of identifying the cells, and we firmly believe that the cells would have been found in a larger number if the whole of the ovaries had been available for examination. Nevertheless, even in this small and imperfect series the incidence of the cells is appreciable. We feel that further investigations should be made, and if specimens are removed and suitable laboratory facilities are not available we hope that the specimens will be sent to us for examination. The number of cases of squamous-celled carcinoma of the portio available for investigation is surprisingly small, and we are not prepared to offer any comment on the association of the cells with the development of this form of cancer of the uterus.

Summary

Unusual cells have been demonstrated in the mesovarium and ovarian medulla of cases of adenocarcinoma of the endometrium and of endocervical carcinoma.

The cells are secretory in type, yet differ morphologically from any ovarian cells known to us.

They have not been discovered in control cases.

They are found only in relatively small numbers and may be extremely difficult to detect. A single section through the ovary is insufficient, as a general rule, to demonstrate them.

The cells may possibly be carcinogenic, secreting a hormone which stimulates some of the cells of the endometrium of the body of the uterus and cervical canal to undergo malignant change. It is hoped that a further search for them will be made in other laboratories.

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REFERENCES

- Banner, E. A., and Dockerty, M. B. (1945). *Surg. Gynec. Obstet.*, **81**, 234.
 Crossen, R. J., and Hobbs, J. E. (1935). *J. Mo. med. Ass.*, **32**, 361.
 Dockerty, M. B. (1940). *Amer. J. Obstet. Gynec.*, **39**, 434.
 Healy, W. P., and Brown, R. L. (1939). *Ibid.*, **38**, 1.
 Hodgson, J. E., Dockerty, M. B., and Mussey, R. D. (1945). *Surg. Gynec. Obstet.*, **81**, 631.
 Hertig, A. (1945). Quoted by J. V. Meigs, *New Engl. J. Med.*, 1945, **233**, 11.
 Jones, H. O., and Brewer, J. I. (1941). *Amer. J. Obstet. Gynec.*, **42**, 207.
 Masson, J. C., and Gregg, R. O. (1940). *Surg. Gynec. Obstet.*, **70**, 1083.
 Novak, E., and Yui, E. (1936). *Amer. J. Obstet. Gynec.*, **32**, 674.
 Randall, C. L. (1945). *J. Amer. med. Ass.*, **127**, 20.
 Schröder, R. (1922). *Zbl. Gynäk.*, **46**, 195.
 Shaw, Wilfred (1925). *J. Obstet. Gynaec. Brit. Emp.*, **32**, 1.
 — (1926). *Ibid.*, **33**, 183.
 — (1927a). *Ibid.*, **34**, 469.
 — (1927b). *Ibid.*, **34**, 300.
 — (1933). *Ibid.*, **40**, 1125.
 Smith, G. Van S. (1941). *New Engl. J. Med.*, **225**, 608.
 Taylor, H. C., jun. (1932). *Amer. J. Obstet. Gynec.*, **23**, 309.
 — and Millen, R. (1938). *Ibid.*, **36**, 22.
 Woll, E., Hertig, A. T., Smith, G. Van S., and Johnson, L. C. (1948). *Ibid.*, **56**, 617.

INFANTILE DIARRHOEA AND VOMITING

A CLINICAL AND BACTERIOLOGICAL INVESTIGATION

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This paper reports the results of a combined clinical and bacteriological investigation of infantile diarrhoea and vomiting, and includes a note by Dr. F. O. MacCallum on his virus investigations. In order that cases of infective origin should be presented for bacteriological study the clinical material was drawn in the main from outbreaks of diarrhoea and vomiting and from intercurrent attacks in hospital wards. In addition there were included two series of cases admitted consecutively to the gastro-enteritis units of a children's hospital and of an infectious diseases hospital. In these groups there was sometimes uncertainty

whether cases should be classified as "infective," "dietetic," or "secondary to parenteral infection."

The first outbreak occurred in the nurseries of a maternity unit, and a number of observers took part in its investigation. Subsequently the course of each outbreak and the clinical progress of each case were followed by one paediatrician. Material for bacteriological examination was dealt with at a centrally placed laboratory as soon as possible after collection. In this way a wide area of field investigation could conveniently be covered. The methods and results of the general bacteriological investigation of the cases are given in a later section.

The detailed study by Crowley *et al.* (1941) left little expectation that a bacterial agent might be determined by a quantitative comparison of the faecal flora of affected and unaffected babies. From the outset of the present investigation it had seemed that bacterial studies would need to follow the modern trend towards subdivision of genera by serological, biochemical, and phage-typing methods. Primary culture plates or subcultured strains were therefore sent to laboratories specially equipped for their detailed examination. Similarly, for virus studies, post-mortem tissues and other material were submitted to a laboratory engaged in virus research.

Previous investigators (Costello and Lind, 1939; Sakula, 1943; Sevvit, 1945; Light and Hodes, 1943; Buddingh and Dodd, 1944) have found a variety of bacterial and virus agents associated with infantile diarrhoea and vomiting. Bray (1945) isolated a particular serological type of *Bact. coli var. neapolitanum* from 42 out of 44 cases of infantile "summer diarrhoea." From an early stage of our investigation, work on a certain type of *Bact. coli* provided a promising line to pursue. Sera for its identification were prepared from the *Bact. coli* strains of infants suffering from epidemic diarrhoea of the newborn. The outbreak in which these cases occurred is described under Group A below. The strain is referred to throughout the paper as *Bact. coli* D 433. A description of the serological work, together with an account of the biochemical activities and other characters of the strain, are given in a later section.

The main purpose of this paper is to report the association of *Bact. coli* D 433 with certain groups of cases of infantile diarrhoea and vomiting and its absence in control groups of healthy and sick babies. Work on similar lines was published by Giles and Sangster in March, 1948. They reported the isolation of a serologically homogeneous strain of *Bact. coli* from over 90% of cases of primary infective gastro-enteritis admitted to hospital during an outbreak in the city of Aberdeen in 1947.

Cases of Infantile Diarrhoea and Vomiting

Clinical and bacteriological investigations were made on the following groups of infants suffering from diarrhoea and vomiting: one outbreak in a maternity unit (Group A); two outbreaks in a mothers and babies' hostel (Groups B and C); one outbreak in a residential nursery (Group D); intercurrent cases of diarrhoea and vomiting in a children's hospital (Group E); routine admissions to two gastro-enteritis units (Groups F and G); and endemic diarrhoea and vomiting in a residential nursery (Group H).

Group A

An outbreak of epidemic diarrhoea of the newborn occurred in February and March, 1947, in the 90-bed maternity unit of a Derbyshire hospital. In all, 20 babies contracted the disease and 14 of them died. At the onset of the illness the age of 19 of the babies was from 6 to 20 days, and that of one baby, retained for the treatment of minor sepsis, was 43 days. All the babies had received early glucose feeds.