trates. The present study extends these experiments by demonstrating the presence of a heat-stable agglutinin in extracts of normal, as well as neoplastic mouse tissues. The identity of the heat-stable agglutinin has not been ascertained, nor has it been possible to determine whether the activity of these preparations is due to more than one agglutinating agent. However, the data support the concept of a hemagglutinin which is not a tumor virus. The heat-stable agglutinin differs from the polyoma virus (*cf.*(6)) in 3 ways: (a) only a slight elution (approximately 15%) of the heat-stable agglutinin occurs at 37°C; (b) mouse red cells give higher agglutination titers than guinea pig erythrocytes; and (c) agglutination takes place both in the cold (4°C) and at room temperature (23°C).

Fundamental differences exist between the heat-stable agglutinin and the hemagglutinating agents reported by Salaman(4). Significant differences are not apparent, however, when a comparison is made between the agglutinating action of passage A leukemic filtrates(5) and our mouse tissue extracts, whether of normal or cancerous tissues. Gross(5) poses the question as to "whether the agglutinating potency of leukemic extracts is actually related to presence of the leukemic agent." Comparison of our results obtained with filtered and nonfiltered extracts of mouse tissues shows that filtration effects a marked reduction in agglutination titers. These findings are contrary to what one would expect if a viral agent were involved.

The agglutinating activity which we observed with extracts of beef and lamb tissues was not anticipated. The data indicate that agglutinin(s) in the beef and lamb preparations is neither a serum antibody, since high titers were obtained with Fraction 3, nor analagous to the heat-stable agglutinin derived from mouse tissues. In addition, these observations point out the need for caution before stating that the agglutinating action of a given tissue extract is due to a tumor virus.

Summary. Extracts of normal and neoplastic mouse tissues were found to agglutinate mouse and guinea pig red blood cells. Characteristics of the heat-stable substance(s) responsible for this phenomenon are discussed and it is proposed that the hemagglutinating agent(s) is not a tumor virus.

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Urobilinogen Excretion After Hemoglobin Infusions in Patients with Normal Hematologic and Hepatic Findings.* (26077)

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According to Watson(1), the normal daily excretion of urobilinogen in feces ranges from 40 to 280 mg, averaging 140 mg in a recent study. In the urine, daily normal excretion is from 0.5 to 1.5 mg. Theoretically, it is possible to calculate rate of hemoglobin destruction from daily excretion of urobilinogen on the assumption that 1 mg of urobilinogen is derived from 28.5 mg of destroyed he-

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moglobin. Thus, a daily total excretion of 140 mg of urobilinogen would represent the catabolism of 3.99 g of hemoglobin. However, this corresponds to less than ⅔ of the approximately 6.25 g of hemoglobin assumed to be destroyed daily. In view of this discrepancy, it was considered of interest to measure the increment in fecal and urinary urobilinogen following slow intravenous infusion of a known amount of hemoglobin over a period of a few hours.

Methods. Subjects. Six patients in their sixth to ninth decades but with no known hematologic disorder and hemoglobin levels of above 14 g% were selected for study. Cases 2, 4 and 5 were confined to bed, the others were ambulatory about the hospital ward. Their liver function tests such as bromsulfalein, cephalin flocculation, thymol turbidity and alkaline phosphatase were normal, as was their subsequently observed urinary excretion of urobilinogen during control periods.

Hemoglobin solution. Fifty to 100 ml of venous blood were drawn from the patient or from a normal individual with compatible blood and mixed with 500 ml of sterile distilled water. The hemolyzed blood was centrifuged and the supernatant introduced into 1000 ml of sterile 1.25 g% NaCl, making a final tonicity of approximately 0.83 g%. Amount of hemoglobin/100 ml of prepared solution was determined by using an Evelyn photoelectric colorimeter.

Hemoglobin infusion. The freshly prepared hemoglobin solution was given to the patient intravenously through an adjustable, constant rate infusion pump(2) at from 10 to 16 mg of hemoglobin/min in amounts ranging from 7.80 to 11.75 g in different patients. In 1 patient, who received the largest amount of hemoglobin, an infusion was given on each of 3 consecutive days; in 4 patients duration of infusion was from 4 to 5 hr on 2 consecutive days; in 1 patient, 9 hr in 1 day. Blood samples diluted 9:1 with 3% sodium citrate solution were collected just before and at the end of each infusion as well as 3 or more times before and at least 5 times after infusion at intervals of 1 to 2 days for 8 days or more. Determinations of plasma hemoglobin and bilirubin levels were made on these samples using the methods described by Ham(3) and by Ducci and Watson(4), respectively.

Stool collections. These were made over 4-day periods, 5 or more times before, and 4 or more times after beginning of infusions. Before the first 4-day collection of the control period and also on resumption of successive 4-day collection periods after any interruption of the series, 500 ml of saline or tap water were given to the patient as an enema. This was also done just before hemoglobin infusion. Fecal material so recovered was discarded. Otherwise, all stools were collected in 8 x 7 inch glass jars with covers and placed in an insulated metal container half-filled with ice and covered tightly. Determinations of urobilinogen were made by the method of Schwartz *et al.*(5) as soon as each 4-day collection was completed.

Urine samples. These were collected as 24-hr samples for at least 5 consecutive days before infusion. During day of infusion, all urine secreted was collected and analyzed as separate samples. Thereafter, 24-hr urine samples were collected again for at least 5 consecutive days. The accumulating samples of urine were stored in a refrigerator until the 24-hr collection was completed, when determinations of urobilinogen and hemoglobin were made immediately. Standard urinalyses were also performed.

Results. Table I presents the data on fecal urobilinogen excretion during 4-day collection periods before and after beginning of hemoglobin infusions in the 6 patients. Table II shows amounts of hemoglobin theoretically catabolized to produce the observed amounts of urobilinogen excreted both during 8 days of control periods and during the 8 days following start of hemoglobin infusions. The percentage efficiency of the conversions of catabolized hemoglobin to urobilinogen is also shown.

Since the fecal urobilinogen recovered after infusion increased most in the first 4-day period, to a lesser degree in the next 4-day period, and not perceptibly thereafter, average daily fecal urobilinogen before infusion of hemoglobin x 8 was subtracted from total

amount of fecal urobilinogen excreted during the first 8 days after infusion. The difference was taken as total increase in fecal urobilinogen excretion resulting from the hemoglobin infused. The total resulting increase in urinary urobilinogen excretion, which usually lasted from 4 to 8 days but was very small (2.4 to 11.4 mg), was not taken into account in the calculations.

None of the injected hemoglobin appeared

TABLE I. Fecal Urobilinogen Excretion (mg) during 4-Day Periods before and after Hemoglobin Infusions in 6 Patients.

Collection period	Case 1 ♂, 66 yr, cancer of prostate	Case 2 ♂, 79 yr, cancer of prostate	Case 3 ♀, 70 yr, cancer of breast	Case 4 ♂, 82 yr, multiple sclerosis	Case 5 ♂, 61 yr, chronic arthritis	Case 6 ♂, 55 yr, muscular atrophy
1	204	111	163	180	267	327
2	301	125	151	205	279	417
3	210	102	95	196	318	340
4	265	108	151	170	218	415
5	286	128	113	193	297	428
6	216	107		216		
7	245			168		
8	221					
9	295					
10	230					
11	290					
Avg/4-day period	252 ± 12	114 ± 11	135 ± 29	190 ± 17	276 ± 38	386 ± 55
Avg/day	63	28	34	47	69	96
			After hemoglobin infusions			
1	405	406	352	392	525	610
2	384	193	249	280	385	456
Total 8 days	789	599	601	672	910	1066
Avg/day	99	75	75	84	114	133
3	222	40	156	120	205	180
4	190	30	143	105	176	370
5		98	167	158	252	305
6		45		188	213	
7					202	
8					224	
Avg/4-day period	206 ± 22	53 ± 30	155 ± 12	143 ± 37	212 ± 25	285 ± 96
Avg/day	51	13	39	36	53	71

TABLE II. Increase in Fecal Urobilinogen Excretion Induced by Hemoglobin Infusions in 6 Patients.

	Avg urobilinogen excretion before infusion		Avg calculated hemoglobin converted to urobilinogen daily before infusion $\frac{A \times 28.5}{8 \times 10^3}$		Amt of hemoglobin infused	Urobilinogen excretion in first 8 days after infusion		Avg calculated infused hemoglobin converted to urobilinogen $\frac{(B-A)\,28.5}{10^3}$	
	1 day	A 8 days				Total B	Due to infusion B − A		
Case No.	mg	mg	g*	%†	g	mg	mg	g*	%
1	63	504	1.79	29	8.84	789	285	8.12	92
2	28	228	.80	13	11.75	599	371	10.57	90
3	34	270	.97	16	7.80	601	331	9.43	121
4	47	380	1.34	21	9.76	672	292	8.32	85
5	69	552	1.97	32	11.25	910	358	10.20	91
6	96	772	2.74	44	8.30	1066	294	8.38	101
Grand avg	56	451	1.60	26	9.62	773	322	9.17	96

* Assuming that 1 mg of urobilinogen is derived from 28.5 mg hemoglobin.
† Assuming that 6.25 g of hemoglobin are destroyed daily.

in the urine. However, transient albuminuria without other urinary abnormality was noted in 3 of the patients. During infusions, plasma hemoglobin reached peak values of from 25 to 45 mg% and afterwards returned rapidly to base line. Plasma bilirubin, initially less than 0.4 mg%, became elevated to from 0.6 to 1.2 mg% after the infusions and returned within 1 to 4 days to its original level. No fever, fall of blood pressure or other adverse symptom developed in any patient.

Discussion. Abnormally increased erythrocyte destruction is accompanied by increased urobilinogen excretion attributed to this cause. However, in hematologically normal patients, despite large variations in daily urobilinogen excretion, on the average far less urobilinogen has usually been recovered than was expected. Thus, whereas estimated normal daily destruction of 6.25 g of hemoglobin should theoretically yield 219 mg of urobilinogen, Gilbertsen, working in Watson's laboratory, recently found an average daily excretion of only 140 mg, *i.e.,* a conversion efficiency of less than ⅔(1). This discrepancy is even more impressive in view of the evidence from normal subjects fed N^{15}-labeled glycine indicating that from 15 to 20% of the resulting N^{15}-labeled fecal stercobilin is not derived from the breakdown of the labeled hemoglobin of circulating red cells(6).

The control urobilinogen excretion values obtained in the present study account on the average for only 26% with a range of from 13 to 44% of the hemoglobin presumed to be catabolized daily. They are thus unusually low, perhaps because of the sluggish bowel habits of these inactive patients. In retrospect, the occasional enemas preliminary to certain stool collection periods were an ill-advised procedure. However, they could not have greatly lowered average values for urobilinogen excretion calculated from collections during at least 8 consecutive days thereafter. Moreover, the enema preceding the first post-infusion period collection would also have lowered its urobilinogen content. Nevertheless, following the hemoglobin infusions, the increase in amount of urobilinogen excretion accounted for an average of 96%, with a range in individual patients of from 85 to 121% of hemoglobin intravenously administered.

This striking increase over the control percentages suggests either that hemoglobin infused into the blood stream was more efficiently converted to bilirubin than when normally derived from effete red cells or that in the intestinal tract a more or less constant amount of potential urobilinogen fails to be excreted as such, irrespective of increased amount of bilirubin presented for conversion. Normally, except for the possible diversion by the kidney of a small amount of hemoglobin to a dipyrrylmethene such as pentdyopent, the evidence favors a quantitative conversion of hemoglobin to bilirubin and its quantitative excretion as bilirubin glucuronide by the liver(1). On the other hand, it is probable that the conversion of bilirubin to urobilinogen measurable by Ehrlich's aldehyde reagent is far from perfect. Thus, although fecal dipyrrylmethenes such as mesobilifuscin are now thought to be largely of anabolic origin(7), oxidative schism of tetrapyrryl bilirubinoid compounds to mesobilifuscin has been demonstrated *in vitro*(8) and this or other form of biochemical diversion might occur in the intestine or as an artifact of the fecal collections. Another possibility for explaining the reduced amount of urobilinogen excreted is that amount of urobilinogen normally reabsorbed from the colon and destroyed by the liver during the so-called enterohepatic circulation is greater than is generally supposed(9). Whatever the explanation, the present observations suggest that, when an increased amount of bilirubin is presented to the intestine, amount of bile pigment failing to appear as colorimetrically measurable fecal urobilinogen remains fairly constant so that relative proportion of bilirubin converted to urobilinogen increases. Thus, under normal circumstances the unknown diversion process must approach its maximum and so be incapable of much further augmentation.

Summary. In 6 adult patients without hematologic or hepatic abnormality the efficiency of conversion of bilirubin to colorimetrically measurable fecal urobilinogen, assuming a daily catabolism of 6.25 g of hemo-

globin, averaged only 26%. However, the efficiency of conversion of from 8.3 to 11.75 g of hemoglobin subsequently given by slow intravenous infusion averaged 96%. It is suggested that some physiological, biochemical or artifactual process normally diverts a more or less constant amount of bilirubin from conversion to fecal urobilinogen measurable by Ehrlich's aldehyde reagent in collected stools even when the bilirubin to be converted is significantly augmented.

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Effects of an Unsaturated and Saturated Lipid on Experimental Cholesterol Atherosis in Rabbits. (26078)

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Much interest has been shown in the relationship between the serum cholesterol and the iodine value of the dietary fat. It has been reported that feeding rabbits a highly unsaturated lipid in a diet that contains cholesterol increases serum cholesterol and amount of cholesterol atherosis(1,2). The purposes of this paper are: (1) to report some effects of adding an unsaturated and a saturated lipid with 2% cholesterol to the diet of rabbits; (2) to advance an explanation for the results.

When either a saturated or unsaturated lipid was fed to rabbits eating a diet containing cholesterol, higher values of serum cholesterol were produced. Increased amounts of cholesterol were also found in the aortas. Highest cholesterol values were observed in rabbits consuming the most unsaturated lipid. With addition of either lipid to the diet, fecal excretion of Lieberman Burchard chromogens and 3-β hydroxy sterols fell.

Method. Adult male rabbits were divided into 4 groups. Group I, the control group, was fed stock rabbit chow (Purina Rabbit Chow Checkers), Group II, the same stock diet with 2% cholesterol; Group III, stock diet with 2% cholesterol and 10% coconut oil, and Group IV, stock diet with 2% cholesterol and 10% linoleic acid (Fisher). The iodine number of the coconut oil fed was 9, and of the linoleic acid 130. These diets were continued for 8 weeks. Body weight and total serum cholesterol were determined at weekly intervals(3). During a 24 hour period of each week of experimental diets, fecal collections were made from the rabbits in Groups II, III, IV and Lieberman-Burchard chromogens, 3-β hydroxy sterols, and bile acids were measured(4). After 8 weeks the animals were killed and liver and aorta removed. The 3 cm of the aorta just distal to the aortic valve and a sample of liver were taken for cholesterol analysis. These tissues were homogenized, extracted with chloroform and total cholesterol estimated by the Kingsley and Shaffert Technic(5).

Results and discussion. The effect of the various diets on serum cholesterol is shown in Fig. 1. With addition of 2% cholesterol to stock diet the usual progressive rise in serum cholesterol was observed. With addition of either a saturated (coconut oil) or an unsaturated lipid (linoleic acid) to the diet con-