fect by providing proteins to aid humoral transport of thyroid hormones(14) or perhaps to aid in reabsorption of thyroid hormones from the gut in the enterohepatic cycle(15). These possibilities raise a question as to whether the soybean goitrogenic effect may be related to, or even identical with, the soybean antitrypsin effect.

Summary. The goitrogenic effect of raw soybeans was seen in significant increases of both fresh and dry weights of thyroid glands when a diet containing 60% of beans was fed to rats, and a significant increase in the dry weight only of the gland when a diet containing 35% of raw soybeans was fed. The goitrogenic effect of 35% or 60% of raw soybeans in rat diets was obliterated by adding casein to the diets. This result was not attributable to iodine in the casein.

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O-Aminophenol: A Urinary Product of Tryptophan Metabolism in the Human.* (27536)

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Recently, interest in the relationship of diseases to the metabolites of tryptophan has intensified(1). 3-Hydroxy-anthranilic acid and 3-hydroxykynurenine, known tryptophan metabolites, have demonstrated carcinogenic behavior in mice(2) and appear in elevated quantities in the urine of patients with bladder tumors, anemias and leukemias(1). More recently, *o*-aminophenol has been identified in the urine of humans(3), and appears in elevated quantities in the urine of bladder tumor patients(3). However, whether tryptophan was the precursor of this urinary aminophenol is not known. We present here evidence of the conversion of tryptophan to *o*-aminophenol (Fig. 1) in the human.

Experimental. A female patient with multiple-myeloma was given orally in a gelatin capsule 39.376 mg of DL-Tryptophan-7a-C^{14}† containing 51.3817 μc of C^{14}. The patient's respiratory $C^{14}O_2$ was collected for 12 hours. Urine was collected for a 24-hour period under toluene in a refrigerated dark bottle. Aliquots of urine were analyzed for C^{14} content (4), and 100 ml of urine was used for isolation of the *o*-aminophenol.

A column of Amberlite IR-45-ag‡ resin 22 mm × 34 cm was used for the isolation. The

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† This DL-Tryptophan-7a-C^{14} was synthesized in the laboratory of Dr. L. M. Henderson, Oklahoma State Univ., Stillwater.

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TABLE I. Rate of Expiration of $C^{14}O_2$ from DL-Tryptophan-7a-C^{14}.

	Time from administration, min.				
	0–45	45–90	90–180	180–360	360–720
% expired per min.	.0025	.0092	.119	.0671	.0596
% expired, cumulative	.114	.526	1.600	2.808	4.953

resin was washed in the column with three 100-ml aliquots of 4N HCl, three 100-ml aliquots of saturated NaOH, and with distilled water until the rinse water gave no color with phenolphthalein. Then, 104.3 mg of carrier *o*-aminophenol was dissolved in the urine sample, and the solution passed through the column at the rate of 10 ml per minute. The column was washed with 500 ml of distilled water, and eluted with 0.1N acetic acid. The *o*-aminophenol comes off in the pH range of 5.9-2.6. Only the fractions containing *o*-aminophenol in the pH range 5.2-3.0 were pooled in a round bottom flask and concentrated to dryness on a flash evaporator. The residue was transferred to a 12 ml centrifuge tube with ethanol and then taken to dryness.

The *o*-aminophenol was converted into the more stable derivative, *o*-hydroxyacetanilide (5). The material in the centrifuge tube was dissolved in 0.88 ml N NaOH and after cooling in a bath, 1 ml of acetic anhydride was added with rapid mixing or agitation. Then, 0.75 ml of HCl was added to the reaction mixture and the tube placed in an ice box overnight. The *o*-hydroxyacetanilide, which precipitated during the night, was filtered on a sintered glass funnel and washed with cold water. After treatment with charcoal in hot ethanol, the *o*-hydroxyacetanilide was recrystallized from ethanol to constant specific activity, M.P. 208.5-209(6). No depression of the melting point resulted when a sample was mixed with authentic *o*-hydroxyacetanilide. The carbon analysis of 63.55% checked well with the theoretical carbon content of 63.6%. An ultraviolet absorption spectrum of the isolated *o*-hydroxyacetanilide and authentic *o*-hydroxyacetanilide showed identical peaks at 283 mμ and 242 mμ.

Results and discussion. When DL-Tryptophan-7a-C^{14} was administered to a human subject with multiple myeloma, *o*-aminophenol-2-C^{14} was excreted in the urine. Analyses of the expired CO_2 (Table I) showed that DL-Tryptophan was not metabolized as rapidly in the human as was prveiously shown in the rat(7). After a time lag to allow for absorption, the specific activity of the expired $C^{14}O_2$ increased rapidly and reached a maximum in 150 minutes. Approximately *4.8%* of the activity was expired in 12 hours while in the rat *37%* was expired in the same period(7). This wide difference in percent conversion to CO_2 may be due to the fact that the dose was in the physiological range. Studies with DL-Tryptophan-2-C^{14} in humans have shown that raising the dosages of tryptophan from 40 mg to 2 g will raise the $C^{14}O_2$ excreted in 24 hours from approximately 5% to 23%(8). Similar effects of the dosage of DL-Tryptophan-7a-C^{14} in rats have been obtained by Henderson(9) who observed that $C^{14}O_2$ levels in the expired air of rats decreased from 30% to less than 1% as the level of tryptophan given was decreased from a high of 100 mg% of the diet.

The *o*-aminophenol-2-C^{14} isolated from the urine (Table II) contained less than 1% of both the administered dose and total activity of the urine. The mechanism of formation of *o*-aminophenol may be direct decarboxylation

TABLE II. *O*-Aminophenol-2-C^{14}: Isolation from 24-Hour Urine.

Tryptophan-7a-C^{14} admin., mg		39.376
C^{14} admin., μc	a	51.38
o-aminophenol		
o-aminophenol added, mg/24 hr urine	b	1564.5
Carbon-14 excreted in urine, μc	c	7.38
Carbon-14 excreted in urine, % (c/a)		14.36
o-hydroxyacetanilide added, mg (1.385 × b)	d	2166.83
Sp. act. isolated *o*-hydroxyacetanilide, dpm/mg	e	17
o-aminophenol activity, dpm (d × e)		36836
o-aminophenol activity, μc	f	.017
Carbon-14 excreted in *o*-aminophenol		
% administered dose (f/a)		.03
% urinary carbon-14 (f/c)		.23

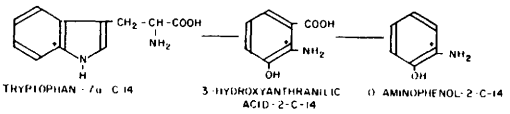

FIG. 1

of 3-hydroxyanthranilic acid, formed in the metabolism of tryptophan (Fig. 1).

Additional evidence for this pathway has been obtained in this laboratory in studies of the metabolism of some impure 3-hydroxyanthranilic acid-1-C^{14} in mammary tumor mice. The urines of such mice receiving impure 3-hydroxyanthranilic acid-1-C^{14} has been shown to contain approximately 0.15% of the administered dose in the form of *o*-aminophenol.

Summary. The metabolic fate of DL-Tryptophan-7a-C^{14} in a human with multiple myeloma was studied. Only 5% of the administered C^{14} was excreted as respired $C^{14}O_2$ and 14% in the urine in the first 24 hours. *O*-aminophenol-2-C^{14} was isolated from the urine.

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Response to Erythropoietin.* (27537)

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Alpen and Cranmore(1) and Erslev(2) have concluded that erythropoietin increases red cell output by accelerating the rate of stem cell differentiation into erythroid precursors. Observations on erythropoiesis in polycythemic mice by Gurney, *et al.*(3) have supported this conclusion. However, observations by several investigators(4-7) seem to indicate that the action of the erythropoietic humoral factor(s) is not limited to regulation of stem cell differentiation. Experiments reported here tested the effect of erythropoietin on later phases of erythroid formation and the results suggest an action here as well as in the early stem cell phase.

Methods. Female Sprague-Dawley rats, weighing 180 to 200 g, were made polycythemic by one or several transfusions of packed homologous red blood cells. Each transfusion equaled 50% of the animal's original red cell mass. At various intervals following the initial transfusion the polycythemic rats were given a single injection of human urine erythropoietin(8) or purified sheep erythropoietin (9).‡ Subsequent changes in erythropoiesis were assessed by reticulocyte counts(10) and measurement of the 18-hour erythrocyte radio-iron incorporation(8). Erythroid elements per 1000 nucleated cells were enumerated in smears of marrow from control polycythemic animals.

In 3 experiments, polycythemic rats were given single intraperitoneal injections of 2 ml of urine concentrate on either the 5th, 15th or 31st day after their first transfusion. Con-

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