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URINARY EXCRETION OF METABOLITES OF STEROID HORMONES BY MEN WITH CANCER OF THE STOMACH

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SUMMARY

The urinary excretion of androsterone (A), aetiocholanolone (E) and 17-hydroxycorticosteroids (17-OHCS) and the A/E ratio have been measured in 22 male hospital controls, 14 men with gastric ulcer, 22 men with cancer of the stomach and 14 men with cancer of the rectum before and after surgery, and also in 25 normal men. There were no significant differences in the excretion patterns of normal men, hospital controls and patients with gastric ulcer. The stomach cancer patients excreted less A ($P < 0.004$) and E ($P < 0.02$) and had a lower A/E ratio ($P < 0.05$) than the hospital controls before surgery. After surgery the excretion pattern was not significantly different from the postoperative values for the hospital controls. The rectum cancer patients excreted less A ($P < 0.004$) and had a lower A/E ratio ($P < 0.01$) than hospital controls before surgery, but after surgery excreted less A ($P < 0.001$) and E ($P < 0.02$) and had a lower A/E ratio ($P < 0.01$) than the postoperative hospital controls.

INTRODUCTION

The possible role of steroid hormones in the aetiology of cancer in hormone-dependent tissues has been widely investigated over the past 20 years. Knowledge of steroid metabolism also greatly increased during that time as more specific and accurate methods have become available for the determination of steroid hormones and their metabolites in plasma and urine. The application of such methods to the study of patients with breast cancer raised hopes that urinary steroid excretion patterns might prove useful in the early diagnosis, treatment and prognosis of this condition [1–4]. Recently the value of this type of investigation has been called into question [5].

Similar investigations have been carried out in patients with cancer of the prostate [6] and endometrium [7]. A relationship between steroid hormones and neoplastic growth in endocrine-controlled tissues would not be entirely unexpected in view of what is known about the effect of steroid hormones on the growth of their target tissues.

However, abnormal urinary steroid excretion patterns have also been observed in patients with cancer of the lung [8, 9], an organ not normally considered to be under the specific control of steroid hormones. It has been claimed that the study of hormone excretion patterns might be of value in the early diagnosis, treatment and prognosis of patients with lung cancer [8].

All workers in this field appear to be agreed that the abnormal steroid excretion patterns seen in some cancer patients are not the result of the general debility caused by the disease, nor of abnormal metabolism of steroids by tumour tissue [3, 8, 9].

The steroids usually implicated in this type of investigation are metabolites of hormones of the adrenal cortex and testes; *viz.* androsterone (andro) and aetiocholanolone (aetio) derived mainly from testosterone or dehydroepiandrosterone, and the 17-hydroxycorticosteroids (17-OHCS), derived mainly from cortisol.

Although the stomach is not generally considered to be a steroid-dependent organ, there is considerable epidemiological and experimental evidence that steroid hormones play some role in the aetiology of peptic ulceration. This condition is much commoner in men than in women, and regression or healing of ulcers occurs when women become pregnant. After the menopause the sex difference in the incidence of peptic ulceration disappears. Adrenocortical hormones also have marked effects on peptic ulceration, and also play a physiological role in other parts of the gastrointestinal tract [10]. However, urinary metabolites of androgenic and adrenocortical hormones are not significantly different in normal men and men with duodenal ulcer [11].

The aim of the present investigation was to carry out a pilot study on the urinary excretion of andro, aetio and 17-OHCS in men with cancer of the stomach, and to compare the urinary excretion pattern of those patients with those of normal men, hospital patients without gastrointestinal or endocrine disorder, men with gastric ulcers, and male patients with cancer of the rectum. Differences have been found in the steroid excretion patterns of these groups of men.

MATERIALS AND METHODS

Steroid analyses

To overcome the difficulty of obtaining reliable

24 h urine samples the urine passed by the subjects on wakening was collected. Samples were frozen at −30°C until processed. Urinary andro and aetio were determined by a g.l.c. method [8], and 17-OHCS by the approved MRC method [12]. Creatinine was measured by Autoanalyser. Results are expressed as μg or mg steroid/g creatinine.

Subjects and patients

The 25 normal men (normals) had no history of gastric or endocrine disorder and carried out their normal duties with no dietary restrictions, except that they were asked to abstain from alcoholic drinks 24 h before the day of collection of urine. The men in hospital (hospital controls) were 22 patients admitted to the surgical unit for minor surgery for hernia or varicose veins. It was thought that a group of patients with cancer of a type not thought to be under endocrine influence should be included; these cancer controls were 20 men with cancer of the rectum. As a control of non-malignant stomach disease 14 men with gastric ulcer (G.U.), established by endoscopy, were also studied. The 22 men with cancer of the stomach were slightly older than the men in the other groups, but the difference was not significant (Table 1). The postoperative assessment of the malignant tissue removed at surgery and the fate of these patients are shown in Table 2.

Table 1. Age of male subjects and patients in years (±S.D.)

Normal men	50.4 (±12.4)
Hospital controls	50.3 (±16.6)
Gastric ulcer	52.4 (± 9.2)
Stomach cancer	57.1 (±14.5)
Rectum cancer	54.0 (± 9.6)

Because of the large catchment area of the Royal Victoria Infirmary, Newcastle upon Tyne, from which the patients were drawn, it was not possible to pursue follow-up studies on most of the patients who were usually transferred to peripheral hospitals or to convalescent homes about 5 days after surgery. Also, the patients were usually admitted only one or two days prior to surgery. Therefore only one or two urine samples were collected preoperatively, and to standardise the procedure the urines were collected on the third to fifth day after surgery. None of the patients had renal complications, but the mortality rate in the stomach cancer patients was high (Table 2).

Statistical analyses

The raw data are presented in Table 3 and in Figs. 1–3. However, since the urinary excretion of these steroids has a log normal distribution [13], statistical analyses were done on the logarithms to the base 10 of steroid concentrations expressed as μg steroid/g creatine. Student's *t*-test was used to assess the probabilities of 2-tail differences between groups of subjects. Paired *t*-tests were also done on the logs of data for the values obtained before and after surgery. The standard of significance was set at $P < 0.05$.

RESULTS

The values for the excretion of andro, aetio and 17-OHCS and of the andro/aetio ratio (A/E) are given

Table 2. Clinical features of stomach cancer patients. Pathology of tumours. Status of patients 6 months after surgery

Number	Age-range	Type of surgery	Pathology of tumour	Status
7	56–59	Total or partial gastrectomy	2 Adenocarcinomas 1 Carcinoma of pylorus 1 Carcinoma of fundus 1 Reticular cell carcinoma 2 Generalised carcinoma	Alive and well
3	53–70	Total or partial gastrectomy	Generalised carcinomas, usually with secondary deposits in lymph nodes	All died within one month
12	53–70	Not resectable	Generalised carcinomas and adenocarcinomas with much secondary tissue involvement	

Table 3. Urinary excretion of androsterone (andro), aetiocholanolone (aetio) and 17-hydroxy corticosteroids (17-OHCS) and the andro/aetio ratio (A/E) in normal men (normals), male hospital controls (hospital controls), and men with gastric ulcer, stomach cancer and rectum cancer. Data expressed as mg steroid/g creatinine (±S.E.M.); n = number in each group. Before surgery (Preop); after surgery (Postop)

	Normals	Hospital controls		Gastric ulcer		Stomach Cancer		Rectum Cancer	
		Pre-op	Post-op	Pre-op	Post-op	Pre-op	Post-op	Pre-op	Post-op
n	25	22	19	8	5	18	14	16	8
Andro	2.74 (0.14)	2.60 (0.37)	2.26 (0.27)	2.64 (0.46)	2.30 (0.59)	1.49 (0.30)	1.85 (0.36)	1.86 (0.20)	1.05 (0.30)
Aetio	1.88 (0.14)	1.87 (0.27)	1.43 (0.12)	2.02 (0.30)	2.09 (0.69)	1.21 (0.17)	1.18 (0.14)	1.66 (0.24)	0.89 (0.19)
A/E	1.53 (0.09)	1.40 (0.14)	1.58 (0.15)	1.52 (0.30)	1.29 (0.13)	1.22 (0.14)	1.48 (0.14)	1.16 (0.16)	1.21 (0.20)
17-OHCS	5.42 (0.28)	6.04 (0.34)	5.74 (0.46)	5.31 (1.84)	6.87 (2.21)	7.21 (1.26)	8.13 (1.49)	7.02 (0.92)	6.49 (2.74)

in Table 3 and in histogram form in Figs. 1–3. These results are expressed as mg steroid/g creatinine to permit comparison with the values obtained by others [1–3, 8]. However, statistical analyses of probabilities of differences between and within groups were carried out on the logs of the data (see Materials and Methods).

There were no significant differences between the data for the normal men and for the values for the hospital controls before and after surgery, although there was a definite, but not significant change in the excretion of the steroids and in the A/E ratio in the hospital controls after surgery. Further statistical comparisons were made between the pre- and post-operative data of the hospital controls and the data for the other groups before and after surgery.

No significant difference was found when the preoperative levels of the hospital controls were compared with the G.U. patients before surgery. Similarly, there were no significant differences between the postoperative data of the hospital controls and the G.U. group. However, before surgery, the stomach cancer patients had significantly different values from the preoperative hospital controls in terms of andro ($P < 0.004$), aetio ($P < 0.02$) and the A/E ratio ($P < 0.05$). In contrast, when the postoperative values of these two groups were analysed, no significant differences were found (Table 4). The preoperative values for the rec-

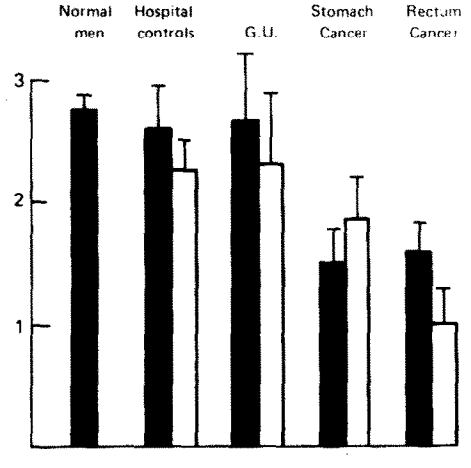

Fig. 1. Excretion of androsterone (mg/g creatine) by normal men and the groups of patients studied. Linear values. In this and other Figs. the dark bars represent the values before surgery, and the open bars the values after surgery.

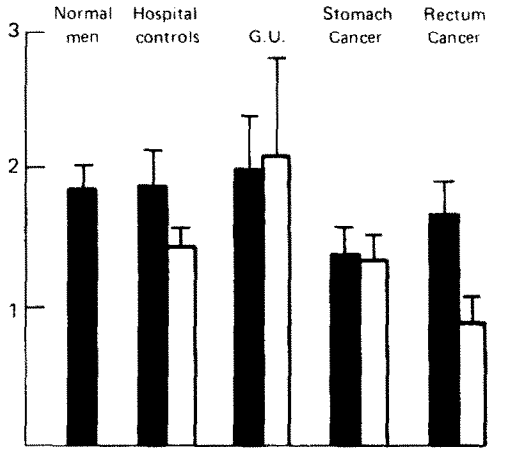

Fig. 2. Excretion of aetiocholanolone (mg/g creatine) by normal men and groups of patients studied (see legend to Fig. 1 for details).

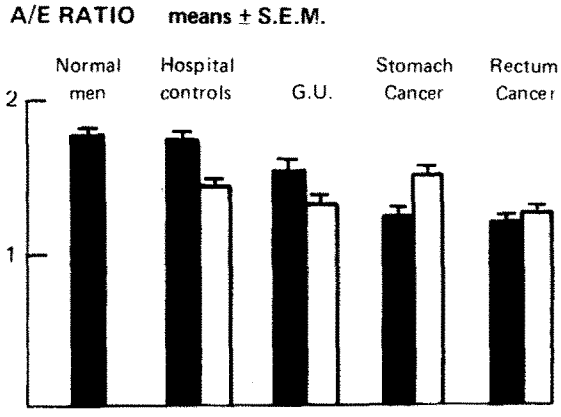

Fig. 3. The androsterone/aetiocholanolone (A/E) ratio for normal men and for the groups of patients studied (see legend to Fig. 1 for details).

tum cancer patients were also significantly lower than those of the hospital controls in terms of andro ($P < 0.04$) and the A/E ratio ($P < 0.01$). After surgery, the rectum cancer patients excreted markedly less and significantly different amounts of andro ($P < 0.001$) and aetio ($P < 0.02$) and the A/E ratio was also decreased ($P < 0.01$) (Table 4).

DISCUSSION

Androsterone and aetiocholanolone are metabolites of testicular and adrenocortical androgenic hormones, while the 17-hydroxy-corticosteroids (17-OHCS) are derived entirely from the adrenal cortex. In discriminant functions derived for cancer at sites other than the gastrointestinal tract [3, 6–7], aetiocholanolone is an important factor in the equation. In the lung-cancer discriminant, however, the androsterone–aetiocholanolone ratio is important, and it has been claimed that this ratio alone has a discriminating power of 80% in lung cancer [8]. It has also been suggested that the excretion of androsterone, aetiocholanolone and 17-OHCS is increased by the stress of hospitalisation and surgery [4, 8]. However, in the present work there was little evidence to support this view (Table 3). There was no significant difference between the levels of steroids excreted by the normal men and the hospital controls before surgery. Indeed there were small, but not significant, decreases in the amounts of steroid excreted by the hospital controls after surgery (Table 3), even though most of the preoperative samples were collected on the morning of the day on which the subjects were to be operated upon.

Comparison of steroid excretion patterns of hospital controls and of patients with gastric ulcer (G.U.) and stomach and rectum cancer before surgery

There were no significant differences between the preoperative excretion patterns of the hospital controls and the G.U. patients (Table 3; Figs. 1–3), but the large S.E.M. and the small number of G.U. patients studied makes this finding equivocal, and a larger number of G.U. patients needs to be studied to clarify this point. However, before surgery, the patients with stomach cancer excreted significantly smaller amounts of androsterone and aetiocholanolone than the hospital controls (Table 4). Lower than normal levels of these steroids have also been found in patients with breast and other cancers [14]. The A/E ratio of the stomach cancer patients was also significantly different ($P < 0.05$) from that of the hospital controls. In contrast, the preoperative values for the patients with cancer of the rectum differed from the hospital controls with respect to androsterone ($P < 0.04$) excretion which was decreased enough to cause a significant decrease in the A/E ratio ($P < 0.01$) (Table 4).

The effect of surgery on steroid excretion patterns

When the steroid levels were compared with those after surgery within the same group of patients, the only significant difference found was a decreased excretion of androsterone by the hospital controls ($P < 0.02$), but this was not large enough to disturb the A/E ratio.

When the postoperative values for the hospital controls were compared with the postoperative values for the patients, the G.U. group did not exhibit any significant difference. This result must also be treated with caution because of the small number of men in this group. However, the steroid excretion patterns of both the stomach and rectum cancer patients did change. The androsterone and aetiocholanolone values and the A/E ratio were greater than the corresponding preoperative values, with the result that these values were not significantly different from the postoperative values of the hospital controls. In marked contrast the cancer of the rectum patients excreted significantly less androsterone ($P < 0.001$) and aetiocholanolone ($P < 0.02$), and the A/E ratio remained significantly decreased ($P < 0.01$) (Table 4).

Therefore it appears that patients with stomach cancer tend to revert to a steroid excretion pattern

Table 4. Probabilities of differences assessed by Student's *t*-test on logs of data for groups of patients when the preoperative (Pre) and postoperative (Post) data were compared with the corresponding values for the hospital controls. Significance of difference set at $P < 0.05$

	Stomach cancer		Rectum cancer	
	Pre	Post	Pre	Post
Andro	0.004	NS	0.04	0.001
Aetio	0.02	NS	NS	0.02
A/E Ratio	0.05	NS	0.01	0.01

similar to that of the hospital controls after surgery, whereas patients with cancer of the rectum developed more abnormal patterns after surgery.

While these changes in androsterone and aetiocholanolone occurred there was no significant or apparent change in the excretion of 17-OHCS in any of the groups. This may be a reflection of the wide range of excretion of 17-OHCS and the small number of subjects studied. One reason for using early morning urine samples rather than 24 h samples was to minimise the effects of the diurnal rhythm of corticoid secretion on the results. Most of the patients were in the same surgical unit and so received common preoperative treatment.

Also, the changes in steroid excretion pattern described here cannot be due entirely to change in diet. It should be stressed that most of the patients were not seriously "ill", and some were apparently fit and well before surgery.

The results indicate that patients with cancer of the stomach excrete smaller amounts of androsterone and aetiocholanolone than normal men and men with non-malignant disease requiring surgical treatment. After surgery, the steroid excretion patterns of these patients reverts to a more normal pattern, but it would be unwise to conclude from this that removal of the tumour had also removed some agent which causes inhibition of steroid secretion. Nor is this due to the presence of malignant disease *per se*, since patients with cancer of the rectum also have low steroid excretion before surgery, but after surgery the steroid excretion decreases further. These changes do not appear to be due to disease of the stomach itself, since men with gastric ulcer do not appear to have abnormal excretion patterns before or after surgery. It has also been found that men with duodenal ulcer do not excrete abnormal amounts of these steroids before or after surgery [11].

The data presented provide no evidence that stomach or rectum cancer is induced or maintained by androgenic or adrenocortical hormones, but the characteristic steroid excretion patterns of patients with cancer of the stomach and rectum is similar in many respects to patients with cancer at other sites. Thus, steroid excretion patterns in patients with cancer of the stomach and rectum are similar to those patterns found in breast and prostatic cancer, i.e. reduced excretion of aetiocholanolone with no abnormality in the A/E ratio. However, the stomach and rectum cancer patients differ from those with breast cancer in that they excrete lower than normal amounts of androsterone. The only factor which is common to stomach (and rectum) cancer and lung cancer patients is the reduced excretion of androsterone, but they differ in the important respect that the A/E ratio, which is significantly reduced in, and appears to be characteristic of, lung cancer is normal in stomach and rectum cancer.

These results are of interest in view of the suggestion that C_{19}-androgenic steroids may act as precursors of tumour-growth promoting hormones [15]. Therefore, it is possible that some common mechanism operates on the secretion and/or metabolism of certain steroids in patients with cancer.

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DISCUSSION

Junghlut. Just a quick question to Dr. Taylor. Was the preoperative treatment and the anesthesia of the cancer patients standardised?

Taylor. The patients were all in the same Surgical Unit and received the same preoperative treatment, anaesthesia and so on. Of course we could have no control over the treatment the patients received before they were admitted to hospital, since they were referred by different medical practitioners.

James. A short comment. I was puzzled, Dr. Taylor, that you chose to collect an early morning urine specimen. Adrenocortical activity is maximal during sleep, and in normal subjects declines during the day. It could well be that it is the level of adrenal activity during the day which is significant, and by looking only at morning samples, you may well have missed any differences between patients.

Taylor. Professor James may well be right, and I agree that analysis of 24-h urine samples might have shown dif-

ferent results. However, the analysis of overnight samples was chosen for a number of reasons: I wished to compare my results with those of Rao who used overnight urine samples in his study of lung-cancer patients (*Lancet* **441** (1970) 1063); also many clinical laboratories use overnight specimens for analysis of steroid metabolites, particularly, for example, urinary oestriol; in the very busy Surgical Unit with which we were involved, we found it very difficult to obtain reliable 24-h specimens; finally, with respect to the point raised by Professor James, it could be argued that we were measuring 'basal' adrenocortical activity, thus avoiding large fluctuations in adrenal secretion which might occur in stressed patients.

There is another possible source of error which I thought someone might mention, namely that radical surgery might affect the production and clearance of creatinine, but we found no evidence that urinary creatinine concentration 3–5 days after surgery was substantially different from the preoperative values.