TABLE V. Effect of Heparin and of Heparin plus Other Acid Mucopolysaccharides upon the Clotting Time of Recalcified Sheep Plasma.

Additional substances in clotting tubes*	Quantity of heparin in clotting tube (ml) 0	.05	.10	.15	.20
	Clotting times in min.				
None (heparin only)	5	12	21	40	60
B-Heparin, .1 ml	6	12	22	40	61
Heparin monosulfate, .1 ml	8	16	23	40	60
Chondroitin sulphate, .1 ml	5	12	21	41	62

* Inhibitors used in following concentrations: Heparin, .01 mg/ml; B-Heparin, heparin monosulfate and chondroitin sulphate at .1 mg/ml.

ides possibly present in the extract were found to be so much less effective than heparin as inhibitors of thromboplastin formation. The relative lack of interference of these substances with the anticoagulant assay of heparin using recalcified sheep plasma indicates that the plasma heparin levels we have reported(8) are substantially correct.

Summary. 1. Heparin monosulfate has 10%, and B-heparin and chondroitin sulphate less than 10% the inhibitory activity of heparin on thromboplastin generation. 2. This action of heparin is decreased in presence of other acid mucopolysaccharides. 3. Extracts of normal human plasma strongly inhibit thromboplastin formation thus confirming either the presence of heparin in plasma or of an unknown substance which acts identically with heparin. 4. The presence of other acid mucopolysaccharides does not substantially alter the assay of heparin when clotting time of recalcified sheep plasma is the method used.

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Effect of Thyroid Hormone on Protein Biosynthesis by Cell-Free Systems of Liver.* (27345)

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Thyroid hormone is required for normal growth and its presence is one of the prerequisites for the secretion and activity of growth hormone(1,2). The availability of cell-free preparations for study of metabolic pathways offered a possibility of localizing more closely one of the effects of thyroid hormone on growth, namely that on protein synthesis. In the following experiments the microsomal system of Zamecnik *et al.*(3), from the livers of normal and thyroidectomized animals was used to study the role of thyroid hormone.

Materials and methods. Chemicals. (ATP) adenosine triphosphate dipotassium salt was obtained from Sigma Chemical Corp. (FDP) fructose diphosphate, barium salt, from Nutritional Biochemicals Corp. was converted to

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TABLE I. *In vitro* Incorporation of Leucine C^{14} into Protein of Rat Liver Cell-Free System.

Group	No. of animals	c/M/mg total protein, mean ± S.E.	Significant differences: Comparisons	t*	P
A. Purina diet	28	361 ± 95	A *vs* B	—	>.05
			A *vs* D	—	>.05
B. I-deficient diet + 5 μg I^-/ml drinking water	9	255 ± 53	A *vs* F	—	>.05
C. Purina diet + PTU (.02%)	7	114 ± 14	A *vs* C	2.57	<.05 >.02
			C *vs* D	2.97	<.02 >.01
D. *Idem* + 5 μg T_3/day s.c.	3	331 ± 72	E *vs* C	2.80	<.01 >.001
			E *vs* B	3.65	<.01 >.001
E. Thyroidectomized + I^- deficient diet	21	50 ± 18	F *vs* B	1	>.05
F. *Idem* + 5 μg T_3/day s.c.	7	309 ± 80	G *vs* E	—	>.05
			G *vs* F	3.41	<.01 >.001
G. " + 2.5 mg G.H./day	12	73 ± 7	G *vs* B	2.93	<.01 >.001

* t—calculated according to Student's t-test.

T_3 = L-Triiodothyronine.

a potassium salt. L-leucine-C^{14} was obtained from the radiochemical center, Amersham, specific activity 7 μc/μM. Hyamine 10× (p-diisobutyl cresoxy-ethoxyethyl dimethyl-benzylammonium chloride) was obtained from Rohm and Haas. The chloride salt was converted into free base using the procedure of Eisenberg(5). POPOP (p-bis 2-(5 phenyloxazolyl)benzene) and PPO (2,5 biphenyloxazole) were obtained from Pilot Chemicals, Mass.

Hormonal preparations. L-triiodothyronine (Smith, Kline and French Laboratories, Philadelphia) was dissolved in a minimal amount of 0.01N NaOH and diluted with saline. The animals were injected subcutaneously with 5 μg per day for 3-4 days before sacrifice. Growth hormone ("Somactone" Ferring, A. B., Sweden) was dissolved in 0.01N NaOH and injected subcutaneously or intraperitoneally, 2.5 mg/rat/day, for 4 consecutive days before sacrifice.

Female albino rats of the Hebrew University strain were used. Thyroidectomy was performed under light ether anaesthesia on rats weighing 60-100 g. Following operation the animals were fed an iodine-deficient diet (4) and 1% $CaCl_2$ in drinking water for 10-12 weeks. One group of thyroidectomized animals was given Purina laboratory chow and 0.02% propyl-thiouracil (PTU) in drinking water. Control groups consisted of non-thyroidectomized animals of the same weight given either PTU or fed the iodine deficient diet and receiving a supplement of iodide in drinking water (5 μg/ml).

Liver homogenates 1:3 by volume, were prepared in 0.25M sucrose, with 0.025 M KCl, 0.035 M $KHCO_3$, 0.02 M potassium phosphate buffer, pH 7.8 and 0.01 M nicotinamide. Nuclei and cell debris were removed by centrifugation at 800 × *g* for 6 minutes and the resulting supernate was then recentrifuged for 15 minutes at 10,000 × *g*, in a refrigerated Lourdes centrifuge. 0.5 ml of the 10,000 × *g* supernate were incubated in 10 ml flasks with 10 μM of FDP, 1.0 μM ATP, 1.0 μM $MgCl_2$ and 0.05 μM L-leucine-C^{14} in a final volume of 1 ml. The incubation was carried out for 40 minutes at 37°C in a metabolic shaking incubator with 95% O_2 and 5% CO_2 as gas phase. The reaction was terminated by TCA precipitation and the proteins were isolated, using the procedure of Siekevitz *et al.*(6). The dried protein powder was weighed and redissolved in a 1M solution of hyamine in methanol. The samples were counted in a liquid scintillation spectrometer using a 10-100 v window at 970 volts, the scintillating fluid being 0.1% POPOP and 3% PPO in toluene. Correction for quenching was effected by the use of an internal standard. Protein determinations were performed according to the method of Lowry(7).

Results. In Table I are summarized the results of studies on the effect of thyroid hormone on protein synthesis by cell-free preparations of rat liver homogenates. It is evident that both PTU treatment (group C) and even more, thyroidectomy plus iodine deficiency (group E) caused a significant depression in the incorporation of labelled

amino acids into protein. This effect can be reversed in both cases by triiodothyronine administration (groups D and F). Neither the iodine-deficient diet nor PTU treatment *per se* appeared to affect the synthetic process, since supplementation with iodide (group B) and thyroid hormone (group D) respectively resulted in preparations in which the leucine incorporation rate did not differ significantly from that of the normally fed controls. It can also be seen that administration of growth hormone (group G) to thyroidectomized animals failed to improve significantly the reduced protein synthetic activity of the cell-free system. When microsomes were separated after incubation it became evident that the specific activity of the microsomal protein accounted for 80% of total incorporated radioactivity in both the normal and thyroidectomized groups.

Preliminary experiments have indicated that the impairment in protein synthesis in thyroidectomized animals is due to a defect of both microsomal and supernatant fraction.

Discussion. The dependence of protein synthesis on normal thyroid function in the whole animal has been discussed in publications dealing with hormonal regulation of growth(8,9,10). In experiments *in vitro* it has been shown that the decrease in incorporation of labelled alanine into proteins in liver slices of thyroidectomized animals could be corrected by pretreating the animal with thyroxine(11). In the present study amino acid incorporation was studied in a cell-free system consisting of microsomes and soluble proteins of liver and again the impairment in protein synthesis was observed in preparations from thyroidectomized animals. Pretreatment of the animals with triiodothyronine was followed by a full recovery of the system; however, addition of triiodothyronine *in vitro* had no effect. The present findings differ somewhat from those of Sokoloff *et al.*, who found that addition of thyroxine to a cell-free system of liver homogenate of normal rats increases the rate of amino acid incorporation into protein, similar to *in vivo* pretreatment with either thyroxine or triiodothyronine(12). The identity of the *in vivo* and *in vitro* effects of thyroxine seems questionable in view of the fact that D-thyroxine was found to be as active *in vitro* as L-thyroxine, but was inactive when administered *in vivo* (13). Sokoloff *et al.*, have indicated also that the stimulation of amino acid incorporation by thyroxine is limited to the mitochondrial fraction (a fraction sedimenting at 15,000 $\times$ *g* for 20 min)(12). The discrepancy between their results and our findings of changes in the rate of protein synthesis in the 10,000 $\times$ *g* supernatant of thyroidectomized animals could be due to differences in experimental conditions. Experiments are in progress to localize further the protein synthetic defect found in the cell-free system of thyroidectomized animals.

The failure of growth hormone to effect a restitution of protein synthetic activity of the microsomal system of thyroidectomized animals might be partly due to the lesser responsiveness of such animals, than of hypophysectomized animals to growth hormone (1). However, even in hypophysectomized animals in which a fall in protein synthetic activity of microsomes *in vitro* was described by Korner(14,15) administration of growth hormone restored only partly this metabolic defect. These findings would indicate that the impairment of protein synthesis in the microsomal system of thyroidectomized animals cannot be solely due to lack of growth hormone, known to occur in thyroidectomized animals.

Summary. The microsomal system derived from the livers of thyroidectomized rats shows a markedly reduced ability to incorporate L-leucine-C^{14} into protein. This effect can be corrected by pretreatment of the thyroidectomized animal with triiodothyronine, but not with growth hormone.

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An Azasterol that Inhibits Cholesterol Synthesis *in vitro*. (27346)

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The site of physiological regulation of cholesterol synthesis has been suggested by Siperstein and Guest(1,2) to be the reductive transformation of hydroxy-methylglutarate (HMG) to mevalonate. These investigators demonstrated that liver slices from rats previously fed cholesterol incorporated C^{14}-labeled acetate into cholesterol at significantly reduced rates. When, however, mevalonate-2-C^{14} was the labeled substrate, utilization of this material for cholesterol synthesis proceeded at near normal rates. Furthermore, they observed that conversion of labeled acetate to fatty acids and to keto-acids was essentially unimpaired by cholesterol feeding. They reasoned, therefore, that the homeostatic mechanism regulating cholesterol synthesis in the liver was a negative feedback system in which the sensitive element, HMG-CoA reductase, was inhibited by greater than normal amounts of cholesterol in the liver-plasma cholesterol pool.

The present report deals with one of a series of azasterols structurally related to cholesterol that seemingly inhibits cholesterol synthesis in a manner similar to that reported for dietary cholesterol. This compound, synthesized by one of us (R.E.C.), is 20α-(2-dimethylaminoethyl) amino-5α-pregnan-3β-ol dihydrochloride, or SC-11952.*

Methods. The homogenate system of Bucher(3) was used in all studies. The livers of female rats (100-120 g) were briefly homogenized in a "loose" glass homogenizer with 2.5 volumes of ice cold phosphate buffer at pH 7.2 and centrifuged at 500 $\times$ *g* for 3 minutes. The supernatant was incubated in the presence of co-factors with or without SC-11952. Animals were either pre-treated with SC-11952 or the compound was added as a suspension to homogenates prepared from untreated animals. After a 5-minute equilibration period, labeled substrate was added, and the incubation was continued in an oxygen atmosphere. When the same homogenate was used for all studies the per cent incorporation of substrate C^{14} was determined. In other studies where different homogenates were compared the results were calculated in terms of the dry weight of the preparations. Further details are included in the data presentation of each experiment. After incubation an aliquot of the incubated mixture was saponified in 10% KOH in 50% ethanol. The non-saponifiable fraction was extracted with n-hexane and an aliquot of this solution was placed on a silicic acid column (10 mm $\times$ 120 mm) that had been prepared according to Hirsh and Ahrens(4). Gradient elution of the non-saponifiable lipids was accomplished using n-hexane in a mixing flask and 15% ethyl ether in n-hexane in a reservoir. A pressure of 60 mm Hg of N_2 was impressed on the reservoir contents. Fractions (10 ml) were collected in a GME Model T-15 fractionator† from 7 columns simultaneously

* SC-11952 has also been termed 22,25-diazacholestanol.

† This fraction collector was purchased from Gilson Medical Electronics Co., Middleton, Wisc.