Excretion of Radioactivity by Human Subjects after Ingestion of Liver from Cattle Treated with Labeled Polydiethylstilbestrol Phosphate. (30359)

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Polydiethylstilbestrol phosphate (PSP) is an effective growth-promoting agent in beef cattle(1). However, following injection of labeled PSP into rats and a cow, considerable residual radioactivity was found at the site of injection and in the liver(2). Although PSP is orally inactive in rodents(3), it was necessary to determine if residues of PSP which might be present in tissues of treated animals could constitute a hazard to human beings ingesting the meat. Hence, the two experiments reported here relate specifically to the question of intestinal absorption by human beings of radioactivity in bovine liver from animals injected with labeled PSP.

Experiment I. Procedure. A Holstein cow was injected with an aqueous solution of 212 mg H^3-PSP (11.0×10^9 cpm) so labeled(4) and purified(2) to give a specific activity of 40 μc/mg as determined in a windowless gas-flow counter. Fifteen days later the animal was sacrificed, and 100 g of the radioactive liver was fried and eaten in a meal by a human subject (TP); his urine and feces were collected for counting. The different materials were analyzed as follows. For liver, 10 g samples were hydrolyzed in hot 0.2 M NaOH. For feces, 5 g samples were suspended in water. Urine was diluted 1:1 with water. Each preparation was then extracted with butanol, samples of which were counted in duplicate.

Results. The cow liver contained 1.4×10^9 cpm of which 2.9×10^7 cpm was ingested. Analysis of 18 urine collections revealed no radioactivity; the method was sensitive to 3000 cpm of either H^3-diethylstilbestrol or H^3-PSP added to 50 ml control urine. All counts were corrected for background, self-absorption, and extraction losses. Thus, each 50 ml sample of experimental urine contained no more than 0.01% of the ingested radioactivity. The data for feces are presented in Table I.

TABLE I. Recovery of Radioactivity from Feces of a Human Being Collected After Ingestion of Cow Liver Containing 2.9×10^7 cpm of Tritium-Labeled Residues of Polydiethylstilbestrol Phosphate.

Fecal sample No.	Wt, g	Hr after ingestion	Recovered radioactivity, cpm $\times 10^3$	% of ingested radioactivity
TP-1	39	26	479	1.7
TP-2	20	41	366	1.3
TP-3	42	50	1,892	6.5
TP-4	230	89	20,900	72.0
TP-5	20	99	686	2.4
TP-6	91	113	779	2.7
TP-7	196	137	0	
Total	638		25,102	86.6

Experiment II. Procedure. A Guernsey steer (190 kg) was injected i.v. with 490 ml isotonic saline containing 39.3 mg C^{14}-PSP synthesized†(3) from diethyl-(ethyl-1-C^{14})-stilbestrol.‡ The radioactivity (479 μc) equalled 5.16×10^8 cpm as counted in a scintillation spectrophotometer. Periodic analyses for C^{14} in the liver were made on samples obtained by biopsy(5). Six days after injection the steer was slaughtered and the liver removed. Slices were fried, ground, and mixed. For counting, 3 samples of 2.0 g each were hydrolyzed in 50 ml 0.2 M NaOH at 120°C for 3 hours. Of the cooked ground liver 100 g was provided with breakfast to each of 3 subjects; 3½ hours later an additional 65 g was eaten. The subjects entered a metabolic ward for 4 days. Urine and feces were collected. Two days before the experiment and 2 and 6 hours after ingestion of the second meal, blood samples were drawn, heparinized, and centrifuged; the plasma was analyzed for C^{14}. Samples of well-mixed feces

* Deceased, April 21, 1963.

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‡ Radiochemical Center, Amersham, England.

TABLE II. Analyses of Urine Samples Collected from Each of 3 Human Beings Before and After Ingestion of Steer Liver Containing 1.25×10^7 cpm C^{14}-Labeled Residues of Polydiethylstilbestrol Phosphate.

Experimental subject	Urine	No. of collections*	Avg cpm, mean ± S.D.
WS	Control	5	103 ± 3
	Experimental	13	104 ± 5†
RM	Control	6	105 ± 5
	Experimental	13	102 ± 5†
DP	Control	5	97 ± 4
	Experimental	13	103 ± 4†

* Volumes varied between 100 and 500 ml. Each collection was analyzed in duplicate.

† The difference in average counts between experimental and control urines is not significant at the 95% level.

collections were hydrolyzed as was the liver tissue. Samples (0.50 and 1.0 ml) of each fluid (urine, plasma, hydrolysates of liver and feces) were counted directly by adsorption on Cab-O-Sil(6); the scintillation fluid was 0.3% diphenyloxazole in toluene. Alkaline digests of fecal samples (5.0 g each) were so dark that to each adsorbed aliquot was added 0.5 ml 2% H_2O_2; counting efficiencies were improved from 15 to 65%. Corrections for quenching were made for all samples, and background curves were constructed for each fluid or extract. Since high accuracy was desired in counting urines, several control samples were collected; samples of all urines were counted in duplicate for long periods in order to determine mean values and standard deviations.

When C^{14}-PSP was added to human urine (2500 cpm per 100 ml), recoveries of 97%, 99%, and 80% were obtained. Recovery of C^{14} mixed with 5 g lots of feces (81,000 cpm and 16,000 cpm, resp.) amounted to 99% in each case. When C^{14}-PSP (48,000 cpm) was added to 10 ml whole blood, a recovery of 59% was obtained.

Results. Biopsies taken from the liver of the steer 24, 70, and 120 hours after injection of C^{14}-PSP contained 20,000 cpm, 50,000 cpm, and 43,000 cpm per g wet tissue, respectively. The cooked, ground liver contained 75,500 cpm per g. Since each subject received 165 g, he ingested 1.25×10^7 cpm. The results of the analyses of control and experimental urines are summarized in Table II. The difference in average counts registered for each set of control and experimental urines is not significant at the 95% level. Since recovery experiments had shown that a sample of urine containing 16 cpm of C^{14}-PSP per ml could be accurately determined, it seems reasonable to state that no radioactivity was excreted in the urine of the experimental subjects. Furthermore, no significant difference in radioactivity was found between the experimental plasma samples and the samples drawn 2 days prior to ingestion. Six fecal samples were collected from each subject. As shown in Table III, in each case more than 92% of the ingested radioactivity was excreted *via* the fecal route within 5 days.

Discussion. It is difficult to prove conclusively that a compound administered orally is not absorbed during passage through the digestive tract. If the methods are sensitive enough, it seems probable that minute amounts of almost any compound will be found to pass the gastrointestinal wall. In the present investigation, the administration of labeled material of high specific activity made

TABLE III. Recovery of Radioactivity from Feces Collected from Each of 3 Human Beings After Ingestion of Steer Liver Containing 1.25×10^7 cpm C^{14}-Labeled Residues of Polydiethylstilbestrol Phosphate.

Fecal sample No.	Wt, g	Hr after 2nd meal	Recovered radioactivity, cpm × 10^3	% of ingested radioactivity
WS-1	153	21	7,245	60.4
WS-2	177	45	4,544	37.9
WS-3	20	69	220	1.8
WS-4	71	93	79	.7
WS-5	110	117	0	0
WS-6	118	141	0	0
Total	649		12,088	100.8
RM-1	26	21	278	2.3
RM-2	58	45	2,840	23.7
RM-3	170	68	6,408	53.4
RM-4	26	93	528	4.4
RM-5	175	117	1,239	10.3
RM-6	112	141	26	.2
Total	567		11,319	94.3
DP-1	44	20	2,470	20.6
DP-2	29	45	2,085	17.4
DP-3	122	68	4,732	39.4
DP-4	126	93	1,661	13.8
DP-5	133	104	190	1.6
DP-6	105	141	0	0
Total	559		11,138	92.8

it feasible to trace very small fractions of ingested PSP. In the first experiment the lack of radioactivity in urine was the only evidence for non-absorption. However, it was considered that the absence of radioactive material in urine would not prove that ingested radioactivity had not been absorbed since absorbed material might be accumulated in the body or be metabolized and excreted *via* the biliary-fecal route. Therefore, analysis of blood samples drawn at 2 different times after ingestion of the radioactive meal was included in the second experiment. It seems very probable that lack of radioactivity in both blood and urine indicates little or no absorption of the labeled material. Quantification of radioactivity in feces was complicated by the difficulty in obtaining homogeneous samples and by the introduction of color into the counting fluid. In the first experiment about 87% of ingested radioactivity was accounted for in the feces of one human subject. In the second experiment the use of C^{14}-labeled PSP and liquid scintillation counting allowed the analyses of radioactivity in feces to be more accurate. The recovery in the feces of 3 subjects of 93-101% of ingested radioactivity lends strong support to the conclusion that little radioactivity had been absorbed. An enterohepatic circulation of labeled material may have occurred, but the lack of detectable radioactivity in either blood or urine argues against this hypothesis.

Summary. Polydiethylstilbestrol phosphate (PSP), an effective growth promoting agent in beef cattle, tends to accumulate in the animal's liver as indicated by studies in which radioactive PSP was used. Upon ingestion of such liver (cooked) by human beings, 93-101% of the radioactivity was found in the feces. No radioactivity could be detected in either blood or urine. It is concluded that neither PSP nor its metabolites ingested under such circumstances are absorbed in detectable amounts during passage through the human intestinal tract.

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Lysine Inhibition of Cell Protein and Viral Synthesis. Reversal by Other Amino Acids.* (30360)

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In virus-infected cells maintained in a minimal medium, addition of an excess of amino acids such as lysine, tryptophane or phenylalanine causes viral inhibition which is reversible by addition of certain other amino acids (1,2). This effect of lysine on replication of influenza virus hemagglutinins was of special interest because a wide variety of amino acids also reversed the moderate inhibition of cell protein synthesis. Krebs 2 ascites tumor cells infected with a high multiplicity of influenza strain PR_8 which give good yields of cell-associated viral hemagglutinins and CF antigen but no additional infectious virus(3) have several advantages as an experimental system. One-step replication and accurate measurement of viral structural protein as the titer of unassembled intracellular hemagglutinins facilitates interpretation of results. In addition ascites tumor cells have been found

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