

EFFECT OF ESTROGENS ON COPPER METABOLISM IN WILSON'S DISEASE *

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Wilson's disease, a rare heritable disorder of copper metabolism, has been the subject of considerable investigation in recent years, and a number of interesting clinical and biochemical facts (1-4) have become known. However, the exact pathogenesis and perhaps even the fundamental metabolic defect involved remain to be elucidated. The inheritance pattern is that of a rare recessive autosomal gene (5, 6) with only the homozygous individual manifesting symptoms. It is clear that there exists a state of positive copper balance, since increased quantities of copper have accumulated in various tissues of the body by the time symptoms appear (7-10). Decreased fecal excretion of orally or intravenously administered copper (11), increased urinary excretion of copper (12), and diminished total serum copper (5, 13) characteristically are detectable. Special attention has been directed toward ceruloplasmin, a blue copper-containing serum protein with oxidase activity which migrates electrophoretically as an α_2 -globulin (13, 14). The quantity of this protein is greatly decreased in the serum of almost every patient with Wilson's disease. Several investigators consider the deficiency of this protein to be the direct result of an abnormal gene as well as the basic defect of the disease (15). A decreased incorporation of radioactive copper into ceruloplasmin has been noted in patients with this disease (11, 16, 17) as well as in some heterozygotes (18); some of the latter have a decreased serum ceruloplasmin concentration (1, 4). The biological role of this protein has not yet been clarified.

In the normal individual both total serum copper and ceruloplasmin levels increase during pregnancy (19-23) as well as during estrogen administration (24, 25). A similar increase has been shown to occur in Wilson's disease (1, 4, 26, 27) following administration of estrogens. The pres-

ent study, in which 11 patients were given estrogen, had a dual objective: 1) to determine whether therapeutic benefit follows the elevation of ceruloplasmin; and 2) by altering in a normal direction certain parameters characteristic of the genetically abnormal state, to obtain further information concerning the disturbance of copper metabolism in Wilson's disease. During the prolonged period of the study, serum copper and ceruloplasmin levels, urinary copper excretion, and clinical status of the patients were observed.

METHODS

Twenty-four-hour urine specimens were collected in acid-cleaned vessels and were stored at 4° C using thymol crystals as preservative. Urine and serum copper determinations were carried out in duplicate by methods previously described (28, 29). Serum ceruloplasmin was estimated either by the method of Bearn and Kunkel (29) and Holmberg and Laurell (30) or by a modification of Broman's method (31), all of which employ para-phenylenediamine as substrate. The serum copper calculated to be incorporated in ceruloplasmin was based on a copper content of this protein of 0.34 per cent (32); the copper present in the serum in excess of this was considered to be loosely bound to albumin (11) and is referred to as non-ceruloplasmin copper. Ethinyl estradiol (Estinyl, Schering Corp.), 5.0 mg daily, was administered during 16 courses to 11 patients (Table I).

Clinical material. The 11 patients with Wilson's disease in this study exhibited varying degrees of neurological and hepatic disorder; Table I (fourth column) indicates clinical severity ranging from 0 (no disease detectable) or 1 (minimally affected) to 4 (severely affected). Four individuals were ambulatory and in a stable phase of the disease (AC, FL, CP, LA); the remainder were severely disabled. Two cases had severe liver disease in addition to serious neurological manifestations; these two patients exhibited the highest pretreatment levels of ceruloplasmin (RE, VC). The symptoms in the remaining 9 cases were predominantly neurological.

Prior to the administration of estrogens, clinical and biochemical evaluation of each patient was made. Other forms of therapy¹ which might interfere with the study

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¹ Most of the patients have also received dimercaprol (BAL) (minimum dosage 2.5 mg per kg per day) or peni-

TABLE I
Clinical and biochemical response to estrogen administration of 11 patients with Wilson's disease

Case and Course #	Sex and Age	Age at Onset	Clinical Type*	Mean pre-estrogen levels				Peak levels with estrogen				Clinical changes during estrogen treatment
				Serum Copper µg%	Ceruloplasmin mg%	Urine Copper mg/24hrs	Days of Estrogen	Serum Copper µg%	Ceruloplasmin mg%	Urine Copper mg/24hrs	Urine Copper mg/24hrs Penicillamine BAL	
AC	F 28	12	N-2	73	8.0	0.30	22	90	8.9	0.71	4.42	Unchanged
FL	M 39	22	N-1	50	7.5	0.58	19	80	6.4	0.73	2.67	Dysarthria slightly worse
BR1†	F 40	13	N-4	60	6.7		14	119	10.2			Slight restlessness
BR2	F 40	13	N-4	72	8.0		36	71	10.2			Unchanged
CP	M 30	20	N-1	41	5.2	0.11	14	50	6.0	0.37	2.28	Slight subjective weakness
LA	M 56	31	N-2	40	7.3	0.73	28	80	10.7	1.07	2.06	Increase of tremor and incoordination
SC	M 19	18	N-3	51	2.8	0.47	14	69	2.0	1.88	4.81	Slight increase in tremor and drooling
YR ‡	F 41	13	N-3	52	4.8	0.83	21	87	8.4	1.50	2.00	Initial improvement. Sudden coma and hemiparesis day 22.
AD	M 32	27	N-3	99	10.1	0.44	28	154	24.0	5.26		Initial improvement in coordination. Then marked increase in belligerence, disorientation, dysphagia, incontinence.
RE	M 18	15	N-2	81	16.4	0.58	30	188	33.0	3.78	7.16	Tremor disappeared. Speech and disorientation worse.
MP1	F 25	17	N-4	68	7.3	0.28	46	123	21.0	1.89	3.71	Slow, mild improvement of rigidity, flapping and speech.
MP2†	F 25	17	N-4	82	6.4	2.74†	30	70	11.5	3.00		Unchanged
VC1	F 27	20	N-3	108	20.8	0.26	16	235	78.8	1.45		Improvement
VC2	F 27	20	N-2	106	26.4	0.22	46	188	48.3	2.10		Striking improvement
VC4	F 28	20	N-4	79	14.4		87	140	30.2	0.65	2.41	Mild improvement
Normal (mean)				126	38.5	0.05						

* Neurological or hepatic disorder prior to estrogen administration. Severity graded 0-4.

† Estrogen and penicillamine administered simultaneously

‡ Identical twins (50)

TABLE II
The effect of estrogens in 11 patients with
Wilson's disease (mean levels)

	Total serum copper	Non- cerulo- plasmin serum copper	Cerulo- plasmin	Urinary copper
	$\mu\text{g}\%$	$\mu\text{g}\%$	$\text{mg}\%$	$\text{mg}/24 \text{ hrs}$
Pre-estrogen level	71	36	10.1	0.44
Estrogen response (peak)	116	46	20.6	1.84
Normal	100-130	0-10	38.5	<0.10

were discontinued for several weeks prior to institution of the hormone, except in the case of two patients (MP2, VC4) in whom the combined effect of estrogen and penicillamine was determined. Foods high in copper content were restricted.

RESULTS

Tables I and II summarize the biochemical status before administration of estrogen and indicate the peak biochemical response to such treatment. The various types of biochemical and clinical response observed in several of the patients are illustrated in Figures 1-4.

Total serum copper. In all but three instances (BR2, CP, MP2) the serum copper concentration rose during or immediately following estrogen. During each of six courses of estrogen there was a mild to moderate increase to levels of 69 to 119 μg per 100 ml. During seven courses the level rose to 123 to 235 μg per 100 ml (AD, RE, MP1, VC1-4); in three of these patients the level prior to treatment was in the higher range exhibited by some individuals with this disorder (81 to 108 μg per 100 ml). There was often a lag of several days following discontinuation of treatment before the serum copper concentration began to decrease.

Ceruloplasmin. Seven patients showed no significant increase in serum ceruloplasmin; all these had very low levels prior to treatment. Six of the seven, however, showed a mild to moderate rise in total serum copper. Of the four cases (AD, RE, MP, VC) who showed increase in ceruloplasmin, two had low concentrations prior to treatment; one (VC) had a fairly high level of cerulo-

cillamine (1.0 g per day of combined *dl*-isomers, Aldrich Chemical Co., or 0.5 g per day of *d*-isomer, Distillers Co., London) but at times other than the test period; the effects of these chelating agents on urinary copper excretion are recorded in Table I.

plasmin (20.8 mg per 100 ml), and her response to estrogen was the most striking of the cases studied, rising to 78.8 mg per 100 ml by Day 13 after treatment was instituted (Figure 4).

Urinary copper excretion. Prior to treatment all ten patients (BR not tested because of incontinence) exhibited elevated cupruria, ranging from 0.11 to 0.73 mg per day. During estrogen administration three cases [FL, CP (Figure 1), LA] showed no significant change in urinary copper excretion while on estrogens.² Two patients approximately doubled their output [AC, YR (Figure 2)]. The remaining five patients showed marked increases in average daily urinary copper excretion in response to estrogen: that of SC increased from 0.45 to 1.71 mg; of MP from 0.36 to 1.68 mg; of VC from 0.32 to 1.85 mg (Figure 4); of RE from 0.58 to 1.9 mg; and of AD from 0.48 to 4.29 mg per day (Figure 3).

Interrelationships of serum copper, ceruloplasmin, and urinary copper. When given oral ethinyl estradiol some patients with Wilson's disease exhibited little or no change in the concentrations of serum copper and ceruloplasmin; such patients showed minimal or absent increase in urinary copper excretion and minimal clinical change (Figure 1). Some patients showed a rise in total serum copper without significant increase in ceruloplasmin, indicating therefore a rise in non-cerulo-

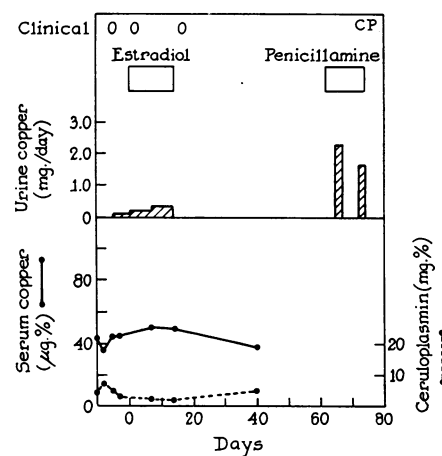


FIG. 1. RESPONSE OF PATIENT CP TO ESTROGEN, SHOWING NO BIOCHEMICAL OR CLINICAL CHANGE.

² During estrogen administration (14 days) to five heterozygotes, two possible heterozygotes, and a normal male individual, significant elevation of urinary copper excretion did not occur.

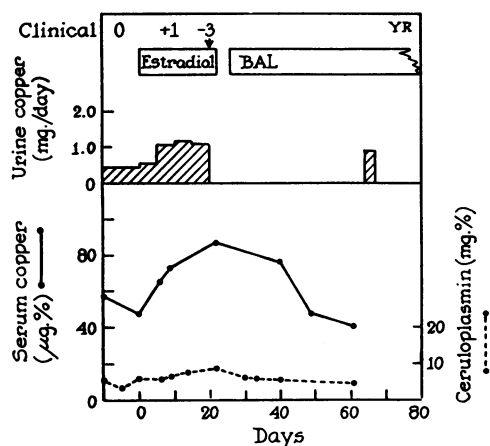


FIG. 2. RESPONSE OF PATIENT YR, SHOWING INCREASE OF TOTAL SERUM COPPER AND URINARY COPPER EXCRETION. INITIAL IMPROVEMENT FOLLOWED BY DETERIORATION.

plasmin serum copper; of this group three showed an increased cupruresis and five deteriorated neurologically (Figure 2, for example; Table III).

Four patients responded to estradiol with not only a marked increase of the serum copper to normal or supernormal levels but also an increase in ceruloplasmin content (AD, RE, MP1, VC). The ceruloplasmin levels of AD (Figure 3) and RE rose to low normal levels, but the non-cerulo-

plasmin copper levels of their serum rose markedly (73 and 76 μg per 100 ml). These two patients showed the greatest degree of cupruresis of all the patients as well as the most severe neurological deterioration.

MP1 initially showed a roughly parallel rise of serum copper and ceruloplasmin with moderate cupruresis and mild clinical improvement. Preceding and during a subsequent course of estrogen (MP2) penicillamine was given; the serum level of ceruloplasmin did not rise, and the serum copper level decreased to 29 μg per 100 ml in contrast to her pretreatment concentration of 68 μg per 100 ml.

VC, during her first two courses of estradiol administration (Figure 4), showed a striking rise of both total serum copper and ceruloplasmin, with moderate cupruresis. It is noteworthy, however, that during the first course the rise in total serum copper could be accounted for by the increase in the copper incorporated in serum ceruloplasmin. Thus, the amount of non-ceruloplasmin copper in the serum was relatively small. (In addition to this unique biochemical response the singular clinical response of this patient is described later.)

There was, in general, a rough correlation between the absolute height of non-ceruloplasmin

TABLE III
Biochemical and clinical alterations associated with estrogen administration in Wilson's disease *

Patients and courses	Total serum copper conc.	Ceruloplasmin conc.	Non-ceruloplasmin copper conc.	Cupruresis	Clinical signs and symptoms
AC	+	0	+	+	0
FL	+	0	+	0	+
BR#1	+	0	+		0
BR#2	0	0	0		0
CP	0	0	0	0	0
LA	+	0	+	+	+
SC	+	0	+	+	+
YR	+	0	+	+	++
AD	+	+	+	+++	++
RE	++	+	++	++	++
MP#1	+	+	+	+	0
MP#2†	-	0	0	++	0
VC#1	++	+++	-	+	
VC#2	+	++	+	+	
VC#3			0		
VC#4	+	+	+	+	0

* + Indicates increase; -, decrease; 0, no detected alteration.

† With penicillamine

copper and the degree of cupruresis. A parallel increase between serum copper and ceruloplasmin was frequently observed during the initial phase of the response. Subsequently, the ceruloplasmin level tended to become stable while both the total copper (and, therefore, the non-ceruloplasmin copper) and the urinary copper increased further. There was also a clinical correlation: patients whose condition deteriorated showed, in general, a high non-ceruloplasmin copper level either prior to or in response to estrogen treatment. Patients whose condition either improved or did not change showed a decrease or little change in the non-ceruloplasmin copper level.

Some patients exhibited cupruresis without a ceruloplasmin rise; however, all of those who showed cupruresis had an increase in total serum copper, which suggests that a rise in non-ceruloplasmin copper is related to an increase in urinary copper excretion.

Clinical results. Table I indicates the predominant clinical type prior to treatment and the clinical response to estrogen administration. Three cases did not change (AC, BR, CP). Three cases deteriorated slightly but returned to pretreatment status within two to three weeks following treatment (FL, LA, SC). One patient (YR) improved initially but abruptly deteriorated, develop-

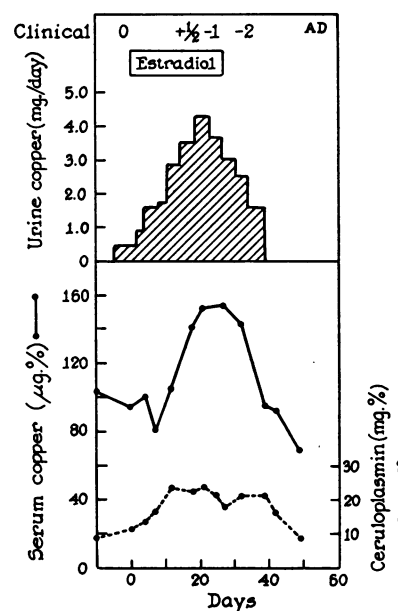


FIG. 3. RESPONSE OF PATIENT AD. INCREASE IN SERUM COPPER, MILD INCREASE IN CERULOPLASMIN, AND A STRIKING CUPRURESIS ACCOMPANIED CLINICAL DETERIORATION.

ing semicoma and hemiparesis, a state very similar to that of her identical twin. However, she returned to a pretreatment status about three weeks after simultaneous discontinuation of the hormone and initiation of BAL. Two patients (AD, RE)

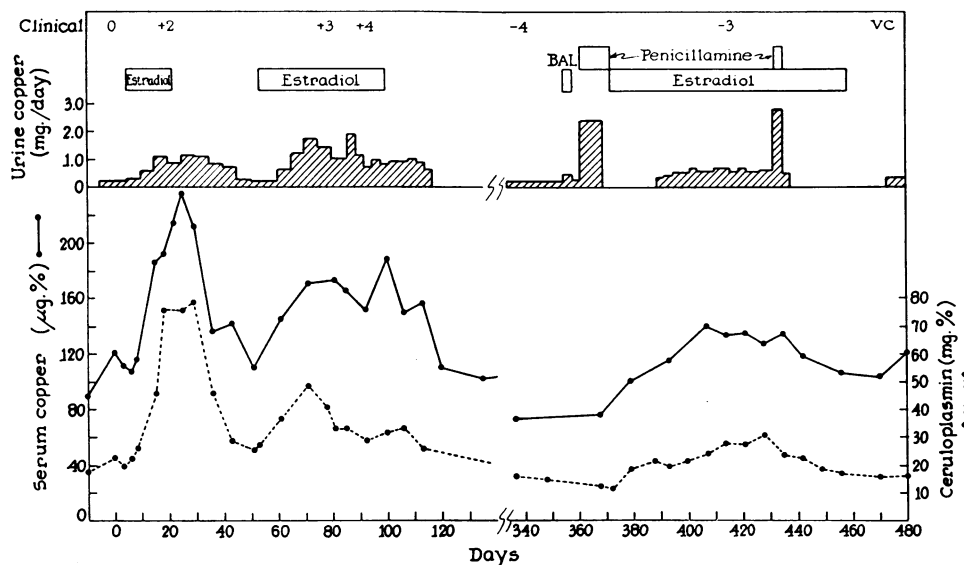


FIG. 4. RESPONSE OF PATIENT VC DURING 3 OF 4 COURSES OF ESTROGEN. During the first courses clinical improvement and only moderate cupruresis occurred in association with a great elevation of total serum copper. Much of the increase in total serum copper could be accounted for by a simultaneous increase in ceruloplasmin during course 1 and the early part of course 2.

exhibited initial neurological improvement but subsequent marked deterioration with only minimal improvement following cessation of treatment. Although both of these patients died of Wilson's disease within months following treatment, they were severely affected prior to the study.

One patient (MP) showed mild improvement during her initial course and no change during the second course which was superimposed upon penicillamine administration. VC (Figure 4) showed striking improvement when given estrogen. Prior to treatment she had become markedly debilitated, displaying severe hepatic and neurological symptoms. Prompt and extensive neurological improvement and weight gain from 35 to 47 kg occurred during the first three courses of treatment. She progressed from being bedridden to a condition in which she could be discharged from the hospital for short periods. Toward the end of each course of treatment and in the period following discontinuation of the hormone, neurological deterioration occurred. When her last course was initiated she showed severe neurological disease, and only slight improvement occurred during estrogen administration. Her biochemical response during the first courses was unique, as previously indicated. Although she is the single instance of striking improvement associated with estrogen therapy, this treatment seemed largely responsible for her survival early in the hospital course when death apparently was impending and, further, enabled her to withstand major surgery (course 3).

During estrogen administration three patients developed phlebotrombosis, in one case associated with pulmonary embolism. No consistent coagulation abnormalities could be detected.³

DISCUSSION

A notable lack of uniformity was evident in the response to estrogen administration of the 11 patients with Wilson's disease. It is clear (Table II) that such treatment usually leads to an alteration of copper metabolism. The nonhomogeneity in response is a further example of the clinical and biochemical variation seen in patients with this disorder (6). This, of course, does not neces-

sarily imply genetical nonhomogeneity, but could equally represent the result of varying internal environments or compensatory physiological mechanisms, as well as modifying genes.

In many respects the patient with Wilson's disease in whom total copper and ceruloplasmin concentrations are brought toward normal by administration of estrogen is comparable with the rare patient who, at some phase of his disease, exhibits total levels in the normal range (33, 34). The reason for the increased concentration of ceruloplasmin in such unusual cases is not immediately apparent. It is noteworthy that two such patients, both males, described by Sass-Kortsak, Cherniak, Geiger and Slater (33) had severe liver disease without neurological symptoms; one showed gynecomastia and delay in maturation of male sexual characteristics. Our observations demonstrate that environmental (in this instance, endocrinal) alterations can increase the concentration of the protein, ceruloplasmin, which is characteristically present in decreased quantities in Wilson's disease.

It seems likely that some of the effects of estrogen administration to patients with Wilson's disease are the result of nonspecific mechanisms affecting protein synthesis without altering the metabolic error which is the result of the primary genetical defect. The manner in which estrogen effects anabolism in the normal individual is not clear at the present time (35-37). It is known that the concentration of a number of serum components increases during pregnancy or estrogen administration; these include fibrinogen, α_1 -, α_2 -, and β -globulins (38-42), ceruloplasmin (43), transcortin (44), and the thyroxine-binding α -globulin of serum (45, 46). Also, the serum copper level may rise during pregnancy (19-23, 47) or estrogen administration (24, 25, 43, 48).

When estrogen is given to patients with Wilson's disease the concentration of two distinct serum compartments may undergo alteration—1) non-ceruloplasmin serum copper and 2) ceruloplasmin. Since these increases, when they occur, do not necessarily either parallel each other or occur in the same patient, the question arises of whether the hormone is influencing one metabolic mechanism only or whether the two serum components increase for unrelated reasons. The former and more attractive possibility implies that as the ceruloplasmin-synthesizing mechanism

³ Dr. Margaret Todd of Cornell University Medical College, New York, N. Y., kindly assisted in these assays.

attempts to increase the production of this protein, simultaneous inordinate increase of the serum non-ceruloplasmin copper concentration occurs. Insufficient data exist for determining whether either of these possibilities is correct.

Since it is unknown whether alteration of the biliary or fecal excretion of copper occurs during estrogen administration, comments concerning total copper balance or actual alteration of tissue content of copper during such treatment can not be made. It is noteworthy in this respect that the normal individual remains in copper balance although he excretes virtually no copper in the urine.

Although the administration of estrogen to patients with Wilson's disease may in some instances result in increase in the serum concentration of copper and ceruloplasmin, it is not usually of therapeutic value. Although only 11 patients have been treated, and although the course of the disease is unpredictable, treatment with estrogens has been associated with increased rate of neurological decline in over half of the patients. Further, some patients in a stable phase of the disease have abruptly shown the onset of deterioration in association with administration of estrogen. Increasing neurological symptoms, increasing cupruresis, and rising non-ceruloplasmin copper concentration are all accentuations of characteristic manifestations of the preexisting disease. In the presence of this specific genetical abnormality, the efforts of the cell to increase ceruloplasmin synthesis in response to estrogen may be partially effective, as observed in 4 of 11 patients, but these efforts are usually simultaneously associated with increase in the disease processes. The lack of improvement seen in some patients whose ceruloplasmin concentrations increase is evidence that the low level of this protein per se is not critical in determining the presence or absence of the clinical and pathological findings of the disease.

Several observations made during the study thus indicate that although a trend toward normality in total serum copper and serum ceruloplasmin may be established, other events may take place simultaneously which are seriously deleterious. The increase in the non-ceruloplasmin component of serum copper (49), though associated with striking cupruresis, is also associated with increase of neurological symptoms. It is unclear

whether the increase in this compartment of serum copper itself is the deleterious event or is only an associated occurrence.

SUMMARY

1. Ethinyl estradiol was administered to eleven patients with Wilson's disease. In four patients the serum copper and ceruloplasmin concentrations and the urinary copper excretion increased. In six patients there was an increase in the serum copper concentration, three of whom showed cupruresis. The levels of serum copper and ceruloplasmin and the urinary copper excretion showed no change in one patient.

2. One patient improved clinically, four were unchanged, and six deteriorated during the estrogen administration.

3. The interrelationships of total serum copper, non-ceruloplasmin serum copper, ceruloplasmin, urinary copper excretion, and clinical change were investigated. Neither ceruloplasmin elevation nor cupruresis necessarily led to improvement. Cupruresis and clinical deterioration could, in general, be correlated with an increase in the non-ceruloplasmin fraction of serum copper.

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ANNOUNCEMENT OF MEETINGS

The Fifty-Third Annual Meeting of THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION will be held in Atlantic City, N. J., on Sunday afternoon and evening, April 30, 1961, in the Chalfonte-Haddon Hall (jointly with the AFCR); and on Monday, May 1, at 9:00 a.m., at the Casino Theater on the Steel Pier.

The Eighteenth Annual Meeting of THE AMERICAN FEDERATION FOR CLINICAL RESEARCH will be held in Atlantic City, N. J., on Sunday, April 30, 1961, at 9:00 a.m., at the Casino Theater on the Steel Pier. On Sunday afternoon and evening, April 30, 1961, a joint sectional meeting with THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION will be held in rooms in the Chalfonte-Haddon Hall.

THE ASSOCIATION OF AMERICAN PHYSICIANS will hold its Seventy-Fourth Annual Meeting in Atlantic City, N. J., at the Casino Theater on the Steel Pier on Tuesday, May 2, 1961, at 9:30 a.m., and in the Carolina Room, Chalfonte-Haddon Hall, on Wednesday, May 3, 1961, at 9:30 a.m.