The oxidation of isovaleryl CoA to the α,β-unsaturated thiol ester (Reaction 1) is postulated by analogy to the corresponding reaction of the CoA derivatives of straight chain fatty acids.[8,9] The conversion of senecioyl CoA to HIV CoA in heart extracts, due to the presence of crotonase,[10,11] and the fixation of radioactive carbon dioxide into the carboxyl carbon of acetoacetate in the presence of either of these synthetically prepared substrates have been described.[12]

It has recently been found that brief heating at 60° at *p*H 7.4 destroys the crotonase present in a dialyzed potassium chloride–ethanol extract of fresh pig heart tissue. With the use of this heated enzyme preparation HIV CoA has been established as the substrate for acetoacetate synthesis and the reaction has been shown to require the presence of ATP as well as bicarbonate (Table I).

TABLE I

ENZYMATIC SYNTHESIS OF ACETOACETATE

The test system contained 500 μM. Tris buffer, *p*H 8.1, 20 μM. $MgCl_2$, 26 μM. cysteine, 10 μM. ATP, 200 μM. $KHCO_3$, 2 μM. CoA ester (prepared by the general method of Wieland and Rueff[13]), pig heart enzyme fraction free of crotonase (6 mg. protein in Expts. 1–3, 32 mg. in Expts. 4–6), and, where indicated, 0.01 mg. crystalline liver crotonase[14]; volume 4.2 ml.; incubation, 60 minutes at 38°.

Expt.	System	Substrate	μM. acetoacetate formed[15]
1	Complete	HIV CoA	0.26
2	Complete	Senecioyl CoA	0.02
3	Complete + crotonase	Senecioyl CoA	0.20
4	Complete	HIV CoA	0.23
5	ATP omitted	HIV CoA	0
6	$KHCO_3$ omitted	HIV CoA	0.05

TABLE II

ENZYMATIC CLEAVAGE OF β-HYDROXY-β-METHYLGLUTARYL COENZYME A

3 μM. HMG CoA[16] was incubated 60 minutes at 38° with 200 μM. Tris buffer, *p*H 8.1, 13 μM. cysteine, 20 μM. $MgCl_2$, and 26 mg. heart enzyme protein, final volume 3.0 ml. After deproteinization with trichloroacetic acid the *p*H was adjusted to 7.4 and aliquots were taken for independent determination of acetoacetate[15] and acetyl CoA (by citrate formation[17] in the presence of oxalacetate and crystalline citrate condensing enzyme[14]).

Total μM. acetoacetate	1.13
Total μM. acetyl CoA	0.85[a]
Ratio, acetyl CoA/acetoacetate	0.75

[a] The lability of acetyl CoA at *p*H 8.1 during the initial incubation at 38° accounts for a value less than that obtained for acetoacetate.

The intermediate product predicted as a result of carbon dioxide fixation (Reaction 3), HMG CoA, has been prepared and has been shown to furnish acetoacetate and acetyl CoA in almost equimolar amounts upon incubation with the enzyme system (Table II). As anticipated, acetyl CoA was found to yield no acetoacetate under these conditions.[18,19] The cleavage (Reaction 4) requires the presence of cysteine (or glutathione), but, unlike the carboxylation reaction, is not dependent upon the addition of ATP and bicarbonate.

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5-HEPTYL-2-THIOHYDANTOIN, A NEW ANTITUBERCULAR AGENT

Sir:

In the course of screening compounds for antitubercular activity it was found that 5-hexyl-2-thiohydantoin, prepared some years ago in these laboratories[1] and tested as an anti-thyroid agent, exhibited a significant therapeutic effect when tested in mice infected with *M. tuberculosis* H37Rv. This result stimulated an extensive program designed to exploit this lead.

At the outset it was found that small structure changes profoundly affected the antitubercular activity. Maximum activity in the 5-alkyl-2-thiohydantoin series was attained with 5-*n*-heptyl-2-thiohydantoin (m.p. 132.8–134.0°. *Anal.* Calcd. N, 13.07; S, 14.96. Found: N, 12.93; S, 14.89). The effectiveness fell precipitously as the 5-alkyl chain was lengthened; the *n*-octyl homolog (m.p. 132.8–134.0°. *Anal.* Calcd.: N, 12.27; S, 14.31. Found: N, 11.90; S, 14.33) was only slightly suppressive and the *n*-decyl-2-thiohydantoin (m.p. 133.8–134.8°. *Anal.* Calcd.: N, 10.93; S, 12.50. Found: N, 10.62; S, 12.45) was an inactive drug. Many isomers of 5-*n*-heptyl-2-thiohydantoin were without effect. The 5-(2-methylhexyl)-, (m.p. 190.2–190.4°. *Anal.* Found: N, 13.22; S, 14.96), 5-(3-methylhexyl)-, (m.p. 124.5–125.4°. *Anal.* Found: N, 13.05; S, 15.02) and 5-(4-methylhexyl)-2-thiohydantoin (m.p. 143–144°. *Anal.* Found: N, 13.20; S, 14.94), were inactive for all practical purposes. Only 5-(5-methylhexyl)-2-thiohydantoin (m.p. 152.1–153.3°. *Anal.* Found: N, 13.14; S, 15.02) showed moderate suppressive activity. The closely related 5-(2-nonenyl)- (m.p. 123.1–125.3°, *Anal.* Calcd.: N, 11.66; S, 13.34 Found: N, 11.78; S, 13.62) and 5-(2-hexenyl)-2-thiohydantoin (m.p. 130.3–133.9°. *Anal.* Calcd.: S, 16.17. Found: S, 15.97) were without appreciable antitubercular activity.

A similar state of affairs was encountered when the thiohydantoin moiety was varied. For example, both 5-heptylhydantoin[2] and 5-hexylhydantoin[2] were inactive. This was also true for 5-*n*-hexyl-2,4-

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dithiohydantoin[3] and 5-hexyl-5-methyl-2,4-dithiohydantoin (m.p. 98.5–99.6°. *Anal.* Calcd.: N, 12.16; S, 27.83. Found: N, 12.20; S, 27.83.)

Doses of the order of 200–300 mg./kg./day of 5-*n*-heptyl-2-thiohydantoin were required to protect all mice infected intravenously with *M. tuberculosis* H37Rv from the lethal effects of the disease. This was true also when a strain highly resistant to streptomycin was used to infect the animals. In both cases postmortem examination of the surviving mice revealed little if any tuberculous pathology. When this drug was fed to hamsters infected with the H37Rv strain at a concentration of 0.1% in the diet a therapeutic effect equivalent to that obtained with a fourfold concentration of *p*-aminosalicylic acid was achieved. In this species, too, the tuberculous pathology in the survivors was quite small. Extensive acute and chronic toxicity studies in rodents, dogs and monkeys showed that the drug is well tolerated by these species. In view of these results it is felt that 5-*n*-heptyl-2-thiohydantoin is worthy of clinical trial as an antitubercular drug.

It seems more than a coincidence that the grouping, —NH—C(=S)—NH— or a tautomeric form thereof occurs so frequently in *in vivo* tuberculostatically active drugs. The thiosemicarbazones, the thioureas reported by Huebner,[4] the mercaptotriazinones of Hagenbach[5] and now the thiohydantoins all have in common the thioureido function.

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DIRECT PRODUCTION OF RADIOACTIVE ALIPHATIC HYDROCARBONS BY PILE IRRADIATION[1]

Sir:

Study of the hot atom chemistry of carbon-14 by the irradiation of nitrogenous organic materials in the heavy water pile at the Argonne National Laboratory (CP-3′) has led us to observe a method of producing saturated aliphatic hydrocarbons in radioactive form in high yield and high specific activity.

A 5 mole per cent. solution of aniline in normal pentane, 20 cc. of which was enclosed in a quartz tube and irradiated for one week in the CP-3′ pile at a flux of 10^{11} neutrons per cm.^{2} per second, proved to yield about 25% of the radiocarbon in the form of radioactive normal pentane, with less than 1% as iso- or neo-pentane; about 15% in the form of radioactive hexane, which apparently is about two-thirds normal hexane; and the remainder in heavier hydrocarbons. The distribution is given in Table I.

(1) This research was supported by the United States Air Force through the Office of Scientific Research of the Air Research and Development Command.

TABLE I

COMPOSITION OF THE RADIOACTIVE HYDROCARBONS FORMED BY THE IRRADIATION OF A 5 MOLE PER CENT. SOLUTION OF ANILINE IN NORMAL PENTANE

5% of total C^{14} was extractable into 12 *N* HCl

Chemical form	Per cent. of the total radiocarbon
Gases (Boiling up to room temperature)	12
n-Pentane	25
i-Pentane	1
n-Hexane	12
Other hexanes	6
Heptane and heavier hydrocarbons, boiling according to following ranges, °C.	
95–125[8]	8
125–155[8]	5
155–175[8]	6
175–215[8]	5
215–245[8]	3
245–290[8]	3
Residue	9

It is clear from these preliminary results, which were duplicated by a second run in which ethylamine was substituted for aniline, that a high velocity carbon-14 on colliding with the liquid aliphatic hydrocarbon has a very good chance of entering the chain. The reasons for this may be debatable, but the facts seem to be clear. It should be realized that the hexane and heavier hydrocarbons produced in the above-described bombardment were essentially carrier free except for any which may have been produced by gamma and fast neutron radiation. It would seem therefore that the process described can produce radioactive hydrocarbons of high specific activity. It is further clear that as far as neutron economy is concerned, this process could well compete with any organic synthesis, for the sole labor involved is the purification of the original chemical, the preparation of the samples for irradiation, and the subsequent distillation and separation. There is reason to believe that the procedure outlined would also serve to introduce radiocarbon into heavy lubricating oils.

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ENZYMATIC REDUCTION OF CORTISONE[1]

Sir:

Previous *in vivo* and *in vitro* studies reveal that the major pathway of cortisone metabolism is the reduction of the Δ4-3 ketone group to the saturated 3-alcohol by the liver.[2,3,4] We should like to report the presence of an enzyme system in rat liver

(1) Abbreviations as used in this communication are: cortisone (Δ4-pregnene-17α,21-diol-3,11,20-trione), dihydrocortisone (pregnane-17α,21-diol-3,11,20-trione), tetrahydrocortisone (pregnane-3α,17α,21-triol-11,20-dione), TPNH and TPN (reduced and oxidized triphosphopyridine nucleotide, respectively), DPNH and DPN (reduced and oxidized diphosphopyridine nucleotide, respectively).
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