MAMMOTROPHIC POTENCY OF HUMAN URINE

BY

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It is now generally believed that progressive cellular multiplication in some cancers of the human breast is strictly conditioned by an adequate supply of an anterior pituitary hormone. The favourable results of hypophysectomy in such tumours is spectacular. On the other hand, it can do nothing for the patient whose tumour is independent of hypophysial control. It is unfortunate, therefore, that we have no reliable laboratory investigation capable of clinical application on even a modest scale which can inform the surgeon whether his patient with breast cancer has a pituitary-dependent tumour or not.

The pituitary trophic hormones appear to be glycoproteins of low molecular weight, and the bioassay of one of them, gonadotrophin, is capable of clinical application. The technique used is the Klinefelter extraction process in which the hormone proteins are precipitated by alcohol, the precipitate purified, redissolved, and injected into suitable animals, the biological end-point being a significant increase in the weight of the uterus (Klinefelter *et al.*, 1943).

Preliminary Experiments

A series of preliminary experiments carried out early in 1955 strongly suggested that Klinefelter extracts of 72-hour specimens of the urine of normal pre-menopausal women powerfully and specifically stimulate the proliferation of mammary epithelium (Scowen and Hadfield, 1955). These experiments have now been repeated, amplified, and controlled, and the original observation has been fully confirmed.

Very early in the course of these confirmatory experiments it was found that, when using Klinefelter extracts for the detection of the mammotrophic activity of normal urine, the results were highly irregular and unpredictable. The final extracts were injected over a period of five days into weanling male mice whose mammary glands were found to be so remarkably sensitive to all the hormones known to be concerned in normal mammatogenesis that the biological side of the experiments could not be blamed for the irregular results. For example, 65 weanlings were given prolactin twice daily for five days; 345 mammary glands were isolated and in all of them a highly significant increase in area and glandular complexity was observed, at least 60% of the glands showing widespread development of glandular acini. Similar responses were observed with oestrogenic hormones.

It was therefore considered justifiable to suspect that the Klinefelter process, instead of increasing mammotrophic potency, was frequently responsible for its loss.

The extraction process may be summarized as follows:

STAGE I: Four Volumes of Absolute Alcohol Added to 72-Hour Specimen of Urine

Deposit A (Largely composed of phosphates. Presumed to contain precipitated urinary and hormone proteins) **Supernatant.** Discarded

STAGE II: Deposit Extracted with Three Successive Aliquots of Distilled Water: Centrifugalized

Supernatant (Presumed to contain all hormone protein in solution) **Deposit B** (Largely phosphatic. Presumed free of hormone protein). Discarded

STAGE III: Dialysed at 0° C. Against Water. Protein Re-precipitated by Alcohol

Deposit C (still largely phosphatic. Presumed to contain all hormone protein) **Supernatant.** Discarded

STAGE IV: Deposit Dried, Powdered, and Extracted with Distilled Water

Supernatant. Presumed to contain protein. Injected into animal **Deposit D** (Presumed inactive). Discarded

A series of experiments was set up in which eight batches of weanling males were injected twice daily for five days with Deposit D from eight normal urines. On the average the deposits were found to be 6.3 times more potent than their supernatants. Several other batches of animals were then injected for a similar period with various samples of Deposit B. The results were as spectacular as they were unexpected. In the intact weanlings of the strain used the average number of end-bulbs or "clubs" per normal gland is 1.14. The average number of clubs in the injected animals was 20.2, the average area covered by the reacting mammae was at least 15 times that of the controls, and in 42% of them there was unmistakable early development of glandular acini (see Fig.).

Whole mammary glands: mounted unsectioned ($\times 18$).

A. Mammary gland of normal weanling male mouse showing average complexity of structure. It consists of a single main duct with a few short unclubbed branches. B. Mammary gland reaction produced by injection twice daily for five days of extract of urine from a normal pre-menopausal woman. Note the long clubbed branches and the branch buds, all of which are already clubbed.

In the course of the control experiments it was found that the amount of prolactin required to produce an optimum acceleration of growth in the rudimentary mammae of the weanling male was 1,000 μ g. per day over a period of five days. This increased the average number of clubs per gland from the normal 1.14 to 23.8. It has been shown by Lyons and his colleagues (1955) that no combination of hormones which does not include prolactin in relatively large quantities will stimulate the growth of the mammae of hypophysectomized and gonadectomized immature male rats. It is at least possible, therefore, that the mammotrophic substance present in human urine is prolactin, which in synergism with oestrogens forms a mammotrophic combination in which prolactin acts at a concentration of several hundred micrograms.

It seems likely that a determination of the mammotrophic potency of human urine can be so simplified that it will eventually find a place in the clinico-pathological investigation of those patients suffering from breast cancer in whom hypophysectomy must be seriously considered.

A detailed account of the experiments outlined in this paper is in course of preparation. Meanwhile, it is hoped that the work described in this communication will be of assistance to other workers in this field.

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Medical Memoranda

Immediate Results of Treating Sun-Blindness with Carbachol Injections

The eclipse of the sun on June 30, 1954, was observable in the north-east of Scotland, and especially in Aberdeen, in perfect cloudless conditions. As a result, during the following few days many patients came with symptoms indicative of sun-blindness. A search for treatment in current and old literature was fruitless, and it was decided to aim at improving the retinal circulation by retrobulbar injections of carbachol, 0.25 mg.

Symptoms.—It was noted that: (a) The patients complained of difficulty in reading and in recognizing faces at a certain distance. In milder cases the complaint was of "letters jumping out." Less introspective patients described a general haze or central blurred spot. (b) The time spent on observing the eclipse, or the inadequacy of protective glasses, or both, had no constant bearing on subjective symptoms, but seemed to be more related to the observable changes at the macula. (c) The symptoms had little relation to the macular changes.

We confirmed the ophthalmoscopic changes as described by Elwyn (1946), but in many cases there was a bright diamond-like reflex at the macula surrounded by an area of exudation and hyperaemia.

Assessing the Injury.—All tests were carried out in a dark-room in standard lighting, allowing the patient about 15 minutes for adaptation. Visual acuity on Snellen's types was noted. The near vision was tested with Jaeger types. If patients experienced difficulty a record was made of the number of letters or words "falling out," of those only blurred, whether constantly or intermittently, and the character of scotomata (blackish, greyish, whitish). This method was found to be quicker and more accurate than using Amsler's (1953) charts.

Treatment.—After assessing the extent of functional damage and recording the ophthalmoscopic appearance a retrobulbar injection of carbachol, 0.25 mg., was given to one eye only. If there was no definite improvement on one of the previously described tests (Jaeger's types were generally used) the patient was classified as not responding to treatment. All responding patients were given daily treatment for two to three days.

Response to Treatment.—Table I shows the response of 55 patients to treatment with carbachol. The "immediate response" embraces those whose condition was clearly

TABLE I.—Response to Treatment with Carbachol

Day After Injury on which Treatment was Started	Immediate Response	Doubtful Reaction	No Response
1-7	28	4	9
8-14	2	—	1
15-21	5	1	1
22-28	4	—	—
Total ..	39	5	11

improved within 2 to 30 minutes after the injection. The immediate improvement was variable: in some cases from 6/36 to 6/12, from Jaeger 4 to Jaeger 1. Twenty-four hours after the single injection the patients could be classified into three categories: (a) further improved (which in early cases was rather to be expected); (b) improvement maintained; (c) a "backslide" to a condition somewhere midway between that of before and that after the last injection.

TABLE II.—Degree of Improvement in 39 Cases After One Course of Treatment

Days After Injury on which Treatment was Started	Recovered	Improved By		
		Snellen (More than 2 Lines) or Jaeger (More than 2 Type-sizes) or Scotoma Diminished by $\frac{1}{2}$	Snellen (1 to 2 Lines) or Jaeger (1 to 2 Type-sizes) or Scotoma Diminished by $\frac{1}{4}$	Less than Previous Standards
1-7	4	9	5	10
8-14	1	1	—	—
15-21	2	2	1	—
22-28	—	1	2	1
Total ..	7	13	8	11

No relation was found between the appearance of the macula and the patient's reaction to treatment.

Complications.—Carbachol caused no serious side-effects. It occasionally gave rise to excessive sweating, salivation, and slight nausea, or to diplopia and pain and throbbing in the head. Some patients developed blurred vision (even to less than 6/60). Ophthalmoscopically, oedema of the retina was found. All disturbances were transient.

CONCLUSION

In many instances in the present series, even in comparatively late cases, it was shown that symptoms of sun-blindness could be alleviated immediately by retrobulbar injections of carbachol.

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Neonatal Diabetes

Probably only 10 cases of neonatal diabetes have been reported (Schwartzman *et al.*, 1947). Until the peculiarities of the disease at this age are better understood, it is desirable that new cases should be reported as they occur.

CASE REPORT

On September 13, 1954, a month-old male child was admitted to hospital suffering from dehydration. He had been reluctant to feed for a few days and had vomited once. He weighed 5½ lb. (2.5 kg.) at birth, and had gained ½ lb. (225 g.) in the first two weeks, but had then been weaned on to artificial feeds. He weighed 6 lb. 4 oz. (2.8 kg.) at 3 weeks, but on admission weighed only 5 lb. (2.3 kg.).

Physical examination on admission revealed no abnormality apart from dehydration and weakness. The temperature was 99° F. (37.2° C.). Although clear indications were absent, infection was suspected and a course of chlorotetracycline was begun. The baby took his feeds well at first. The blood picture was normal, but sugar was found in the urine. On the morning of September 15 he vomited and became collapsed. Four blood-sugar estimations were carried out, with the following results: 12.45 p.m., 1,680 mg. per 100 ml.; 4.30 p.m., 1,980 mg.; 5.30 p.m., 1,620 mg.; and 9.30 p.m., 1,200 mg. The cerebrospinal fluid contained 1,200 mg. of glucose per 100 ml. Two specimens of urine contained more than 2% of sugar, with small amounts of acetone. Glucose was identified by fermentation and osazone tests and by chromatography.

After the first blood-sugar report, an intravenous dextrose-saline infusion (4% dextrose, 0.18% saline) was set up to run at 25 ml. an hour, and soluble insulin was given: 2 units at once, 4 units at 7 p.m., and 10 units at 11 p.m. The latter dose was repeated at 3 a.m., and at 7 a.m. the following day. The baby's condition had improved, but he then began to perspire freely, and at 10 a.m. a specimen of urine contained no sugar. No more insulin was given, and 5% dextrose was administered through the intravenous drip. At 11.30 a.m. sudden collapse and death occurred.

Post-mortem Findings.—The blood sugar estimated shortly after death was 325 mg. per 100 ml., and the urine