dice although transient symptoms and minor deviations in liver function tests did occur in 2 out of 3 volunteers in each group from 41 to 64 days after oral administration of the plasma and in one volunteer a serum bilirubin of 2.16 mg/100 ml was observed on the 44th day. The failure to demonstrate the agent of I.H. in these 3 acute phase blood samples can not be accepted as unequivocal evidence that it was not present since the number of volunteers employed was small. The results obtained lend some weight to those of experiment 1, *i.e.*, that serum hepatitis is at least in part responsible for jaundice in U.S. troops in post-war Germany.

Discussion. Our knowledge of the viruses of I.H. and S.H. is extremely limited and to date has been accumulated almost entirely from work with volunteers. The previous experiments have been well reviewed in a recent report by Havens(6).

The results of the present experiment establish the existence of serum hepatitis, or at least "long incubation period" hepatitis, in U.S. troops stationed in Germany in 1949 where viral hepatitis has been endemic and highly prevalent among U.S. soldiers(1). There is no evidence presented by this or other published studies which indicates the extent to which serum hepatitis may be present among these troops although there is considerable circumstantial evidence from other sources which would suggest that serum hepatitis is common(1).

The soldier whose plasma contained an icterogenic agent of the long incubation period type had been exposed in the 2 months preceding his jaundice to dental instruments for a tooth extraction, as well as to suture needles, either of which may have been contaminated with icterogenic blood. Possible transmission of this disease by dental equipment as well as by surgical instruments should thus be borne in mind.

The occurrence of clinical symptoms and of tenderness over the liver was noted 17-20 days after inoculation in 2 of 3 volunteers who developed definite jaundice at a much later date and similar findings were also noted in 2 of 3 volunteers who never developed definite jaundice. These early changes in the course of serum hepatitis have previously been noted in volunteers studied by Neefe *et al.*(9) and it is known that the causative agent is present in the blood of such volunteers during the first months after inoculation, as demonstrated by Paul, *et al.* and others(6).

Summary. The presence of serum hepatitis (SH), or at least of long incubation period hepatitis, among the hundreds of cases of acute hepatitis which have been occurring annually in U.S. troops stationed in Germany has been established by human volunteer experiments using as the inoculum acute phase plasma obtained from a soldier who had contracted hepatitis in that area. In one experiment, one of 2 sera so tested produced clinical jaundice in 3 of 3 volunteers following incubation periods of 53, 68 and 87 days, respectively. Symptoms suggesting mild hepatitis occurred about 3 weeks after inoculation in 2 of these same 3 volunteers.

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Retardation of Tumor Growth in Mice by Oral Administration of Methyl Androstenediol and Methyl Testosterone. (18352)

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The retardation of growth of transmissible tumors of mice by steroid hormones has been described by several investigators(1-4), and favorable results in the treatment of metastatic breast cancer in women by the parenteral injection of methyl androstenediol have been reported by Homburger, Kasdon and Fishman(5). The present report presents ex-

TABLE I. Retardation of Tumor Growth in Mice by Methyl Androstenediol and Other Compounds.

Tumor	Measurement after, days growth	Compound	No. of animals	Sex	Dosage	Avg tumor size (cu mm)	Range
Sarcoma 180	13	Methyl androstenediol	20	♂	.06*	603	(413-817)
		" "	5	♂	.03*	618	(507-778)
		" "	5	♀	.03*	629	(569-728)
		" "	6	♂	.015*	1530	(1200-2200)
		" "	5	♀	.015*	1517	(1065-2196)
		" "	12	♂	100†	589	(373-687)
		Methyl testosterone	6	♂	100†	645	(406-702)
		Urethane	6	♂	.15*	746	(504-1144)
		Controls	25	♂	—	2130	(1508-3558)
		"	5	♀	—	1635	(1490-2980)
Mammary gland adenocarcinoma C3H-BA		Methyl androstenediol	4	♀	.06*	205	(120-390)
	11	Controls	4	♀	—	1560	(1170-2400)
	15	Methyl androstenediol	6	♀	.06*	576	(430-730)
		" "	6	♀	100†	333	(120-420)
		Controls	6	♀	—	1383	(1050-1872)
	18	Methyl androstenediol	6	♂	.06*	505	(336-840)
		" "	6	♂	.03	1345	(847-1843)
		Controls	6	♂	—	2852	(2345-3183)
	15	Methyl testosterone	5	♂	100†	468	(162-686)
		Controls	5	♂	—	1295	(1081-1860)
Adenocarcinoma EO-771	11	Methyl androstenediol	5	♂	.06*	245	(125-330)
		Controls	5	♂	—	600	(400-1092)
Sarcoma 37	8	Methyl androstenediol	4	♂	.06*	375	(140-616)
		Controls	4	♂	—	699	(480-896)

* % in diet. † mg/kg/day orally.

periments in which the growth of transplantable mouse tumors, sarcoma 180 and adenocarcinoma C3H-BA, were retarded when methyl androstenediol (3B-17 B-17 methyl androstenediol ("Methostan") or methyl testosterone ("Oreton M") was given orally to mice bearing subcutaneous implantations of the tumors.

Sarcoma 180 and sarcoma 37 were implanted in albino mice, adenocarcinoma C3H-BA in C3H mice, and adenocarcinoma EO-771 in C57 black mice.* Minimal implantations were made subcutaneously with an 18 gauge trocar in the right axillary region of young adult (18-20 gram) mice, and administration of the test substances was started 24 hours later. Tumor volume was calculated from measurements made in 3 diameters with vernier calipers. Methyl androstenediol or methyl testosterone was dissolved in warm corn oil and mixed with the pulverized stock diet (Wayne Fox Food) and fed *ad libitum* or administered daily orally in 0.5 ml of corn oil by stomach tube and syringe. Urethane, used as a control, was dissolved in a small quantity of water, mixed with the diet, and fed *ad libitum*. It was estimated that each mouse ate about 3 grams of food a day. Thus the daily intake of the steroids by mice fed the 0.06% diet was approximately 100 mg/kg a day. No marked loss of weight of the animals was noted during the treatment. Results showing the tumor retarding effect of the compounds when given by the oral route are shown in Table I.

From the data in Table I, it is seen that methyl androstenediol, when given orally, has

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* The tumors and specified strains of mice were obtained from the R. B. Jackson Memorial Laboratory, Bar Harbor, Me.

a distinct retarding effect on the growth of both sarcoma 180 and adenocarcinoma C3H-BA. This effect is apparently dependent upon the amount administered, little retardation being noted when the diets containing 0.015% was fed. Methyl testosterone, likewise, retarded the growth of these tumors when 100 mg/kg daily was given by the oral route. Growth of adenocarcinoma EO-771 and sarcoma 37 was not greatly retarded by methyl androstenediol given in concentrations which markedly impaired the growth of the other tumors.

The retarding effect of the compounds on the tumors is confined essentially to the first two weeks of treatment; after this time, growth proceeds at a rapid rate in spite of continued treatment. When implantations were allowed to grow for 5 days before oral treatment with methyl androstenediol was begun, little or no inhibition of growth was seen.

No retardation in growth of sarcoma 180 has occurred in repeated experiments in which either methyl androstenediol or methyl testosterone has been administered *subcutaneously* in 100 mg/kg daily doses for 13 days.

Summary. The growth of sarcoma 180 or adenocarcinoma C3H-BA is markedly retarded in mice by *oral* administration of methyl androstenediol or methyl testosterone in doses approximating 100 mg/kg daily, when treatment is started the day following implantation. Inhibition of sarcoma 37 and adenocarcinoma EO-771, under similar conditions, is slight and of short duration. No retardation of growth of sarcoma 180 was noted when 100 mg/kg doses of the compounds were administered daily by *subcutaneous* injection.

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Use of Bromsulphalein for the Measurement of Proteolytic Activity. (18353)

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Disodium phenoltetrabromophthalein disulfonate (Bromsulphalein), widely used as a test of hepatic function(1), has been found to be an enzyme inhibitor(2). In the study of this property, it was noted that protein could be precipitated completely from an acid solution by addition of this indicator, and that the color intensity of an alkaline solution of the precipitate was proportional to the amount of protein present. Carroll(3) by reporting recently the effect of pepsin on the binding of bovine albumin with the dye orange I, suggested the present investigation.

Experimental. 1. *Relation of protein concentration to amount of Bromsulphalein in precipitate.* Casein prepared according to Hammarsten(4) (Kahlbaum) was dissolved in aqueous 0.1% Na_2CO_3 solution so that 1 ml contained the amount of protein shown on the abscissa of Fig. 1. In 9.8 x 1.2 cm test tubes, 1 ml of casein solution and 1 ml of distilled water were mixed with 2 ml of indicator solution made by adding 3 ml of 5% aqueous Bromsulphalein (Hynson, Westcott, and Dunning) to 197 ml pH 2.5 citrate-phosphate buffer (McIlvaine)(5). The tubes were centrifuged at 2500 rpm for 10 minutes, inverted, and allowed to drain for several minutes, after which the inner walls of the tubes were wiped dry with absorbent cotton on an applicator stick. The precipitates at

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