6. A comparison of the maternal and fetal thyroids shows no similarity for the first seven months. In the last months of pregnancy, however, the similarity in the histological appearances of the fetal and maternal thyroid is striking. This observation, however, is based on a small number of cases and requires further study.

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The histological sections for this work were prepared by Mr. Teale in the Department of Pathology of the Winnipeg General Hospital under the supervision of Prof. William Boyd. Miss Nason prepared all the micro-photographs for this and all previous publications.

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REFERENCES

1. ABBOTT, A. C. AND PRENDERGAST, J.: Histological variations in animal thyroids in western Canada, *Canad. M. Ass. J.*, 1934, **31**: 465.
2. ABBOTT, A. C., GOODWIN, A. M. AND MELTZER, S.: The effect of tracheal compression on the thyroid gland, *Canad. M. Ass. J.*, 1933, **28**: 481.
3. WILSON, G. E.: The thyroid follicle in man: its normal and pathological configuration, *Anat. Rec.*, 1927-1928, **37**: 31.
4. NORRIS, E. H.: The morphogenesis of the follicles in the human thyroid gland, *Am. J. Anat.*, 1916, **20**: 411.
5. COOPER, E. R. A.: The Histology of the More Important Human Endocrine Organs at Various Ages, Oxford Medical Publication, 1925.
6. ABBOTT, A. C. AND BALL, R. P.: Pathology of the thyroid gland of the human fetus and newborn infant, *Canad. M. Ass. J.*, 1931, **24**: 347.
7. MURRAY, I.: The thyroid gland in the full-time human fetus and in the newborn infant, *Brit. M. J.*, 1927, **1**: 5.

THE PRODUCTION OF TUMOUR AND TUMOUR-LIKE GROWTHS IN RATS

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THE production of tumours in rats was attempted on the basis of the factors shown in a preceding paper* to be characteristic of a strain of mice with a tumour history. The low oxygen consumption of their excised tissue, the low hæmoglobin of their blood, their low red cell count, and their low red cell volume all pointed in the direction of an oxygen deficiency of the cells. The high calcium content of their soft tissues seemed to indicate an excess of base. Although these conditions were compatible with tumour, it was not shown whether they were the cause or the result of tumour. Therefore an attempt was made to bring about oxygen deficiency and excess of base in certain tissues of the rat in order to see whether tumours would result.

As to oxygen deficiency, it has been shown by Barcroft *et al.*[1] that the oxygen tension of the blood which supplies the rabbit fetus is very low, ranging from 20 to 30 mm. of mercury. From this it is apparent that the fetal cells subsist and multiply on a much smaller oxygen consumption than is required by the cells in post-uterine life. From the work of Warburg[2] and others it would seem that excised tumour and embryonic tissue have somewhat the same type of respiration. It would seem probable, therefore, that if in post-uterine life the cells were subjected to the same low oxygen tension as existed in uterine life they might be able to revert to the fetal type of respiration, but in so doing run the danger of becoming tumour cells.

As to excess of base in the tissues, it has been shown by Bálint and Weiss[3] that alkalosis, irrespective of its cause, always leads to an increase of the proliferative process. Thus they found that alkalinity promoted growth of seedlings, healing of wounds, assimilation process of inflammation, tissue-culture growth, and growth of carcinoma. Others[4] have found more potassium in young, actively growing tumours than in slowly growing or old tumours. Roffo,[5] in particular, found that the potassium content of rat tumours was about twice that of the rest of the rat, and was about the same as that of rat embryo. It would seem probable, therefore, that excess of base in the tissues might furnish the predisposing condition for cells that have been forced into the fetal type of respiration to proliferate, and, under favourable conditions, to become cancerous.

The apparent oxygen deficiency and excess of calcium in the tissues of the tumour-strain mice of the preceding study seemed to be part of a general condition. If the same factors occurred in man it was thought they would more likely be part of a local rather than a general condition. Since the significance to the

* See *Canad. M. Ass. J.*, 1937, **36**: 27.

human situation was the foremost consideration in this study, an attempt was made to produce these conditions locally. The uterus was the organ chosen for this purpose, partly because of its comparatively greater isolation, and partly because one horn might serve as a control for any condition produced in the other horn. Oxygen deficiency was brought about by ligating the artery supplying either horn of the uterus. This would probably result not only in deficiency of oxygen but also of substrate to be oxidized, for the ovary as well as the uterus. Nothing was done to bring more base into the tissues other than the injury they would suffer through malnutrition. It was thought that this injury would result in the feeble circulation and retrogressive changes that Wells[6] says are always conducive to calcification. Such a deposition of calcium was counted upon to take place in the tissues affected and to furnish the base thought necessary for the initiation of cell proliferation.

The rats used in these experiments were mostly the common white strain, but also included some descendants of a cross between the white and a hardier black and white strain. This stock had been bred and raised in our laboratories for 7 years, during which time no case of spontaneous tumour was ever observed. Two series of rats were studied. The first consisted of 12 which had the artery to one or both horns ligated near the bifurcation of the uterus, and the second, of 12 litter mates kept as controls. The horn with the unligated blood supply also served as a control. All but two of the rats were kept in small cages to prevent much exercise and to minimize its effect upon the circulation of the blood and the lymph. The remaining two were kept in a large cage which permitted plenty of exercise.

About three months after the operations on the first series, Rat 1, having the left artery ligated, died at the age of 18 months, having on the left side the uterus and ovary growth shown in Fig. 1. This growth had the appearance of a tumour, but in the microscopic section shown in Fig. 2 its cells appear to be more characteristic of inflammatory than tumour growth. The death of this rat seemed to be due to intestinal obstruction brought about not only by the size of the growth but by its absorption of liquid from the intestinal tract.

About 5 months after the operation, which in this case involved the ligation of the arteries to both horns, Rat 2 at the age of 24 months developed the 10 g. growth on the breast shown in Fig. 3. The microscopic section shown in Fig. 4 indicates that this growth was an adenocarcinoma of the mammary gland. This rat died under anæsthesia while the tumour was being removed.

About 6 months after the operation, also involving the ligation of both arteries, Rat 3, a litter mate of Rat 2, also developed the 15 g. breast tumour shown in Fig. 5. The microscopic section shown in Fig. 6 indicates that this growth was an adenofibroma of the mammary gland. This tumour was removed, but about five months later appeared a little lower down another tumour (shown in Fig. 7), of which microscopic sections indicated that it also was an adenofibroma of the mammary gland. Death intervened before the second tumour reached the size of the first. Autopsy did not reveal any pathological conditions other than the tumour and the ligated arteries.

Both of the rats allowed more freedom in a larger cage also developed breast tumours, but not until after a lapse of about twice the time taken by those kept in the smaller cages. One of these, Rat 4, having both arteries ligated when about 16 months old, had 12 months later the breast tumour, weighing 96 g., shown in Fig. 8, microscopic sections of which indicated that it was an adenofibroma of the mammary gland. This rat died under anæsthesia while the tumour was being removed. The other, Rat 5, having both arteries ligated when about 6 months old, had 12 months later the breast tumour shown in Fig. 9. This rat was still living at the time of writing.*

The other 7 rats were sacrificed at intervals of 4 to 8 months after the operation, at ages ranging from a year to a year and a half. Five of these had developed abnormal abdominal growths, the most common being the cyst-like growths. One had set up an accessory circulation by forming adhesions between the uterus and intestines. One had succumbed in the apparent effort to compensate by absorption of liquid from the intestinal tract. This rat pre-

* This rat has since died, having an adenofibroma of the mammary gland, weighing 201 g., or 23 g. more than the rest of the body.

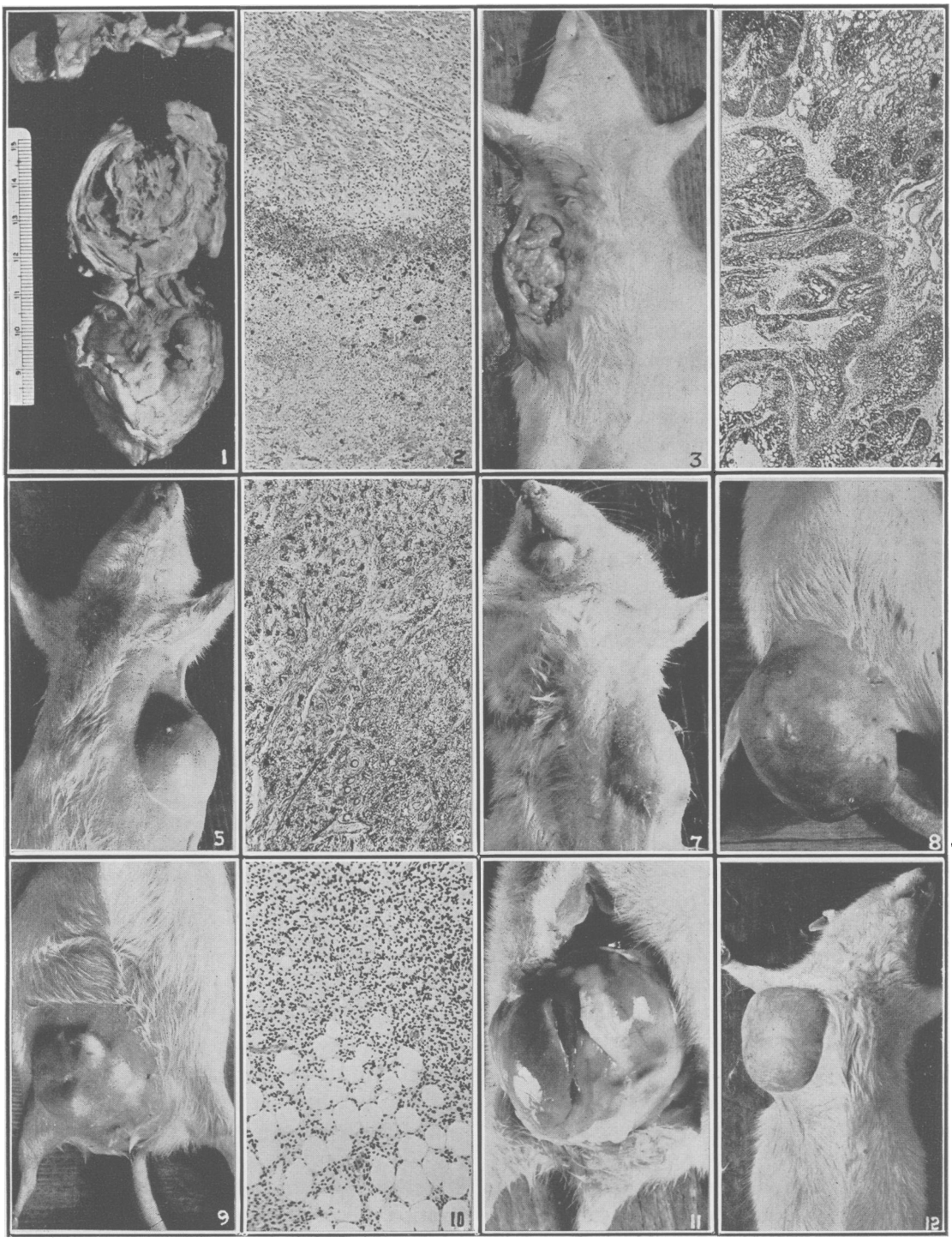

Fig. 1.—Abscess in Rat 1, Series I. **Fig. 2.**—Photomicrograph (x 140) of abscess in Rat 1, Series I. **Fig. 3.**—Adenocarcinoma of Rat 2, Series I. **Fig. 4.**—Photomicrograph (x 45) of adenocarcinoma of Rat 2, Series I. **Fig. 5.**—First adenofibroma of Rat 3, Series I. **Fig. 6.**—Photomicrograph (x 45) of adenofibroma of Rat 3, Series I. **Fig. 7.**—Second adenofibroma of Rat 3, Series I. **Fig. 8**—Adenofibroma of Rat 4, Series I. **Fig. 9.**—Breast tumour of Rat 5, Series I. **Fig. 10.**—Photomicrograph (x 140) of sarcoma of Rat 3, Series II. **Fig. 11.**—Sarcoma of Rat 3, Series II. **Fig. 12.**—Breast tumour of Rat 5, Series II.

sented three nodular growths in the broad ligament with intestinal obstruction. Apparently the other six rats either were examined too soon or were better able to fight off the effects of ligation.

Since during the development of these tumours none of the 12 litter-mate controls exhibited any sign of tumour growth, 6 of these controls were also subjected to the ligation of both arteries. The 6 rats operated on, with the remaining 6 controls, constituted the second series. About 3 months after the operations on the second series Rat 1 of this series died of gangrene, and Rat 2 of intestinal obstruction similar to that already observed in the first series. About the same time Rat 3 died at the age of 19 months, having the abdominal growth shown in Fig. 11. This growth involved the uterus, ovary, and broad ligament, and completely enclosed the caudal portion of the bowel. The microscopic section shown in Fig. 10 indicates that this was a neoplasm, probably a sarcoma. About 6 months after the operation Rat 4 died at the age of 30 months, having in the ligated areas many nodular growths. Rat 5 at the age of about 30 months developed the breast growth shown in Fig. 12. This rat and Rat 6, which was without external sign of tumour, were both living at the time of writing.*

That an oxygen deficiency occurred as a result of these operations was shown by metabolic determinations made on slices of excised uterus by the Warburg differential method.[7] It was found that the oxygen consumption of the horn whose blood supply had been obstructed was less and its glycolysis greater than that of the other horn or of the horns of its litter mate not operated on. This, it should be noted, is the type of respiration characteristic of tumour and of the fetus.

Whether the initiation of tissue proliferation resulted from a deposition of calcium could not be easily determined. However, it seemed probable that the tumour-type of metabolism observed indicated degenerative changes such as Wells[6] found favourable for calcification. In tissues with this type of metabolism Warburg[2] had observed the elimination of much ammonia, which would also serve to increase the base in these tissues. No increase was observed in the pH of the blood.

These conditions of malnutrition and supposed consequent deposition of base were probably responsible for the inflammatory growth of the uterus and ovary of Rat 1 of the first series. Probably the rapidity of the growth in an otherwise healthy rat was responsible for the inflammatory character of the tissues. The extreme measures used apparently brought about growth so quickly that the rat did not live long enough for the tissue to become anything but inflammatory. If the measures adopted had been less extreme, so that growth had taken place very slowly with the rat becoming less healthy, then it is probable that the tissue would have been more tumour-like. That such a transition may take place is supported by Ewing, MacCallum and most pathologists. Some authors[8] have gone so far as to maintain that there is only a difference in degree between the healing of wounds, inflammatory hyperplasia, and tumour-formation. Ewing[9] says,

> A process beginning as a simple inflammatory hyperplasia may in the same individual gradually assume neoplastic qualities. In the thyroid gland of goitre, especially in fish, in the prostate gland of hypertrophic prostatitis, in the uterine mucosa of a glandular endometritis, in the mammary gland of chronic mastitis, and in the lymph nodes of Hodgkin's granuloma are occasionally seen transformations of a functional or inflammatory hyperplasia into a more or less typical neoplasm. . . . . All these considerations strengthen the view that the precancerous condition is of wide occurrence and of much theoretical and practical importance in oncology. In this condition one finds tissues and cells in a state of overgrowth intermediate between inflammatory and neoplastic hyperplasia, exhibiting certain tumour characters which must be judged from different standards for each tissue, and which experience shows are often followed by genuine and usually malignant tumours.

That somewhat different conditions might affect the nature of the growth was shown in the case of Rat 3 of the second series. In this 19-month-old rat the ligation of both arteries had resulted in a growth that enclosed the caudal portion of the bowel without causing bowel obstruction. In the other 18-month-old rat the ligation of one artery resulted in a growth that caused intestinal obstruction. Perhaps the different conditions to be met and, perhaps more important, the different individual health and resistance characteristics with which to meet the different conditions may have accounted for the growth in the latter case being an abscess, while in the former it was a neoplasm.

* These rats have since died, No. 5 having an adenofibroma of the mammary gland, and No. 6, small uterine growths.

That injury to the uterus and ovary should have resulted in tumour of the mammary gland may not appear at first sight consistent with the assumptions that have been made. That a relationship exists between the uterus and ovary, on the one hand, and the mammary gland, on the other, is evidenced by the following facts quoted from Dr. Turner:[10]

1. From the time of puberty there are changes of a cyclic nature taking place in the mammary gland of the female correlated with the ovarian cycle.
2. During early pregnancy the growth and proliferation of the gland is greatly increased, while toward the end of pregnancy the growth phase is gradually superseded by secretory activity which becomes intense about the time of parturition.
3. With the cessation of secretory activity the alveoli and ducts shrink in size, resulting in an enormous decrease in the size of the entire gland. Further growth and renewed secretion normally take place only with another cycle of reproduction.

These relationships between the uterus and the mammary glands were formerly explained on the basis of direct nervous connection, more recently on the basis of hormonal rather than nervous connection. Whatever the explanation, there can be no doubt of the relationship and of the probability that injury to the uterus and ovary might affect the mammary gland and *vice versa.*

The evidence in support of this probability in the case of women has been examined by Taylor, of the Memorial Hospital, New York City. He has studied the etiology of 66 cases of chronic mastitis,[11] and of 217 cases of neoplasms of the breast.[12] From an etiological point of view he considers that tumours of the breast, including carcinoma, are closely related to those of the endometrium, thyroid and ovary, and somewhat also to myomas of the uterus and tumours of the prostate. It will be noted that these are the same tissues referred to by Ewing as being often found in a state of overgrowth intermediate between inflammatory and neoplastic hyperplasia. Taylor found chronic mastitis and neoplasms of the breast often correlated with pelvic lesions, particularly of the uterus and ovaries. He concluded that a functional disturbance of the physiological relationship between the ovary and the breast was an important factor in the genesis of many forms of tumour of the breast. He was able to assemble a lengthy bibliography in support of his observations and conclusions.

SUMMARY

This study is so far too limited to permit definite conclusions on most of the issues involved. It is desired, therefore, only to summarize the facts and leave their explanation to future study. The facts are as follows.

1. In the 18 rats operated on there developed 1 adenocarcinoma, 3 adenofibromas, 1 sarcoma, and 2 mammary gland growths, probably neoplastic, in rats still living.

2. In 5 of the rats operated on, sacrificed in order to make early observations, cyst-like growths were found which might have become tumours or caused tumour development elsewhere if the rats had been allowed to live longer.

3. These tumours and tumour-like growths did not occur elsewhere than on the parts directly affected or closely related.

4. None of the litter-mate controls developed any similar growths, and no tumour has ever occurred spontaneously in the colony of which these rats formed a part.

REFERENCES

1. BARCROFT, J., HERKEL, W. AND HILL, S.: Rate of blood flow and gaseous metabolism of the uterus during pregnancy, *J. Physiol.*, 1933, **77**: 194.
2. WARBURG, O.: The Metabolism of Tumours, Constable, London, 1932, pp. 129, 229.
3. BALINT, R. AND WEISS, S.: Tissue Proliferation and Acid Base Equilibrium, Constable, London, 1932, pp. 94, 159.
4. SHEAR, N. J.: The rôle of sodium, potassium, calcium, and magnesium in cancer, a review, *Am. J. Cancer*, 1933, **18**: 924.
5. ROFFO, A. H.: Action of certain metallic ions on the growth of normal and neoplastic tissues *in vitro*, *Bol. Inst. Med. Exp.*, 1925, **1**: 307.
6. WELLS, H. G.: Chemical Pathology, Saunders, Philadelphia, 1925, p. 492.
7. WARBURG, O.: Verbesserte Methode zur Messung der Atmung und Glykolyse, *Biochem. Zeitschr.*, 1924, **152**: 51.
8. BROSCH, A.: Theoretische und experimentelle Untersuchungen zur Pathogenesis und Histogenesis der malignen Geschwülste, *Virchow's Arch.*, 1900, **162**: 32.
9. EWING, J.: Neoplastic Diseases, Saunders, Philadelphia, 1928, p. 42.
10. TURNER, C. W.: Sex and Internal Secretions, Williams & Wilkins, Baltimore, 1932, p. 544.
11. TAYLOR, H. C., Jr.: Gynæcological aspects of the etiology and treatment of chronic mastitis, *Surg., Gyn. & Obst.*, 1933, **17**: 627.
12. TAYLOR, H. C., Jr.: The etiology of neoplasms of the breast, *Arch. Surg.*, 1930, **21**: 412, 597.

"Whatever your career may be, do not let yourselves become tainted by a deprecating and barren scepticism; do not let yourselves be discouraged by the sadness of certain hours which pass over nations. Live in the serene peace of laboratories and libraries. Say to yourselves first: 'What have I done for my instruction?', and, as you gradually advance, 'What have I done for my country?', until the time comes when you may have the immense happiness of thinking that you have contributed in some way to the progress and to the good of humanity. But, whether our efforts are or are not favoured by life, let us be able to say, when we come near the Great Goal, 'I have done what I could.'"—*Pasteur.*