ABNORMALITY OF ESTROGEN METABOLISM IN HUMAN SUBJECTS WITH MYOCARDIAL INFARCTION[1]

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Abstract

Estrogen metabolism has been investigated in male subjects with and without previous myocardial infarction. The urinary excretion of estriol, estrone, and estradiol-17β has been measured 1 day before and 4 days after intramuscular injection of estradiol-17β. The excretion of the individual estrogens resulting from the administration of estradiol was determined by subtracting preinjection values from the daily excretion following injection. The resultant values of estriol (T), estrone (O), and estradiol-17β (D) were expressed as the following ratios:

$$\Delta \frac{T}{O} \text{ and } \Delta \frac{T}{O+D}.$$

These urinary estrogen ratios were found to be significantly higher in subjects with previous myocardial infarction than in control subjects. The ratios in the infarction group were not influenced by bed rest nor by the duration of time following infarction which varied from 1 week to 2 years.

Introduction

Myocardial infarction is rare in women before the menopause. Oliver and Boyd (20) in a study of 1000 consecutive patients with clinical and electrocardiographic evidence of myocardial infarction or ischemia found that coronary artery disease is eight times more frequent in men than in women under the age of fifty and that this sex difference decreases markedly in the older age group. Adlersberg, Schaefer, Steinberg, and Wang (1) state that coronary artery disease in subjects under the age of fifty occurs about nine times more frequently in men than in women but that the proportionate number of females with this disorder increases after the menopause. The decrease with age in the preponderance of men over women in the incidence of coronary artery occlusion is also borne out by the pathological studies of Schlesinger and Zoll (29). These data suggest a protective action of the functioning ovary.

Previous studies on the mechanism of this protective action have mainly been confined to the effect of estrogens on plasma lipids because of the relationship between abnormalities of plasma lipids and coronary artery disease (12, 17). The concentrations of plasma lipids (21) and the urinary estrogen excretion (5) both vary during the normal menstrual cycle. Moreover,

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TABLE I

DIAGNOSIS AND AGE DISTRIBUTION OF SUBJECTS STUDIED

Control group			Myocardial infarction group			
Subject	Clinical status	Age	Subject	Site and duration of infarction		Age
IL	Normal subject	48	MAC	Posterior infarction	10 days	60
EG	Pulmonary emphysema	68	SA	Anterolateral infarction	4 weeks	67
RO	Normal subject	28	MCL	Posterior infarction	2 weeks	68
WA	Peripheral atherosclerosis	69	WI	Anterior infarction	10 days	50
ME	Pneumococcal pneumonia	70	HA	Anterior infarction	2 weeks	63
RA	Normal subject	39	DE	Posterior infarction	4 weeks	66
BE	Coarctation of the aorta	25	WY	Anterior infarction	4 weeks	52
YA	Mitral stenosis—commisurotomy	51	PAR	Anterior infarction	1 week	52
FO	Rheumatoid arthritis	63	TH	Anterior infarction	4 weeks	36
MIL	Normal subject	32	SM	Anterolateral infarction	4 weeks	59
BA	Normal subject	38	RE	Anterior infarction	4 weeks	55
WE	Bronchogenic carcinoma	81	FR	Anterior infarction	2 years	63
AR	Duodenal ulcer	26	CO	Posterior infarction	2 weeks	42
PO	Interstitial pulmonary fibrosis	55	REO	Anterior infarction	4 weeks	55
WIM	Pulmonary emphysema	58	MAR	Posterior infarction	11 weeks	41
SH	P.T.A. deficiency*	61	HO	Posterolateral infarction	4 weeks	64
MI	Normal subject	35	PAQ	Posterior infarction	2 weeks	57
HAN	Gastric ulcer	64	GH	Anterior infarction	4 weeks	60
CH	Hemoptysis of unknown origin	44	LA	Anterior infarction	4 weeks	41
BR	Pulmonary emphysema	62	ST	Anteroseptal infarction	4 weeks	69

*Plasma thromboplastin antecedent deficiency.

abnormal concentrations of plasma lipids and lipoproteins in male subjects with myocardial infarction are corrected by administration of ethinyl estradiol (22). The effect of estrogens in the prevention of recurrent myocardial infarction is now being studied in a number of centers.

The present investigation is a different approach to the problem of the relative immunity to coronary artery disease of women before the climacteric. The availability of a specific method of known sensitivity, accuracy, and precision for the determination of urinary estrogens (2) has made possible a study of estrogen metabolism in human subjects. In order to avoid difficulties arising from changes in the endogenous output of estrogens, which occur to a marked extent during the menstrual cycle and may occur to a lesser extent after the cessation of menses, this initial study has been confined to males. Two groups of men, with and without clinical and electrocardiographic evidence of previous myocardial infarction, have been studied. The urinary excretion of estriol, estrone, and estradiol-17β was determined before and after the parenteral administration of estradiol-17β.

Material

Selection of Cases

Myocardial Infarction Group

These patients showed the typical clinical syndrome of myocardial infarction and a Q wave pattern in the electrocardiogram indicative of transmural infarction. None had congestive cardiac failure and all showed normal liver and kidney function by the standard biochemical tests. The time of initial study following infarction varied from 1 week to 2 years (Table I). In four cases (SM, FR, MAR, ST) there had been more than one episode of myocardial infarction. Patients studied within the first 4 weeks following infarction were on complete bed rest and anticoagulant therapy. The individual ages are shown in Table I. The mean age of the group was 56.2 years (S.E. 2.1).

Control Group

The control subjects consisted of six healthy laboratory personnel and 14 hospital in-patients free from hypertensive-vascular and coronary artery disease. The electrocardiogram in each case was normal. The clinical status of the in-patients of this group is shown in Table I. Ambulant and bed patients were included. The therapeutic regimes of the in-patients were continued during these studies. The mean age of the control group was 50.9 years (S.E. 3.7). The individual ages are shown in Table I. The mean age of the control group is not significantly different from that of the infarction group.

Experimental

Procedure

A 24 hour specimen of urine was collected (Control, Day 1). Estradiol-17β (350–500 μg. in 0.4 ml. of peanut oil) was then injected intramuscularly and

urine collected for this and the subsequent three 24-hour periods (Days 2, 3, 4, 5). Each specimen was analyzed in duplicate for estriol, estrone, and estradiol-1β by the method of Bauld (2).

Calculations

The amounts of estradiol-17β, estriol, and estrone excreted were converted to the following ratios to show the relative quantitative significance of these three urinary metabolites.

1. Endogenous estriol/estrone (T/O)

$$\frac{T}{O} = \frac{\mu\text{g. of estriol excreted on Day 1}}{\mu\text{g. of estrone excreted on Day 1}}$$

2. Endogenous estriol/estrone + estradiol ($T/O+D$)

$$\frac{T}{O+D} = \frac{\mu\text{g. of estriol excreted on Day 1}}{\mu\text{g. of estrone and estradiol excreted on Day 1}}$$

3. Endogenous estrone/estradiol (O/D)

$$\frac{O}{D} = \frac{\mu\text{g. of estrone excreted on Day 1}}{\mu\text{g. of estradiol excreted on Day 1}}$$

4. Exogenous estriol/estrone, $\triangle\frac{T}{O}$, is the ratio of increase of estriol to increase of estrone resulting from the injection of estradiol-17β.

$$\triangle\frac{T}{O} = \frac{\text{estriol excreted on Days 2, 3, 4, 5} - 4(\text{estriol excreted on Day 1})}{\text{estrone excreted on Days 2, 3, 4, 5} - 4(\text{estrone excreted on Day 1})}$$

5. Exogenous estriol/estrone + estradiol, $\triangle\frac{T}{O+D}$, is the ratio of increase of estriol to increase of estrone and estradiol-17β resulting from the injection of estradiol-17β.

$$\triangle\frac{T}{O+D} = \frac{\text{estriol excreted on Days 2, 3, 4, 5} - 4(\text{estriol excreted on Day 1})}{\text{estrone + estradiol excreted on Days 2, 3, 4, 5} - 4(\text{estrone + estradiol on Day 1})}$$

6. Exogenous estrone/estradiol, $\triangle\frac{O}{D}$, is the ratio of increase of estrone to increase of estradiol-17β resulting from the injection of estradiol-17β.

$$\triangle\frac{O}{D} = \frac{\text{estrone excreted on Days 2, 3, 4, 5} - 4(\text{estrone excreted on Day 1})}{\text{estradiol excreted on Days 2, 3, 4, 5} - 4(\text{estradiol excreted on Day 1})}$$

7. Percentage recovery is the percentage of administered estrogen appearing as estriol, estrone, and estradiol-17β in the urine during the 4 days following injection.

$$\%\text{ recovery} = \frac{\triangle T + \triangle(O+D)}{\text{estradiol injected}}$$

Results

Fig. 1A shows the typical pattern of estrogen excretion during the experimental period in a control subject (RA). Before injection (Day 1) the 24 hour urine specimen contained 5 μg. of estriol, 3.5 μg. of estrone, and 2 μg. of estradiol-17β. Estrone and estradiol-17β reached a maximum excretion on the day of injection (Day 2) but the maximum excretion of estriol was delayed until the day after the injection (Day 3). The excretion of all three estrogens returned to preinjection levels by the fifth day of the experiment. The increases above control levels occurring in response to the intramuscular injection of estradiol-17β (500 μg.) were 37 μg. for estriol, 39 μg. for estrone, and 16 μg. for estradiol-17β. The recovery of injected estrogen as estriol, estrone, and estradiol-17β was therefore 18%. The increase in excretion of estriol was approximately two-thirds that of estrone and estradiol combined and approximately equal to that of estrone alone.

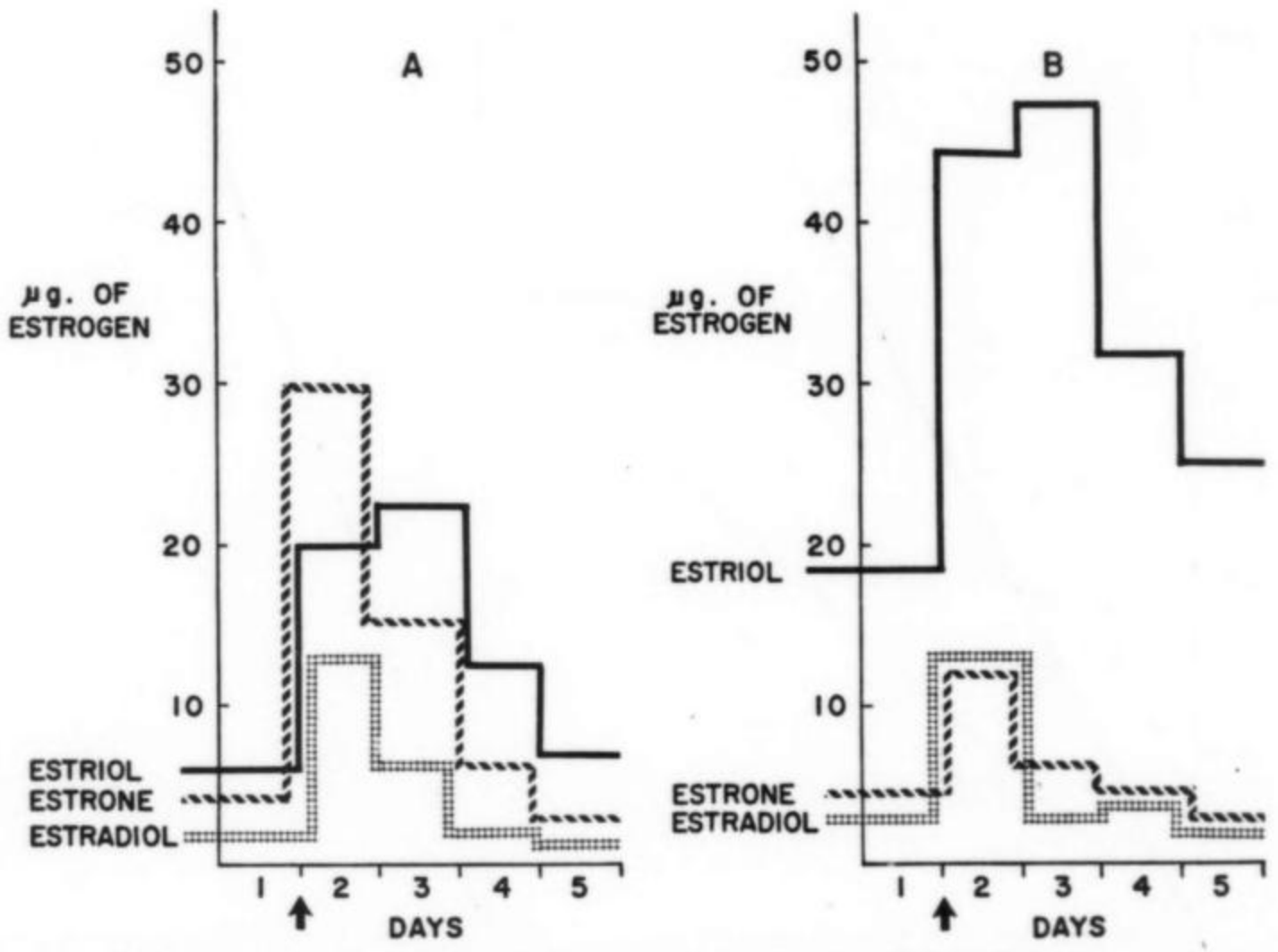

FIG. 1. Urinary excretion of estriol, estrone, and estradiol-17β before and after the injection ($\uparrow$) of estradiol-17β in a control subject (A) and in a patient with previous myocardial infarction (B).

Fig. 1B shows the typical pattern of estrogen excretion during the experimental period in a case (MAR) of myocardial infarction. Before injection (Day 1), the 24 hour urine specimen contained 17 μg. of estriol, 4 μg. of estrone, and 3 μg. of estradiol-17β. Again estrone and estradiol-17β reached a maximum excretion on the day of injection (Day 2) and the maximum excretion of estriol was delayed until the day after injection (Day 3). The excretion of estrone and estradiol-17β returned to preinjection levels by the fourth day of the experiment but estriol was still elevated by the fifth day. The increases above control levels occurring in response to the intramuscular injection of estradiol-17β (410 μg.) were 75 μg. for estriol and 10 μg. for

estrone and for estradiol-17β. The recovery of injected estrogen as estriol, estrone, and estradiol-17β was therefore 23%. The increase in excretion of estriol was approximately 3.5 times greater than that of estrone and estradiol combined and approximately 7.5 times greater than that of estrone alone.

The patterns of estrogen excretion (Fig. 1) resulting from the administration of estradiol-17β are typical of the two groups. Fig. 2 shows the data obtained on the individual subjects. The increments in excretion of estriol ($\triangle T$), estrone plus estradiol ($\triangle O+D$), and estrone ($\triangle O$) were calculated from the total excretion and the endogenous level, as described in "Methods". In Fig. 2A, $\triangle$ estriol is plotted against $\triangle$ (estrone + estradiol). In Fig. 2B, $\triangle$ estriol is plotted against $\triangle$ estrone. While two of the 'control' subjects and three of the 'myocardial infarction' patients show anomalous patterns, there is a clear difference in the relative amounts of estrogens excreted by the two groups.

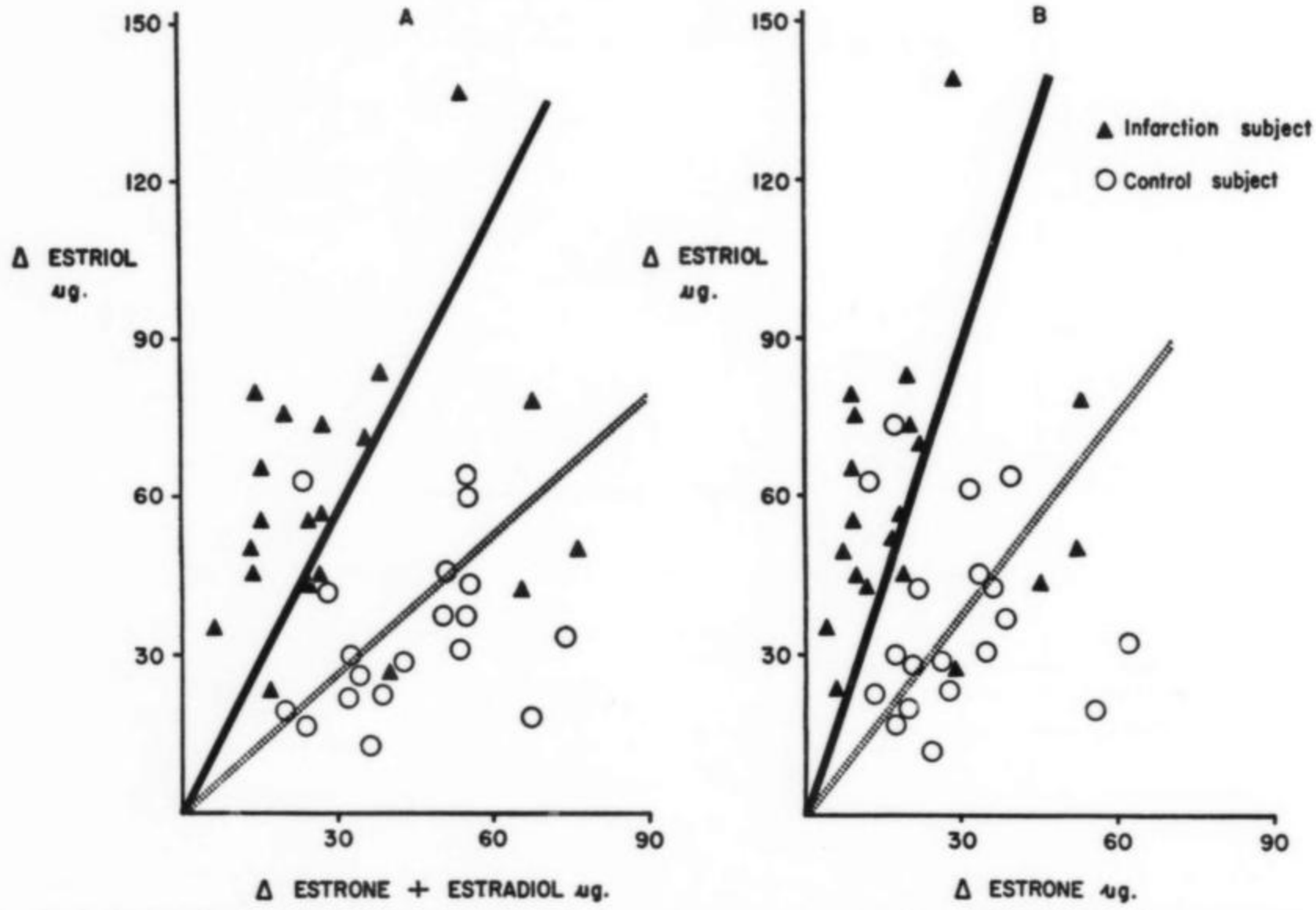

FIG. 2. Relation between increases in urinary excretion of the individual estrogens resulting from injection of estradiol-17β in the subjects of the control and myocardial infarction groups.

A. Relation between increases in urinary excretion of estriol and of estrone + estradiol for the control ○ and the myocardial infarction ▲ groups.

B. Relation between increases in urinary excretion of estriol and of estrone for the control ○ and the myocardial infarction ▲ groups.

Table II lists the values of $\triangle\frac{T}{O+D}$, $\triangle\frac{T}{O}$, and $\triangle\frac{O}{D}$ for the individual subjects together with the percentage of the administered estradiol recovered as estriol, estrone, and estradiol-17β in the urine in the 4 days following injection. Mean and standard error are also shown. For $\triangle\frac{T}{O+D}$, the mean for the

TABLE II

RATIOS OF ESTRIOL, ESTRONE, AND ESTRADIOL EXCRETED IN THE URINE IN RESPONSE TO INJECTION OF ESTRADIOL

Control group					Myocardial infarction group				
Subject	$\Delta \frac{T^*}{O+D}$	$\Delta \frac{T}{O}$	$\Delta \frac{O}{D}$	% recovery	Subject	$\Delta \frac{T}{O+D}$	$\Delta \frac{T}{O}$	$\Delta \frac{O}{D}$	% recovery
IL	0.3	0.3	4.7	17	MAC	0.6	0.9	2.8	29
EG	0.3	0.5	2.2	11	SA	0.6	0.9	2.3	27
RO	0.5	0.5	5.2	21	MCL	0.7	0.9	2.9	15
WA	0.6	0.8	2.6	16	WI	1.1	1.5	3.6	28
ME	0.6	0.9	1.9	20	HA	1.4	3.4	6.5	10
RA	0.7	0.9	2.5	18	DE	1.8	3.8	0.9	17
BE	0.7	0.9	4.2	8	WY	1.9	2.4	4.6	13
YA	0.7	1.1	1.7	17	PAR	1.9	3.2	1.5	27
FO	0.8	1.3	1.5	18	TH	2.0	2.9	2.1	17
MIL	0.8	1.3	1.8	24	SM	2.1	3.0	2.1	15
BA	0.9	1.3	2.1	22	RE	2.2	4.2	1.1	29
WE	0.9	1.4	1.8	15	FR	2.6	3.6	2.7	24
AR	0.9	1.6	1.2	11	CO	2.7	4.9	1.2	44
PO	1.0	1.0	1.0	9	REO	3.4	4.1	4.6	12
WIM	1.1	1.6	2.2	13	MAR	3.4	7.6	1.0	23
SH	1.1	1.9	1.3	30	HO	3.6	6.0	1.3	14
MI	1.2	1.6	2.9	24	PAQ	4.2	7.2	1.4	19
HAN	1.5	2.1	2.7	17	GH	4.6	6.3	2.3	12
CH	2.6	4.1	1.8	23	LA	4.9	9.2	1.1	24
BR	2.8	5.3	1.0	21	ST	5.3	10.5	1.2	8
Mean	1.0	1.5	2.3	18		2.6	4.3	2.4	20
S.E.	0.2	0.3	0.3	1.3		0.3	0.6	0.3	2.0

*For explanation of symbols see Experimental.

'control' group is 1.0 and the mean for the 'myocardial infarction' group is 2.6. The difference between means of the two groups is highly significant ($P<0.001$). For $\Delta\frac{T}{O}$, the mean for the 'control' group is 1.5 and the mean for 'myocardial infarction' group is 4.3. The difference between these means is highly significant ($P<0.001$). For $\Delta\frac{O}{D}$, the mean for the 'control' group of 2.3 is not significantly different from the mean of the 'infarction' group of 2.4. The difference between the mean percentage recovery of the two groups is not significant ($P<0.5>0.1$).

The endogenous excretion of the three estrogens, as determined by analysis of the urine excreted on the day prior to injection, was not significantly different for the two groups. The values have been expressed as ratios of estriol to estrone + estradiol $\left(\frac{T}{O+D}\right)$, estriol to estrone $\left(\frac{T}{O}\right)$, and estrone to estradiol $\left(\frac{O}{D}\right)$ and are listed in Table III.

TABLE III

RATIOS OF ESTRIOL, ESTRONE, AND ESTRADIOL EXCRETED IN THE URINE ON THE DAY PRIOR TO INJECTION OF ESTRADIOL

	Control group			Myocardial infarction group		
	$\frac{T}{O+D}$ *	$\frac{T}{O}$	$\frac{O}{D}$	$\frac{T}{O+D}$	$\frac{T}{O}$	$\frac{O}{D}$
Mean	1.7	3.2	2.5	2.8	4.0	2.3
S.E.	0.3	0.8	0.4	0.5	0.6	0.4
P	0.05	0.10	0.50			

*For explanation of symbols see Experimental.

Fig. 3 shows the consistency of pattern in estrogen excretion following injection of estradiol-17β in a subject at different periods after an acute myocardial infarction. The first experiment was conducted 1 month after infarction when the patient was at bed rest and on anticoagulant therapy. The injection and the urinary estrogen analyses were repeated $1\frac{1}{2}$ months later when the patient was ambulant and at home without anticoagulants. He was then put on a 30 g. fat diet and 2.5 mg. Premarin *R* orally for a 2 month period. Premarin was then discontinued and 5 days later the third experiment was conducted. The relative amounts of the estrogens excreted in response to an intramuscular injection of estradiol-17β were similar in the three periods. The $\Delta\frac{T}{O+D}$ for the three periods was 2.2, 1.8, and 1.9 respectively.

The precision of the analytical method was determined by submitting 464 consecutive duplicate determinations obtained during 18 months of this study to statistical analysis (10). This is shown in Table IV for the three estrogens at different urinary concentrations.

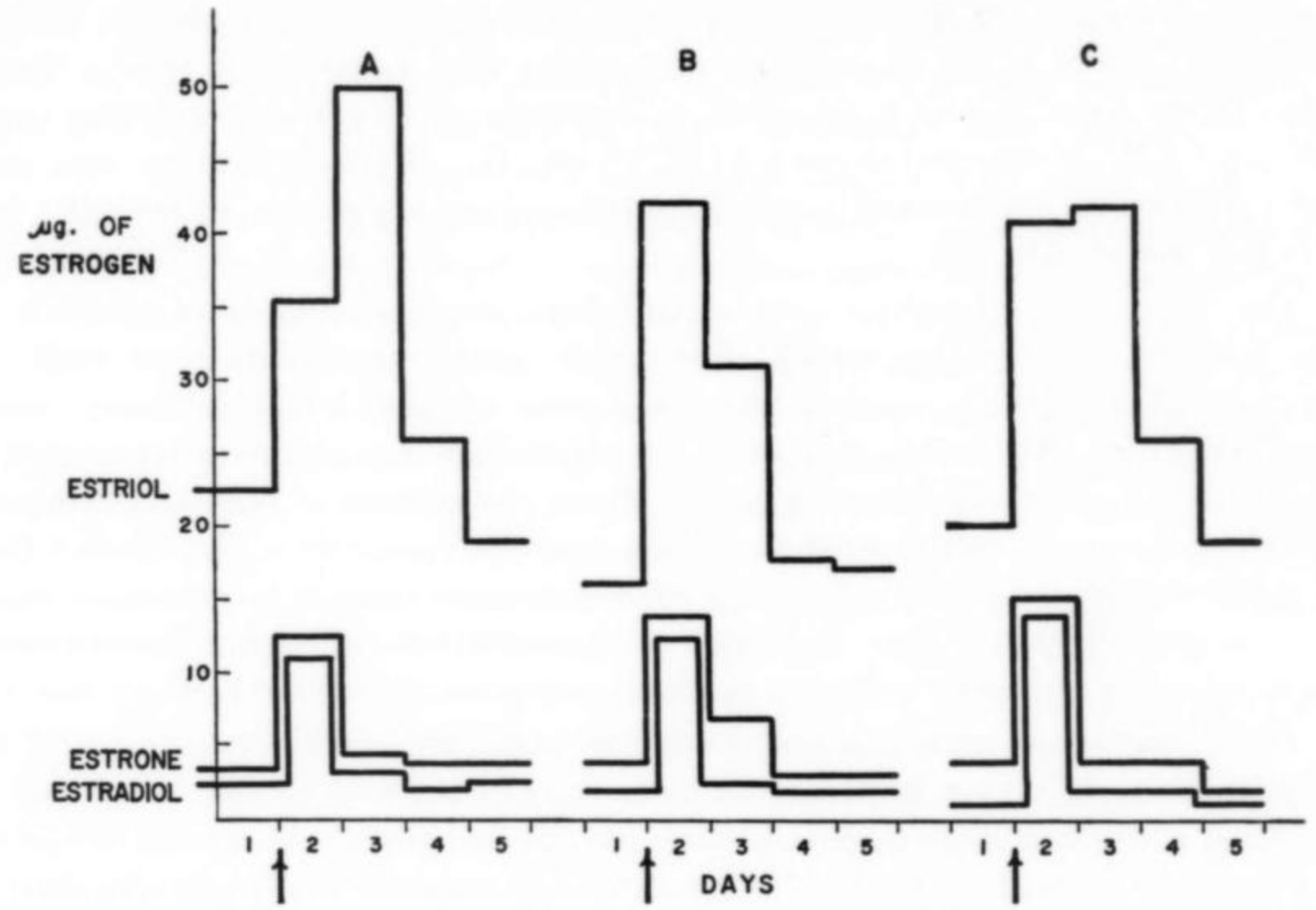

FIG. 3. Urinary excretion of estriol, estrone, and estradiol before and after the injection (↑) of estradiol-17β on three successive occasions in a subject with previous myocardial infarction.

TABLE IV

STANDARD DEVIATION BETWEEN DUPLICATES OF CONSECUTIVE ANALYSES

(μg./24 hours)

Urinary estrogen range (μg./24 hr.)	Estriol	Estrone	Estradiol
0 – 2.5	—	0.38	0.48
2.5 – 5.0	0.22	0.45	0.63
5.0 – 10.0	0.57	0.54	0.74
10.0 – 20.0	1.08	0.88	0.49
20.0 – 40.0	2.09	1.69	—

Discussion

Previous work has established the interrelationships of the three estrogens—estradiol-17β, estrone, estriol—in the human subject. Administration of estradiol-17β causes an increased urinary excretion of estrone (14, 28, 31, 3, 4) and of estriol (28, 3, 4). Administration of estrone causes an increased urinary excretion of estradiol-17β (31, 23) and of estriol (24, 23, 3). On the other hand, administered estriol is not converted into estrone or estradiol-17β (31) but is recovered almost quantitatively from the urine (6). Two of these three findings are supported by *in vitro* studies. Firstly, estradiol-17β and estrone are interconvertible in a variety of human tissue slices (26), in human red blood cells (13), and on perfusion of placenta (16); in fact, a

specific estradiol-17β dehydrogenase has been obtained from placenta (15). Secondly, estriol is not changed on incubation with tissue slices (26). However, many investigators have failed to demonstrate the *in vitro* conversion of estradiol-17β (or estrone) to estriol (15, 25, 26, 27), although this has recently been claimed on the basis of paper chromatographic separation of isotopically-labelled metabolite (9).

The formation of estrone and estriol from estradiol-17β is confirmed in the present investigation which shows that estriol and estrone, as well as urinary estradiol-17β, increase after injection of the latter hormone into male subjects. Moreover, this study establishes the quantitative relationship between the three compounds under these conditions. The proportional increase in urinary estriol to the increase in urinary estrone and estradiol-17β is significantly greater in patients with previous myocardial infarction than in a control group. This indicates a quantitative difference in estrogen metabolism in subjects with and without previous myocardial infarction.

The difference in estrogen metabolism is not a function of age as there is no significant difference between the mean ages of the two groups. Similarly, the time since infarction does not influence the response. As shown in Table I, the period from the episode of myocardial infarction to the beginning of the experiment ranged from 1 week to 2 years. Two cases (DE, MAC) have been studied at repeated intervals without change in the proportions of the three estrogens excreted in response to injection of estradiol-17β. The abnormality of estrogen metabolism seen after myocardial infarction cannot therefore be explained on the basis of age or therapy, nor can it be dismissed as a transient phenomenon of the immediate post-infarction period.

Impairment of liver function also causes an abnormality of estrogen metabolism. This abnormality, however, is not one of increased conversion of estradiol to estriol as seen in this investigation of subjects with previous myocardial infarction. In fact, in cirrhosis of the liver, administered estradiol-17β is largely excreted unchanged, as first shown by Glass, Edmondson, and Soll (11) using bio-assay. These findings were confirmed by Stealy and Stimmel (31) using a chemical method of assay; they showed that the conversion of estradiol to estriol is less in cirrhosis than in the normal subject.

A high level of urinary estriol relative to estrone and estradiol was reported by Diczfalusy and Luft (8) in adrenal cortical carcinoma. In 250 ml. of urine they found 891.5 μg. of estriol, 68.5 μg. of estrone, and 18.4 μg. of estradiol-17β. These authors felt that the high level of estriol was due to the increased production of progesterone demonstrated in this case. This interpretation is in accord with the concepts of Smith and Smith (30). Pearlman, Pearlman, and Rakoff (23), however, were unable to demonstrate any marked difference in estrogen metabolism between men and pregnant women. It is therefore highly improbable that progesterone alters estrogen metabolism. In any event, it is worth noting that increased estrogen production by the adrenal in this one case (8) resulted in an excretion of 10 times as much estriol as estrone and estradiol.

The relative proportions of estriol, estrone, and estradiol-17β excreted in the urine in response to administration of estradiol-17β have been stated by Beer and Gallagher (3, 4) to depend upon the amount administered. These authors reached this conclusion after careful studies with small (0.25 mg.) and large (140–350 mg.) doses of estradiol-17β-16-C^{14} administered to female patients. They found that estriol was the principal urinary excretion product with the small doses and that estrone was the principal urinary excretion product with the large doses. In these experiments, however, the larger doses were given intramuscularly or orally while the small dose, in the subjects whose urine was fractionated, was given intravenously. Possibly the route of administration is important in determining the extent of estrogen catabolism. May and Stimmel (19) found more estrone than estriol in the urine of normal subjects after the ingestion of 2 mg. of estradiol-17β. Similar findings were reported by Brown (7) after the intramuscular injection of 20 mg. of estradiol-17β to a normal subject. These latter findings are consistent with our data on subjects without coronary artery disease.

No significant difference between the 'control' and 'myocardial infarction' group of the present study was found in two indices of estrogen metabolism. Firstly, the mean percentages of administered estrogen appearing in the urine as estriol, estrone, and estradiol during the 4 days after injection do not differ statistically (see Table II). This suggests that the difference between the two groups is not due to alterations in renal function and confirms the conventional tests. Secondly, the relative proportions of estriol, estrone, and estradiol-17β excreted on the day before injection are not significantly different for the two groups (Table III). The difference between the mean $\frac{T}{O+D}$ of the two groups may have been obscured by the lack of precision of the method at values of less than 3 μg./day (see Table IV). On the other hand, it may be that the abnormality in the myocardial infarction group applies only to increased conversion of estradiol-17β to estriol. The hypothesis that a portion of urinary estriol arises by a metabolic pathway other than the conversion of estradiol-17β has been advanced by Marrian (18) and by Brown (5).

This study has demonstrated a significant difference in estradiol-17β metabolism between groups of subjects with and without previous myocardial infarction. Individual cases, however, showed anomalous behavior. Thus, two of our control group (CH, BR) excreted estrogens after the administration of estradiol-17β in the proportions characteristic of the myocardial infarction group (Fig. 2, Table II). Further clinical follow-up is required to determine the significance of increased conversion of estradiol-17β to estriol in these control subjects. On the other hand, present data show that three subjects with previous myocardial infarction metabolize estradiol-17β in a normal manner (Fig. 2). Myocardial infarction can thus occur in subjects not showing an increased conversion of estradiol-17β to estriol. Further study is clearly needed to define the relation of estrogen metabolism to one or more of the pathological mechanisms involved in myocardial infarction.

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