may have an appreciable influence on their growth *in vivo*. In this connexion it is pertinent to point out that when pure cultures of malignant cells are grown side by side with cultures of normal tissues, infiltrative growth is very slow or does not occur at all. Earle (1937), in comparing the slow infiltration of fragments of muscle by the Walker rat carcinoma No. 256 *in vitro* with "the extreme rapidity of infiltration and destruction of surrounding tissues by the tumour grown *in vivo*, particularly in intramuscular injections," remarks that "there are as yet important, unanalysed factors which influence or control the infiltration of other tissues by the living tumour cell." The results of my experiments suggest that these factors may be the presence of monocytes and macrophages in the tumour *in vivo* which are absent from pure cultures growing *in vitro*. One naturally hesitates before applying results obtained from experiments *in vitro* to the explanation of processes occurring in the animal body. So far as I am aware, however, there is no record in the literature of a specific cellular reaction occurring *in vitro* which has not also been found to occur under appropriate conditions *in vivo*. One might expect, therefore, that the results of interaction between leucocytes and macrophages with malignant cells in the animal body would be of the same kind as in tissue cultures. On this assumption one might expect that leucocytes and macrophages would stimulate not only the growth of the malignant cells but also their proteolytic capacity, and thereby facilitate the infiltration of normal tissues.

Summary

The results of these experiments confirm the work of Carrel in demonstrating that cultures of leucocytes produce substances which stimulate the growth of fibroblasts and sarcoma cells.

When fibroblasts or sarcoma cells are grown together with leucocyte cultures their growth begins sooner and is ultimately more extensive than when they are grown alone. Growth stimulation occurs without actual contact between the two types of cells.

When fibroblasts or sarcoma cells are grown in contact with the cells from leucocyte cultures not only is the growth of the former stimulated but also their capacity to digest clotted plasma (proteolysis).

In mixed cultures both kinds of cells remain in a more active functional state.

On the assumption that the same cellular reactions occur in the animal body as have been described in tissue cultures, it is suggested that monocytes and macrophages by stimulating the growth and proteolytic activity of malignant cells may facilitate their invasive growth into normal tissues.

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Prolonged Coma.—According to Lester prolonged coma occurring in the course of insulin shock therapy is a complication which should be avoided whenever possible, as it is not usually followed by improvement in the patient's mental state. Such improvement as may be obtained in a few cases does not justify attempts to produce prolonged coma, as the mortality rate is too high (16 per cent. in this series).—Study of Prolonged Coma following Insulin Shock. D. Lester.—*Amer. J. Psychiat.*, March, 1939, **95**, 1083.

EFFECT OF OESTRADIOL BENZOATE ON THE AMOUNT OF GONADOTROPHIN FOUND IN THE PITUITARY GLAND AND URINE OF POST-MENOPAUSAL WOMEN

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The effect of oestrogens on the gonadotrophic activity of the pituitary gland has been studied by several methods. First, observations have been made on the gonads of the injected animal or on the gonads of another animal united to it in parabiosis (Martins, 1929; Kallas, 1930; Bunster and Meyer, 1938). These methods are not applicable to man. Secondly, the amount of gonadotrophic substance present in the urine and blood has been investigated. Frank and Salmon (1935) and Jones and MacGregor (1936) found that the gonadotrophic substance disappears from the urine of ovariectomized or post-menopausal women treated with oestrogen. Thirdly, indirect information has been obtained from the histology of the pituitary gland (Severinghaus, 1939). Fourthly, the amount of gonadotrophic hormone in the anterior pituitary gland has been estimated directly. Several workers (Meyer, Leonard, Hisaw, and Martin, 1930, 1932; Bialek-Laprida, 1933) found a diminished amount of gonadotrophic hormone in the pituitary glands of gonadectomized rats which had been treated with oestrogen. Henderson and Rowlands (1938) and Noble, Rowlands, Warwick, and Williams (1939) showed that there is an increased amount of gonadotrophic hormone in the pituitary gland of post-menopausal women. In this paper we describe the effect of the injection of large amounts of the oestrogenic substance oestradiol benzoate on the pituitary gland of similar subjects.

Methods

Administration of Oestrogen.—Four patients, whose ages ranged between 54 and 70 years, were injected intramuscularly daily with 10 mg. (100,000 I.B.U.) of oestradiol benzoate in oil solution. One patient was injected with 30 mg. daily for three days. These five patients were suffering from incurable disease, and the cause of death in the order shown in Table II was right heart failure, cirrhosis of the liver, chronic nephritis, carcinoma of the breast, and carcinoma of the cervix. At the post-mortem examination the endocrine glands appeared normal in each case. The pituitary gland was removed and the anterior lobe weighed, desiccated in acetone, and afterwards dried in a stream of hot air. Each gland was then ground to a fine powder and weighed. The pituitary glands of five other women of about the same age who had not been treated with oestrogen were used as controls.

Extraction of Urine.—Another woman, who had been ovariectomized twenty-five years previously, was injected with the same amount of oestradiol benzoate for six days. The urine of this woman was collected for three periods—namely, before, during, and ten days after the injections ceased. Crude extracts, which are referred to as CU1A,

CU1B, and CU1C, respectively, were prepared from these collections by precipitation with alcohol (Noble *et al.*, 1939). The data concerning the collections and extraction of the urine are given in Table I.

TABLE I.—*Data of Collection and Extraction of Urine of Ovariectomized Woman*

Extract (CU1)	Dates of Collections	Total Volume (litres)	Amount of Extract (gm.)	No. of c.cm. of Urine = 1 mg. of Extract
A	4/7-8/7/38	7	38.0	0.18
B	13/7-17/7/38	4	3.6	1.10
C	29/7-5/8/38	5.5	17.7	0.32

The extract CU1A was afterwards concentrated by the method of Thomsen and Pedersen-Bjergaard (1936). One milligramme of this extract (CU1A conc.) was then equivalent to 6.4 c.cm. of urine.

Biological Assay.—The dried tissue from each pituitary gland was assayed separately for gonadotrophic activity by its capacity to stimulate the ovaries of the immature hypophysectomized rat. Rats weighing 40 to 45 grammes were hypophysectomized, and ten days later, when the average weight of the ovaries had decreased from 10 to about 5 mg. (Noble *et al.*, 1939), they were injected subcutaneously with the pituitary tissue once daily for five days. For injection the tissue was dissolved in slightly alkaline water and afterwards neutralized with HCl.

The rats were killed twenty-four hours after the last injection, and the ovaries and uterus were dissected, fixed in Bouin's fluid overnight, and weighed the following day. The sella turcica of each rat was examined for the presence of anterior pituitary tissue, and any rat which was found to be incompletely hypophysectomized was discarded from the test. The ovaries and uterus were prepared for histological examination. Serial sections of all ovaries were cut and were stained in Meyer's haemalum and eosin.

The total gonadotrophic activity of each gland was expressed in terms of the equivalent amount of an extract (AP91D) prepared from a large mixed sample of human pituitary glands by the method of Wallen-Lawrence and Van Dyke (1931). For this purpose a dose-response curve was constructed for the effect of AP91D on the ovaries of hypophysectomized rats. A fivefold increase

(from 5 mg. to 25 mg.) in the weight of the ovaries was produced in five days by the injection of 1 mg. of this extract.

Results

The Effect of Oestrogen on the Gonadotrophic Hormone of the Pituitary Gland.—The results given in Table II show that oestradiol benzoate in even very large doses does not cause any enlargement of the pituitary gland. The total amount of gonadotrophic hormone contained in the gland of the patients injected with the oestrogen is, however, greatly diminished. No gonadotrophic activity was demonstrable in the pituitary gland of the patient injected for fifty-four days. It has already been shown that after the menopause, in untreated patients, there is a tendency to an increased content of the follicle-stimulating hormone and a decreased content of the luteinizing hormone (Noble *et al.*, 1939). Histological examination of the ovaries of the hypophysectomized test rats failed to indicate that the treatment with oestradiol benzoate affects in any way the relative proportions of the follicle-stimulating hormone and luteinizing hormone in the human pituitary gland. The large amounts of oestradiol benzoate which were injected depress both components of the gonadotrophic complex. The general assumption that oestrogens control the relative production of these two substances by stimulating the secretion of luteinizing hormone and by inhibiting that of the follicle-stimulating hormone implies that the qualitative response of the pituitary gland should be influenced by the dosage of oestrogen.

The Effect of Oestrogen on the Gonadotrophic Hormone in the Urine of an Ovariectomized Woman.—The results of the assays given in Table III show that the extract CU1A, prepared from the urine before treatment with oestradiol benzoate was begun, contained a small amount of substance capable of stimulating the ovaries of the intact immature rat. The ovaries of the hypophysectomized rats treated with the same amount of extract, however, remained unstimulated; so it appears that the effect on the normal ovaries is brought about through the intermediation of the animal's own pituitary gland. The extracts prepared from the urine which was collected during and after the treatment with oestrogen, however, failed to stimulate the ovaries even of normal rats. These results are somewhat disappointing on

TABLE II.—*Effect of Oestradiol Benzoate on Gonadotrophic Activity of Pituitary Gland of Post-menopausal Women*

No. of Pituitary Gland (HP.)	Age (years)	Weight of Anterior Pituitary Gland		Duration of Treatment with Oestradiol Benzoate (days)	Biological Assay in Hypophysectomized Rats				Histology of Ovaries
		Fresh (mg.)	Dried (mg.)		Amount of Dried Gland Injected Daily (mg.)	Weight of Organs		Equivalent Activity in Terms of Extract (AP91D) (mg.)	
						Ovaries (mg.)	Uterus (mg.)		
858	64	—	60	3 (30 mg. daily)	0.4	30	73	18	F.S. and I.C.S.
1013	58	640	77	3 (10 mg. „)	0.4	26	54	19	F.S. and I.C.S.
1030	54	570	81	23 „	2.0	50	63	10	F.S. and C.L.
1029	68	730	67	45 „	2.0	12	73	2	F.S. only
907	70	—	77	54 „	2.0	4	15	0	No response
Controls									
727	61	750	117	0	0.2	56	65	164	F.S. and C.L.
739	62	550	81	0	0.2	44	58	81	F.S. and C.L.
755	65	450	57	0	0.2	57	64	80	F.S. and C.L., M.G.L.
737	67	500	90	0	0.2	31	62	56	F.S. and I.C.S.
678	75	700	53	0	0.4	40	107	22	Mainly F.S., but some slight M.G.L. and I.C.S.

F.S. = follicle stimulation; M.G.L. = luteinization of the membrana granulosa; C.L. = corpora lutea; I.C.S. = stimulation of the interstitial tissue.

TABLE III.—Assay of Extract (CUI) of Urine of Ovariectomized Woman Before (A), During (B), and After (C) Treatment with Oestradiol Benzoate

Extract Given (CUI)	Test Animal (Immature Rat)	No. of Rats per Group	Amount of Extract Injected Daily (mg.)	Amount Given Daily in Terms of Original Urine (c.cm.)	Average Weight of :		Histology of Ovaries
					Ovaries (mg.)	Uterus (mg.)	
—	Normal	10	0	0	10	20	
A	"	5	10	1.8	13	36	Very slight F.S.
"	"	5	20	3.6	20	72	F.S. and M.G.L.
A (conc.)	"	5	2	12.8	23	68	
—	Hypophysectomized	5	0	0	5	14	
A	"	3	20	3.6	8	13	No response
A (conc.)	"	4	2	12.8	6	14	" "
B	Normal	5	10	10.1	13	21	" "
"	"	5	20	20.2	12	24	
C	"	5	10	3.2	10	24	" "
"	"	5	20	6.4	11	23	

F.S. = follicle stimulation. M.G.L. = luteinization of membrana granulosa.

account of the comparative inactivity of the extract prepared from the urine before the patient was treated, but it seems that the slight activity initially present undoubtedly disappeared as the result of treatment with oestradiol benzoate.

Discussion

The dose of oestrogen used in this investigation was that shown by Schrire and Sharpey-Schafer (1938) to diminish the excretion of urinary creatinine in acromegaly; smaller doses failed to do so. The importance of dosage is emphasized by the effect of oestrogens on urinary gonadotrophic hormone. Our results on the urine of an ovariectomized woman agree with those of Frank and Salmon (1935) and Jones and MacGregor (1936). On the other hand, Heller and Heller (1939), while clearly demonstrating the increase in urinary gonadotrophic substance that occurs either after the menopause or after castration, were unable to show any effect of injected oestrogen on the amount of gonadotrophic hormone excreted. The maximum daily dosage of oestrogen used by them was 8,000 I.U. of amniotin (Squibb) given orally or, in two cases, 10,000 I.U. injected intramuscularly. This dosage is considerably less than that used by workers who have observed a disappearance of the hormone from the urine as the result of the administration of oestrogen.

Our results show that no gross enlargement of the human pituitary gland follows the injection of large amounts of oestradiol benzoate. Although relatively enormous haemorrhagic tumours can be produced in the pituitary gland of the rat in this way, treatment has to be continued for periods of six to nine months; so it is extremely unlikely that tumours of the pituitary gland would be produced in women by even massive doses of oestrogen in the space of two months. It has, in fact, been shown by Dynes (1939) that the administration of 5 mg. of oestradiol benzoate three times a week for six weeks caused no histopathological changes in the human pituitary gland.

The simplest interpretation of our results is to suppose that, following diminished function of the gonads, the pituitary gland elaborates a greater amount of gonadotrophic hormone and that this excess activity can be inhibited by the administration of large amounts of oestrogen. However, the fact that the pituitary gland has a decreased content of gonadotrophic hormone after treat-

ment with oestrogen does not necessarily imply that secretory activity has diminished. Indeed, it might well be increased and the gland become thereby emptied of its hormone. Severinghaus (1939) has taken the view on histological grounds that the action of oestrogens on the pituitary gland is one of stimulation and release of secretion. The disappearance of gonadotrophic hormone from the urine of patients treated with oestrogen, in the absence of evidence that the renal threshold is altered or that these substances come from elsewhere than the pituitary gland, suggests, however, that the final result is diminished pituitary activity, whether this be due to immediate inhibition of the formation of the hormone by the pituitary cells or to an exhaustion following initial stimulation.

Summary

Investigations have been made of the amount of gonadotrophin in the pituitary glands of post-menopausal women, (a) untreated, and (b) after the administration of large amounts (10 mg., or 100,000 I.B.U.) of oestradiol benzoate daily over periods lasting from three to fifty-four days.

The oestrogen causes a decrease in the content of gonadotrophic substance as assayed on the hypophysectomized rat.

It has also been shown that the presence of a small amount of a gonadotrophic substance in the urine of an ovariectomized woman was abolished by the same dose of oestradiol benzoate.

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