exceeded normal without any resulting marked increase in excretion. In the present experiments, the rate of disappearance of leucine from the blood is much greater than that of histidine but these differences are not reflected in the urine. It is therefore felt that studies of amino acid changes in the blood plasma may be more valid for the purpose of an investigation of utilization of various mixtures.

A comparison of tolerance to the same quantities of amino acids when infused at a constant rate over a one-hour period is now in progress. The results will be reported in a later communication.

Summary. A mixture of the 10 indispensable amino acids has been injected intravenously in normal human male subjects and the plasma levels of these 10 acids as well as the urinary excretion, prior and at various intervals subsequent to the injection, were determined microbiologically. The pattern of amino acids excreted is shown to bear no relationship to that infused.

The work here reported has been carried out with the active cooperation of Drs. H. J. McCorkle, of the University of California Medical School, and H. L. Silvani, Frank Choy, and their associates at The Veterans' Hospital, Fort Miley.

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Intravenous Administration of Massive Dosages of Estrogen to the Human Subject; Blood Levels Attained. (17373)

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The employment of estrogens in the management of cases of cancer of the breast[1] and prostate[2] necessitates our further knowledge of the biological effects of the estrogens when administered in varying dosage and by various routes.

Few data are available concerning the effects of intravenous administration of estrogen in the human subject. Loeser[3] injected as much as 10 mg estrone, 25 mg estradiol, or 150 mg stilbestrol intravenously in propylene glycol and observed an elevation of intrauterine temperature presumably attributable to increased uterine blood-flow. He noted no toxic side reactions and indicated that the medication was well tolerated. Abarbanel[4] reported the administration of small doses of estrone sulfate for the control of uterine bleeding.

We wish to report on the clinical tolerance of intravenous infusions of massive dosages of an aqueous solution of conjugated natural estrogens.* We have also determined by bioassay the blood levels attained immediately following infusion and at fixed intervals after treatment.

Materials and methods. Thirteen female and 9 male subjects have been studied. Their age, race, and clinical diagnosis are indicated in Table I. All patients were at bed rest when treated. Intravenous infusions in the dose and volume indicated were carried out in the customary manner employing the antecubital vein. A buffered aqueous solution of conjugated estrogens in the form of a concentrate from pregnant mare's urine was mixed with normal saline so that a final concentration of from 0.5 to 2 mg equivalents of estrone sulfate per cc was obtained for injection. The rate of flow was so adjusted that the total dose was administered in from 35 to 60 minutes. Each patient's temperature, pulse,

1 Nathanson, I. T., *Surg. Clin. N. A.*, 1947, **27**, 1144.

2 Huggins, C., Stevens, R. E., and Hodges, C. V., *Arch. Surg.*, 1941, **43**, 209.

3 Loeser, A. S., *J. Obst. and Gynec. Brit. Emp.*, 1948, **55**, 17.

4 Abarbanel, A. R., paper No. 71; read by title; Assn. for Study of Internal Secretions, June, 1949.

* The preparation used was "Premarin" (injectable) kindly supplied by the Ayerst, McKenna & Harrison Ltd., through the courtesy of Drs. G. H. C. McKeown and E. C. Reifenstein, Jr.

TABLE I. Intravenous Estrogen Administration.

Patient	Sex and color	Age	Date treated	Total dose (mg)	Serum level of estrogens (μg) Pre.	Post	2 hr	4 hr	6 hr	8 hr	24 hr	Reaction	Remarks
E.T.	C.F.	62	2/16/49	200*	.0	36	8.0	3.2				0	Cancer of breast, with wide local extension; arteriosclerotic degeneration of the brain
			18	200*	.0	24.4	12.0	3.2				0	
			3/ 4	200*		38				.0		0	
			9	200*	.0	30	5.6	.0	.0	.0	.0	0	
			15	300*	.0	35	7.6	4.0	.0	.0	.0	0	
			18	300*	.0	39	4.3	2.4		.0	.0	0	
			5/17	100†	.0	23.2				.0	.0	0	
			25	300*	.0	37		5.6		.0	.0	0	
			27	400*	.0	35	18.4	8.6	6	3.8		0	
			31	400*	.0	38	16.0	7.0		.0	.0	0	
N.H.	C.F.	76	2/16	200*	.0	21.8						0	Cancer of breast, pulmonary and osseous metastases; hypertensive heart disease
			18	200*	.0	22.4						0	
			3/ 3	400*								(e)	
R.A.	W.F.	53	3/ 4	200*	.0	28.4	5.6	5.4	2.4	.0		0	Bilateral, inoperable breast cancer with metastases
			9	200*	.0	19.0			2.2	.0	.0	0	
			15	300*	.0	23.8		3.6		.0	.0	0	
			18	300*	.0	39	5.6	3.2	3.2	.0	.0	0	
J.F.	C.M.	55	3/17	200*	.0	24.4	7.2				.0	0	Carcinoma of prostate; osseous metastases; moribund
			21	200*	.0	25.8	6.0			7.4	.0	0	
J.S.	W.M.	67	3/17	200*	.0	23.8	7.0					0	Carcinoma of prostate
L.H.	W.F.	73	3/25	200*	.0	22.4	3.6	.0	.0	.0		0	Massive, fungating breast cancer; severe anemia
			28	240*	.0	21.2	2.0	.0	.0	.0	.0	0	
			31	200*		17.6			.0		.0	0	
			5/ 5	200*	.0	25.6		2.4		.0	.0	0	
			10	250*	.0	21.2		2.0		.0	.0	0	
J.W.	W.F.	41	3/28	100†	.0	22.4	.0	.0	.0		.0	0	Cancer of breast; pulmonary metastases with pleural effusion
			30	132‡	.0	22.0	3.2		.0	.0	.0	0	
C.D.	W.M.	65	4/ 4	200*	.0	24.0	10.0		7.6			0	Cancer of prostate, huge abdominal masses
L.S.	W.M.	59	4/ 4	200*	.0	21.6	6.0		.0			0	Cancer of prostate
A.T.	W.M.	68	4/ 8	200*	3.2	25.6	9.6	.0		.0		0	Carcinoma of prostate with metastases to bone and lymph node
P.C.	C.M.	81	4/11	200*	.0	23.6	8.4	4.2		.0		0	Carcinoma of prostate

C.M.	W.M.	63	4/15	200*	.0	28.4	7.6	3.2		.0	.0	0	Cancer of prostate with metastases
			22	200*		26.4	8.4	2.4		.0	.0	0	
			24	200*	.0	23.8					.0	0	
			26	200*	.0	21.0	6.8		2.8		.0	0	
			28	200*	.0	24.4		6.4		2.4	.0	0	
			5/ 3	200*	.0	26.4	4.0			3.8		0	
			5	250*	.0	25.6		6.8		2.8		0	
M.W.	C.F.	58	5/10	200*	.0	22.4	6.8	3.6		.0	.0	0	Massive, fungating breast cancer; marked debility
			17	200*	.0	21.2	7.6	4.8		5.0	.0	0	
			25	300*	.0	33.0	14.0	8.4	4.0	7.2	.0	0	
L.J.	C.F.	79	5/25	200*	.0	23.8	6.0	.0		.0	.0	0	Advanced breast cancer; marked senile degeneration
			27	300*	.0	38.0	12.8		2.6	2.0	.0	0	
			31	400*	.0	38	14.4	7.6		3.4	.0	0	
A.F.	W.F.	45	6/ 2	300*	.0	31	.0	.0		.0	.0	0	Breast cancer with osseous metastases
			9	400*	.0	32	13.2	3.2		.0			
M.M.	C.F.	71	6/ 2	300*	.0	35	13.6	6.8		3.4	.0	0	Breast cancer with osseous metastases
			9	300*	.0	45	14.8					(a)	
J.F.	C.M.	66	6/ 9	175§	.0	35	3.6				.0	0	Cancer of prostate
C.P.	W.F.	73	6/10	250*	.0	35	10.0	4.2		.0	.0	0	Severe rheumatoid arthritis
			14	300*	.0	39.8	14.4	7.6		2.8	.0	0	
			15	300*	.0	33						0	
			16	300*	.0	32						0	
			17	300*	.0	37						0	
F.J.	C.M.	44	6/10	250*	.0	38	14.0	6.0		2.0	.0	0	Severe rheumatoid arthritis
			14	300*	.0	36	12.8	5.6		2.0		0	
			15	300*	.0	39.8						0	
			16	300*	.0	34						0	
			17	300*	.0	35						0	
J.D.	C.F.	43	6/10	250*	.0	39	12.8	3.2				0	Severe rheumatoid arthritis
			14	300*	.0	32	13.2	6.6				0	
			15	300*	.0	48						(b)	
			16	300*	.0	35						0	
			17	300*	.0	35						0	
D.M.	C.F.	59	6/16	207‖	.0	19		1.5		.0		0	Advanced cervical cancer; frozen pelvis
F.E.	W.F.	68	6/15	250*	.0	45	14.8	8.0		6.0		0	
			16	225*	.0	38				7.8		0	
			17	300*	5.8	43		18.0		7.8		0	

* Given in 200 cc normal saline.
† " " 100 " " "
‡ " " 132 " " "
§ " " 175 " " "
‖ " " 165 " " "

(a) Mild chill; infusion discontinued.
(b) Nausea for 6-8 hours after infusion.
(c) Died following infusion—see text.
Values less than 1 μg doubtful and indicated by 0.0.

respiration and blood pressure were recorded before, during and after the infusion. Blood samples were drawn for estrogen bioassay just prior to the beginning of the infusion, at the conclusion of the injection, and at stated intervals throughout the subsequent 8 to 24 hours. The drawn blood was permitted to clot and the serum promptly prepared.

The estrogen content of the sera was determined by a modification of the uterine weight method of Lauson *et al.*[5] Twenty-one day-old female rats of the N.I.H. or Holtzman strain were ovariectomized and were given the proper dilution of serum in 0.25 cc twice daily for 2 days. Twenty hours after the last injection the test animals and uninjected controls were autopsied, the uterus dissected out, freed of fluid and weighed to the nearest milligram on a Roller-Smith torsion balance. The estrogen content of the patients' sera was calculated on the basis of the activity of an aqueous dilution of the same preparation of conjugated estrogens that had been employed for the clinical studies. Duplicate and multiple determinations at various levels have indicated an approximate error of 10% in our estrogen bioassays.

Results and discussion. The clinical reaction of 67 infusions of from 100 to 400 mg each was observed. All but 3 infusions were attended by no subjective reactions on the part of the patient. One patient (J.D.) who had received 200 mg complained of nausea for 6 hours following the injection. Another patient (M.M.) who had received 350 mg suffered a mild chill necessitating the discontinuation of the infusion. The third patient (N.H.) who was in a badly debilitated state from advanced metastatic breast cancer and hypertensive heart disease died shortly after the administration of 400 mg of conjugated estrogens. The terminal clinical picture simulated that of cerebrovascular accident. Careful autopsy examination, including study of the brain, revealed no apparent basis for this patient's death. In view of her general debility and the total lack of reaction on the part of other patients to identical treatment, it may be considered that her death was not causally related to the estrogen administration.

The pulse, temperature, blood pressure and respiration in the remaining cases were not significantly affected. The apprehension attendant upon the venipuncture induced a slight but transient rise of blood pressure in some cases, particularly upon the first infusion. This momentary effect subsided rapidly and in most cases was not observed during subsequent infusions. Hyperpyrexia was observed only in the one patient (M.M.) who had experienced a reactive chill.

On the whole, the infusions were remarkably well tolerated in spite of the very high doses employed. Moreover, it should be noted that most of our subjects were all bedridden, debilitated individuals with advanced cancer of the breast or prostate and even greater tolerance might be anticipated from patients in better general clinical condition.

The blood levels of estrogen attained immediately following the infusion varied from 17.6 to 48 μg per cc and rapidly dropped off to negligible levels in most cases after 8 hours. This indicates a fairly ready dilution of the estrogen in the blood and a rapid removal therefrom. Studies on the urinary excretion and metabolic fate of the injected estrogen will be reported subsequently.

These preliminary observations indicate the feasibility of procuring an inordinately high blood level of estrogen in a short time. The quantitative data will permit an estimate of the rate of estrogen infusion which may be required for the maintenance of such levels for longer periods of time. The potential usefulness of such intensive estrogenization in patients with prostatic and breast cancer remains to be determined. We have noted no material clinical effect on the malignant process from the limited number of infusions thus far administered. The evaluation of such potential effects will require more prolonged periods of sustained treatment and observation.

Summary. Thirteen female and 9 male subjects have been given a total of 67 intravenous infusions of from 100 to 400 mg of conjugated natural estrogens dissolved in 200 cc of normal saline. In the main, these in-

[5] Lauson, H. D., Heller, C. G., Golden, J. B., Severinghaus, E. L., *Endocrinology*, 1939, **24**, 35.

fusions were well tolerated. Serum levels of estrogen as determined by bioassay varying from 17.6 to 48 μg were obtained and only negligible quantities of estrogen remained in the blood after 8 hours.

The feasibility of rapidly inducing a high serum estrogen level by intravenous infusion is demonstrated. The clinical usefulness of this form of estrogen therapy in carcinoma of the breast and prostate remains to be evaluated.

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Blood Pressure in the Rat.* (17374)

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The work of Kersten *et al.* on the indirect measurement of blood pressure in the rat[1,2] will increase the use of rats for studying various phases of the hypertension problem. If rats of different ages are to be used it should be remembered that "a tendency to a slight rise in blood pressure with age" has been noted.[3] It was the scope of the following investigations to establish accurate data which the literature does not provide.

Rats of the Long-Evans strain were kept on Friskies and water. The blood pressure was measured in unanesthetized, unheated animals with the foot method described by Kersten, Brosene, Ablondi and SubbaRow.[1] Readings obtained about 1 minute apart in the same animal rarely differed more than $\pm$ 3 mm Hg.

Fig. 1 shows that the systolic blood pressure increases with age in the growing rat. Within the first 2 months up to a weight of 150 g the pressure increases rapidly. A slower, but still progressive rise is noted in rats weighing 200 to 350 g.

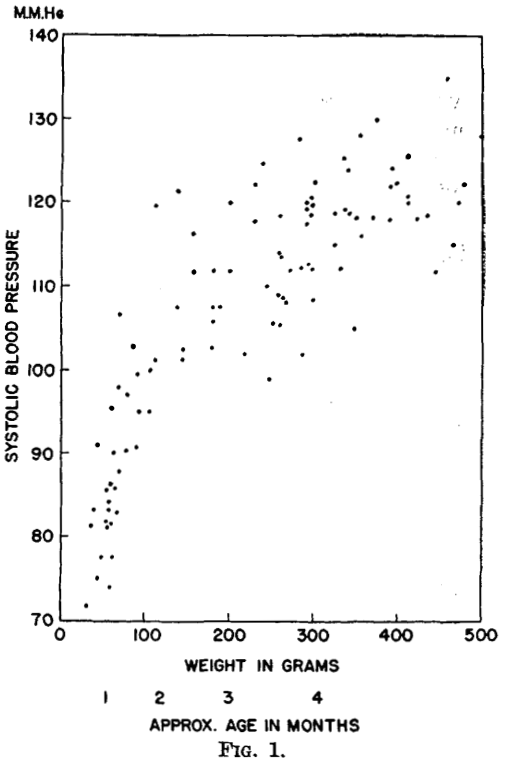

FIG. 1.

Systolic blood pressure in rats of different weights and ages.

The age conditioned differences in the sys-

* Aided by a grant from the Life Insurance Medical Research Fund.

1 Kersten, H., Brosene, W. G., Jr., Ablondi, F., and SubbaRow, Y., *J. Lab. and Clin. Med.*, 1947, **32**, 1090.

2 Ablondi, F., SubbaRow, Y., Lipchuck, L., and Personens, G., *J. Lab. and Clin. Med.*, 1947, **32**, 1099.

3 Griffin, John A., and Parris, E. J., The Rat in Laboratory Investigation, J. B. Lippincott Co., Philadelphia-Montreal-London, 1942, p. 286.

† An apparatus was obtained from the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of the late Dr. Y. SubbaRow.