of epinephrine action led not only to a normal accumulation of liver glycogen, but to a decreased net loss of muscle glycogen. As a result the overall carbohydrate balance sheet was of normal proportions. These results suggest that at least a part of the normal response of the carbohydrate metabolism of fasted rats to the injection of epinephrine is a consequence of the concomitant effect of this hormone in stimulating the release of ACTH from the anterior lobe of the pituitary.

Summary. 1. The subcutaneous injection of epinephrine (0.02 mg/100 g body weight) into fasted adrenalectomized rats is followed by a twofold greater loss of muscle glycogen than in intact rats, and by little or no increase in liver glycogen. Consequently, there is a sixfold greater disappearance of carbohydrate from the body that is not accounted for by the accumulation of glucose or lactic acid in the body fluids. 2. The injection of large quantities of adrenal cortical extract, prior to and during the period of epinephrine action, brings about a restoration of the carbohydrate balance to that found in intact rats.

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Experimental Porphyria. III. Hepatic Type Produced by Sedormid.* (19987)

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An experimental porphyria in rabbits treated with phenylhydrazine, lead, and light has been described in previous reports(1,2). This porphyria was shown to have many features characteristic of the rare erythropoietic (congenital or photosensitive) type of porphyria seen in humans(3). More recently, attempts have been made to find compounds which would produce an experimental porphyria similar to the human hepatic type(3). Ellinger and Riesser(4) described the occurrence of ether-insoluble (uro-type) porphyrins in the urine of patients with trional intoxication, and Fischer and Duesberg(5) later reported the finding of trace amounts of a similar porphyrin in the urine of rabbits treated with sulfonal. Conflicting, and generally negative results have been reported from other laboratories investigating these and similar substances in animals(6,7).

Duesberg(8) reported the presence of porphyria in a patient treated with large amounts of Sedormid (allylisopropylacetylcarbamid). It has now been found that this compound markedly increases the excretion of porphyrins and porphobilinogen in normal rabbits. These and other findings which demonstrate the hepatic nature of this new type of experimental porphyria, form the basis of the present report.

Methods. Rabbits of either sex weighing from 1500 to 3000 g were used, though best results were generally obtained in rabbits weighing less than 2 kg. Sedormid‡ was given orally at the rate of approximately 200 mg per kilo body weight per day in a single dose

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TABLE I. Daily Urinary Porphyrin Excretion in Rabbits with Experimental Sedormid Poisoning.

Days of Sedormid admin.	#162 μg/day		#166 μg/day		#167 μg/day		#168 μg/day	
	Uro-*	Copro-	Uro-*	Copro-	Uro-*	Copro-	Uro-*	Copro-
Control	10	23	—	—	—	—	24	36
1	—	—	12	10	16	24		
2							23	38
3	9	33	63	121				
4					17	36		
5	258	75					2050	223
6			1590	329				
7					25	78		
8	12820	240					—	—
9			33150	761	1150	308	35250†	384†
10	321000†	600†	56250	756				
12					13500	447		
					8030‡	512‡		

24 to 72 hr urine collections combined for determination.

* This includes all ether insoluble uro- type porphyrins.

† These animals were killed before completion of the last 24 hr urine collection. Terminal porphyrin values are therefore expressed in μg/100 ml urine.

‡ This animal died as the result of gastric rupture before completion of the last 24 hr urine collection. Terminal values are expressed in μg/100 ml urine.

or in two divided doses. Attempts made to administer the material in an exact amount parenterally have thus far been unsuccessful because of its relative insolubility in innocuous solvents, including water. The *urine* was collected over 24 to 72 hour periods in metabolism cages permitting separate collection of urine and feces. Porphyrin and porphobilinogen concentrations were determined by previously described methods(2,9,10). Certain modifications employed in some of these analyses will be described in a separate communication. Fluorescence microscopy studies employed a water-cooled General Electric AH6 mercury arc lamp as the light source. One or two Corning filters No. 5-58 (5113) were generally used as primary filters to isolate the 405 mμ line to excite porphyrin fluorescence. An orange Corning filter No. 3-67 (3482) or a light red Corning filter No. 2-63 (2424) was placed between the objective and the eyepiece of the microscope to isolate the fluorescent light.

Results. I. *Urinary porphyrins.* Quantitative data on the excretion of urinary porphyrins have been obtained on 20 rabbits in which treatment with Sedormid was continued for at least 6 days. Peak values for urinary uroporphyrin in these rabbits ranged from 3500 to 60,000 μg per day with an average of

TABLE II. Porphyrin Concentrations in Various Organs and Excreta of 7 Rabbits with Sedormid Intoxication.

Organ or excreta	No. of rabbits studied	μg per 100 g or 100 ml					
		Uroporphyrin*		Coproporphyrin		Protoporphyrin	
		Range	Mean	Range	Mean	Range	Mean
Erythrocytes	5	0- 1.8	.9	tr. - 6.6	1.9	10 - 42	32
Bone marrow	5	0- 1.5	.5	tr. - 14	6.4	15 - 64	42
Spleen	4	0- 13.9	5.8	tr. - 18	7.8	24 - 30	27
Liver, fresh	7	9- 233	108	72 - 167	132	176 - 1700	819
Liver, heated	7	19- 1042	425	65 - 224	139	133 - 1820	585
Kidney	4	30- 120	76	34 - 162	116	46 - 74	66
Brain	3	0- 1.9	.6	tr. - 2.8	1.6	3.8- 5.5	4.4
Plasma	4	0- tr.	tr.	2.5- 8.1	5.1	5.7- 10.4	8
Bile	5	383- 5480	2122	4600 -39600	19800	13800 -86400	40000
Feces	4	90- 2180	870	1980 - 5900	3560	5500 -12800	9400
Urine	7	13800-76130	24750	260 - 780	490	0	0

* This includes all ether insoluble uro-type porphyrins.

17,000 μg per day. Peak values for urinary coproporphyrin ranged from 165 to 756 μg per day with an average of 390 μg per day.

Representative data for 4 rabbits treated with daily oral doses of Sedormid are given in Table I. The urinary excretion of coproporphyrin generally rose more rapidly at first, but levelled off after 5 to 7 days. As pointed out previously(2) significant amounts of uro-type porphyrins are excreted in the urine of normal rabbits. From the fifth to eighth day the uroporphyrin values increased sharply to levels sufficient to give the urine a Burgundy red color and a brilliant red fluorescence when exposed to ultra-violet light. Temporary decreases in dose or discontinuation of the drug altogether resulted in a prompt and marked diminution especially in the uroporphyrin excretion.

Spectroscopic analysis (absorption and fluorimetry) of the uro- and coproporphyrin revealed both to be a mixture of free porphyrin and metal complex. The free coproporphyrin was removed by 0.12 N HCl from the primary ethyl acetate extract of the urine. The remaining coproporphyrin was then freed from the metal by extraction with 1.5 N HCl(11). While a marked variation was found in the amount of metal complex, in some samples over 80% of the total was combined with metal, which, on the basis of the absorption spectrum, the complex is probably zinc.

Over half of the total uroporphyrin was excreted in the form of non-fluorescing precursors which developed fluorescence after heating or treatment with iodine.

At least 10 distinct red fluorescing bands were found by calcium carbonate chromatography of the methyl esters. The strongest of these consisted of a uro-type porphyrin crystallizing in long curved hairs with a melting point of 262-265° consisting mainly of type III isomer. Smaller amounts of uroporphyrin I (M.P. 284°) and of other uro-type porphyrins melting at 242-245° and 252-255° respectively, were also isolated. These and the coproporphyrin were predominantly type III isomers. A detailed report on the physical-chemical properties of the porphyrins will be published separately.

The urinary excretion of porphobilinogen roughly paralleled that of the uroporphyrins. Peak values generally ranged from 20 to 60 Ehrlich units per 100 ml, where one Ehrlich unit represents the color intensity of one mg of urobilinogen.

II. *Tissue, biliary, and fecal porphyrins.* Seven rabbits were killed by cardiac bleeding at the time of marked porphyrinuria. Porphyrin concentrations in various tissues, along with values in bile, urine, and feces are summarized in Table II. Among the most interesting findings were: 1) the high values for liver porphyrins, 2) the increase of liver uroporphyrin on heating, 3) the preponderance of uroporphyrin in urine as compared to bile and feces, 4) the high biliary and fecal coproporphyrin concentrations, 5) the presence of high concentrations of protoporphyrin in bile and feces and its absence from urine, and 6) the relatively high ratio of kidney to urine coproporphyrin as compared to the low ratio

of kidney to urine uroporphyrin.

At autopsy, upon exposure to ultra-violet light, the gallbladder and the liver surface adjacent to the gallbladder, the bile ducts, and the duodenal region around the orifice of the common bile duct showed an intense red fluorescence. This was mainly protoporphyrin. On cut sections of the liver, the fluorescence intensity of the tissue taken from areas near the porta hepatis, was much stronger than that of tissue taken from more peripheral parts of the liver. However, even in these peripheral areas, pin-point red-fluorescing spots could be recognized which, on fluorescence microscopy, appeared to be within the liver cells of the central half of the liver lobules. On the other hand, fluorescence microscopy examination of bone marrow and peripheral blood smears revealed no red fluorescence.

The porphobilinogen reaction was strongly positive in homogenates of liver and kidney, weak in bile, and negative in bone marrow.

III. *Course.* When the urinary uroporphyrin excretion reached levels of approximately 7,000 μg per day or more, the rabbits became lethargic and lost their appetite. Transient paralysis of the hind legs and the bladder was observed in many of the animals. X-ray examination showed marked gastric dilatation, constriction of the pylorus and upper small bowel, and distention of the large bowel with gas. Continued administration of Sedormid resulted invariably in sudden death due to rupture of the markedly distended stomach. Undigested Sedormid tablets found among the gastric contents at autopsy may explain the frequent fall in porphyrin excretion during the last day or two of life.

Discussion. The present findings are of special interest in relation to the separation of human porphyria into erythropoietic and hepatic types, evidence for which has recently been accumulated in this laboratory(3). In erythropoietic porphyria one finds large amounts of uro- and coproporphyrin in the bone marrow and circulating red cells, whereas the liver porphyrin concentrations are relatively small. In the hepatic (intermittent acute, cutaneous, and mixed) types of porphyria, on the other hand, bone marrow and red cell values are normal, while the liver contains large amounts of porphyrin and porphyrin precursors in varying combination.

It is evident that the porphyria induced in rabbits by Sedormid corresponds more nearly to the "hepatic" type, because 1) liver porphyrin concentrations are high whereas bone marrow porphyrin values are normal, 2) large amounts of porphobilinogen are formed, probably in the liver, and excreted in the urine, 3) a substantial portion of the urinary porphyrin is excreted as a metal complex, 4) the uro-type porphyrins consist of a mixture of type I and III isomers, however with much more type III than in the human disease, 5) considerable amounts of non-fluorescing precursors of uro-porphyrin are present in the urine and in the liver, and 6) the urinary coproporphyrin is chiefly the type III isomer. Clinically, too, the transient paresis or paralysis of the hind legs and the functional gastrointestinal disturbances in these rabbits are reminiscent of similar findings in patients with the intermittent acute type of hepatic porphyria.

Studies to be published later have shown a rapid and marked decline in the concentration of liver catalase in these Sedormid-treated rabbits. Since there is a marked accumulation of porphyrins in the liver, this would suggest that Sedormid interferes with the metabolism of the porphyrins and their conversion to iron-porphyrin compounds such as catalase in the liver. It is interesting that no such block is apparent in the formation of hemoglobin in the bone marrow as shown by normal bone marrow and red cell porphyrin values.

Though uro-, copro-, and protoporphyrin concentrations in the liver are all high, their excretory patterns are quite different. The uro- type porphyrins with eight or less carboxyl groups are excreted chiefly in the urine, the 4 carboxyl coproporphyrin is found in the highest concentration in the bile and feces, and the 2 carboxyl protoporphyrin is absent from the urine and excreted only in the bile and feces. It may be that these excretory patterns are related to an increasing number of COOH groups resulting in increasing water solubility of these porphyrins or their sodium salts. Further studies are required to determine

whether the difference in porphyrin concentration in the kidney is due to differences in tubular reabsorption of uro- and coproporphyrin.

The high concentration of protoporphyrin in the liver parenchyma adjacent to the gallbladder suggests that considerable amounts of this porphyrin are reabsorbed from the gallbladder by way of the lymphatics.

Summary and conclusions. 1. Allylisopropylacetylcarbamid ("Sedormid") produces an hepatic form of porphyria in rabbits. 2. The livers of these animals contain large amounts of proto-, copro- and uroporphyrin, the latter chiefly in the form of non fluorescing precursors. Porphobilinogen is also present. 3. The protoporphyrin is excreted only, and the coproporphyrin chiefly, in the bile, most of the uro- type porphyrins appearing in the urine, in amounts ranging up to 60 mg in 24 hours. The latter consists mainly of type III isomers, but type I has also been identified in small amount. Large amounts of porphobilinogen are excreted in the urine. 4. The porphyrin content of erythrocytes, bone marrow, spleen, and brain is within normal limits. 5. Transient paresis of hind limbs, dilatation of the stomach, and constriction of the pylorus are noted, with irregular small bowel spasm. Death is invariably due to rupture of the stomach. 6. The possibility is discussed that the genesis of the porphyria is related to a primary effect of the Sedormid on the formation of iron porphyrin enzymes in the liver cell.

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Histamine Sensitivity and Anaphylaxis in the Pertussis-Vaccinated Rat.* (19988)

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The intact, white rat is known to be relatively insensitive to both histamine and anaphylactic shock. The resistance to both these insults can be significantly diminished by adrenalectomy(1-3) or hypophysectomy(3,4). This behavior is not unlike that exhibited by the mouse(5,6). In the latter species it has been reported that a preliminary inoculation of *Hemophilus pertussis* likewise diminishes the resistance to both histamine(7) and anaphylactic shock(8). The present report is concerned with the effect of *H. pertussis* vaccination on these insults in the white rat.

Methods and results. (1) Female, white rats of about 100 g weight were injected intra-

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