virus propagation. Furthermore, she has found that cysteine can replace the 6 other amino acids for poliovirus propagation. The combined evidence strongly indicates that cysteine and/or its disulfide derivative cystine play an important role in the process of poliovirus propagation in monkey kidney tissue cultures. These results suggest the possibility of finding cystine antimetabolites which would delay poliovirus action *in vitro* and perhaps *in vivo*. With only the information available at this time it is somewhat premature to speculate as to the reason for this cystine requirement. One reasonable working hypothesis is that the available endogenous cystine, either free or in peptide bondage, simply does not meet the demand for the cystine residues of the protein portions of the newly-forming poliovirus particles.

Summary. The cytopathogenic action of the poliovirus strains Akron, Brooks, and Mabie on either tube or Petri plate cultures of monkey kidney cells is considerably delayed when cystine is omitted from the maintenance medium. Omission of all the vitamins or of any one of the 12 other amino acids except glutamine normally present in the maintenance medium resulted in no delay in the appearance of the cytopathogenic effect of Akron PPl. Omission of glutamine resulted in a short delay or no delay. It has further been shown that the propagation of Akron is slower in the absence of cystine. The lowest concentration of L-cystine which will give optimal or near-optimal cytopathogenic action by Akron is about 10^{-4} M. Glutathione and L-cysteine hydrochloride can replace cystine at about the same concentration; DL-homocysteine thiolactone hydrochloride and DL-cystathionine can replace also but only at higher molar concentrations. None of 13 other compounds was found to replace cystine. The adsorption of Akron onto monkey kidney cells is not affected by the absence of cystine.

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Received July 23, 1956. P.S.E.B.M., 1956, v93.

Role of the Adrenal in Response of Rats to *Orinase*.* (22686)

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(Introduced by M. H. Kuizenga.)
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Adrenocortical and medullary hormones both counteract the hypoglycemic effects of insulin(1,2,3). Excessively high doses of the hypoglycemic agent *Orinase* do not produce a hypoglycemic response(4).† These observations suggested that the adrenals may respond to high doses of *Orinase* with increased secretions. Therefore, experiments were designed to study the role of the adrenal cortex and medulla in modifying the hypoglycemic reaction of rats to this drug.

Methods. Male rats obtained from the Upjohn colony (Sprague-Dawley ancestry) were used throughout these experiments. The intact animals weighed 140-160 g at the time of treatment with *Orinase*. Adrenalectomies and

* Upjohn tradename for 1-butyl-3β-tolylsulfonylurea.

† Personal communication from W. L. Miller.

adrenal demedullations were performed at an average body weight of 150 g. Adrenalectomized rats were maintained on 1% NaCl as drinking fluid and were given the hypoglycemic drug on the 7th day postoperatively except in one experiment. In this case, as shown in Table II, it was administered on the 9th day after adrenalectomy. The O day represents the day of *Orinase*. The 500 μg/day dose of hydrocortisone was started on the 4th day postoperatively and given for 5 days, with the last injection given immediately prior to the *Orinase*. The 5 mg dose of hydrocortisone was given as one injection immediately prior to the sulfonylurea on day O. All hydrocortisone injections were given in a volume of 0.2 cc of CMC vehicle(5). When epinephrine‡ was used it was given subcutaneously 1 hour following the oral administration of the hypoglycemic agent. The adrenal demedullated rats were kept at least 4 weeks before *Orinase* treatment in order to insure regeneration of the adrenal cortices. The functional activity of the adrenal cortices has been indicated§ by the fact that these animals can withstand stress induced by intraperitoneal egg albumin and the glands weighed between 40-50 mg after 4 weeks. These rats were maintained on 1% sodium chloride as drinking fluid for the first week and then on tap water. *Orinase* was given orally in all cases. Doses up to 275 mg/kg were given in 0.5 cc of CMC vehicle(5) and higher doses in a volume of 2 cc. All animals were fasted 24 hours prior to *Orinase* administration. Blood samples were obtained from the vena cava 2½-3 hours after *Orinase* treatment while the animals were under sodium cyclopal anesthesia. The blood was oxalated and glucose was estimated by the Folin-Wu method(6). Liver glycogen was determined by the anthrone method on tissues removed 7 hours following treatment.

Results. The minimal effective hypoglycemic dose of *Orinase* in intact rats was approximately 100 mg/kg (Fig. 1). These data also show that the maximal fall in blood sugars was 36% at a dosage of 266 mg/kg.

‡ Upjohn Epinephrine Hydrochloride 1:1000.

§ Unpublished data.

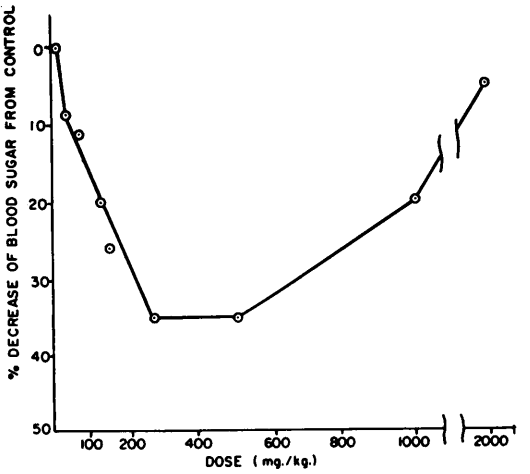

FIG. 1. Dose-response of intact rats to the hypoglycemic effects of *Orinase*. Each point represents 8 controls and 8 treated animals. Animals were bled 2½-3 hr following treatment.

At the high dose of 2 g/kg there was no demonstrable depression of blood sugar in the intact rats. The same type of response was manifested in the deposition of liver glycogen under the influence of *Orinase* (Table I). While 266 mg/kg of the sulfonylurea produced a marked increase in liver glycogen, the higher doses caused less deposition.

In adrenalectomized rats as little as 9 mg/kg of *Orinase* caused a fall in blood sugar (Fig. 2). At dosages of 45 and 90 mg/kg the adrenalectomized rats were subject to convulsions and blood sugar levels were depressed by 45 and 57% from the controls. To establish that the convulsions were due to hypoglycemia an experiment was performed in which *Orinase* at a dosage of 100 mg/kg was administered orally to 20 adrenalectomized rats. One-half of these animals received 1 g of glucose orally after they were in

TABLE I. Effects of Varying Doses of *Orinase* on Fasting Liver* Glycogen.

Treatment	Dose	No.	Liver glycogen
Vehicle	2 cc	7	.24
Orinase	266 mg/kg	6	.66
	523	6	.57
	1048	6	.35
	2000	6	.34

* Livers were removed 7 hr following treatment.

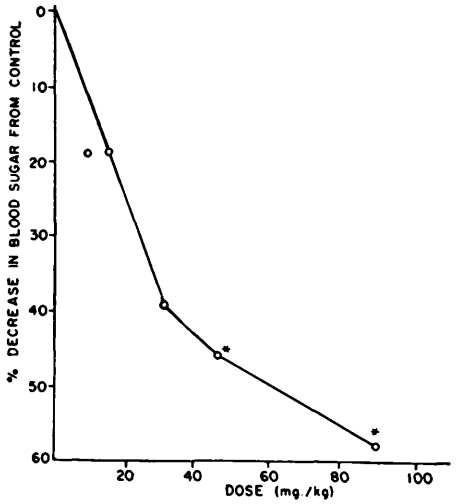

FIG. 2. Dose-response of adrenalectomized rats to *Orinase*. Each point represents 8 controls and 8 treated rats. Animals were bled 2½-3 hr following treatment.

* All animals were convulsing.

convulsions. In every instance the glucose stopped the convulsions and the animals were on their feet within 10 minutes. If glucose was not given, convulsions continued for several hours and usually ended in death.

In adrenal demedullated animals the minimal dose of *Orinase* causing hypoglycemia was between 15 and 30 mg/kg (Fig. 3). This is compared with 9-15 mg/kg for the hypoglycemic dose in adrenalectomized rats and 100 mg kg for intact rats. Therefore, the adrenal demedullated animals are considerably more sensitive to the hypoglycemic effects of *Orinase* than the intact rat but do not seem to be quite as sensitive as the adrenalectomized animals. In addition, the dose of 2 g/kg produced essentially as much depression of blood sugar as the lower doses in the adrenal demedullated animals. It is important to note that the 2 g/kg dose was the maximum dose in this animal preparation since it killed approximately ½ of them.

To delineate further the role of the adrenal cortex and medulla in the response to *Orinase*, experiments were carried out to study the effects of hydrocortisone and epinephrine on blood sugar response of adrenalectomized rats to this drug. Treatment with hydrocortisone at 0.5 mg per rat per day for 5 days

TABLE II. Effects of Hydrocortisone on Response* of Adrenalectomized Rats† to *Orinase*.

No. rats	Treatment	Days	Dose	Blood sugar (mg %)
4	Subq. saline	5	.2 cc	91
	Oral CMC	0‡		
4	Subq. saline	5	.2 cc	
	Subq. F	0	5.0 mg	
	Oral CMC	0	.5 cc	103
4	Subq. F	5	.5 mg	
	Oral CMC	0	.5 cc	100
4	Subq. F	5	.5 mg	
	Oral *Orinase*	0	66.0 mg/kg	58
5	Subq. saline	5	.2 cc	
	Subq. F	0	5.0 mg	
	Oral *Orinase*	0	66.0 mg/kg	53§
5	Subq. saline	5	.2 cc	
	Oral *Orinase*	0	66.0 mg/kg	38§

* Blood was taken 2½-3 hr following *Orinase* treatment.

† Avg body wt at time of *Orinase* was 160 g.

‡ 0 day was 9th day after adrenalectomy and at end of indicated treatment. The F 0-day and last inj. of F 5 days was given immediately prior to the *Orinase*.

§ Convulsing.

prior to *Orinase* administration in the adrenalectomized rats partially reversed the hypoglycemic effects of *Orinase* and increased the blood sugar somewhat over that of the controls (Table II). It is also interesting that a

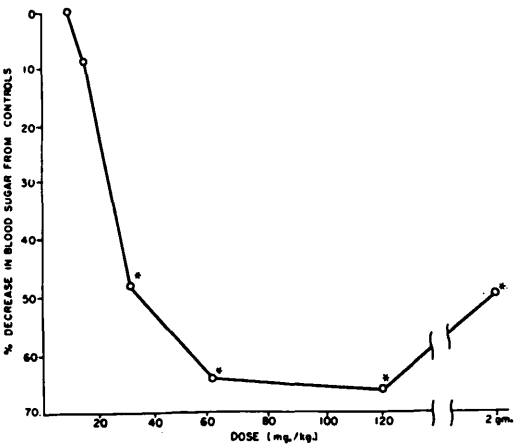

FIG. 3. Dose-response of adrenal demedullated rats to *Orinase*. Each point represents 8 controls and 8 treated animals except at the 2 g/kg dose. In this group there were 15 controls and 8 *Orinase*-treated as 7 treated rats died before they were bled. Animals were bled 2½-3 hr following treatment.

* All animals were convulsing.

TABLE III. Effect of *Orinase* plus Epinephrine on Blood* Sugar in Adrenalectomized Rats.

No. rats	Treatment	Dose	Blood sugar
5	CMC	0.5 cc	76
5	*Orinase*	66 mg/kg	40
7	*Orinase* +Epinephrine	66 " 200 μg/kg	116

* Blood was taken 2½-3 hr following the *Orinase*. Epinephrine was given 1 hr after the hypoglycemic drug.

single dose of 5 mg of hydrocortisone per rat given simultaneously with *Orinase* also partially reversed the extreme hypoglycemic effects with *Orinase* in this animal preparation. The blood sugar levels of the animals which received 5.0 mg of hydrocortisone were also somewhat higher than the untreated control adrenalectomized animals. The effect of epinephrine in reversing the hypoglycemic effect of *Orinase*, however, was more striking than that seen with hydrocortisone (Table III). When 200 μg/kg of epinephrine was administered to adrenalectomized rats 1 hour after oral administration of *Orinase*, the hypoglycemia was completely reversed and the blood sugar levels rose significantly above those of the untreated adrenalectomized controls.

Discussion. The difference in sensitivity of intact and adrenalectomized rats to *Orinase* shows that the adrenal is involved in the response of rats to this drug and agrees with previous observations(7) that adrenalectomized rats were more sensitive to the hypoglycemic effects of other sulfonamides than intact animals. The failure of high doses of *Orinase* to induce hypoglycemia or liver glycogen deposition in the intact rat may be the result of stimulation of epinephrine secretion by the adrenal medulla since epinephrine is known to increase blood sugar and deplete liver glycogen. The observations reported here which support this explanation are as follows: (1) the adrenal demedullated animals are more sensitive to the hypoglycemic effects of *Orinase* than the intact rats. (2) The hypoglycemic activity of 2 g/kg of *Orinase* was nearly as marked as that produced by lower doses in the demedullated rats. (3) Epinephrine completely reversed the hypoglycemic response of adrenalectomized rats to the sulfonylurea.

Since hydrocortisone partially reversed the hypoglycemic response of the adrenalectomized animal to *Orinase* and since adrenalectomized animals seem to be more sensitive to it than demedullated animals, it is probable that the adrenal cortex is also involved, but to a lesser degree. Another observation which suggested that the adrenal cortex may be stimulated with the high doses of *Orinase* was that there was slightly less hypoglycemic response at the 2 g/kg dose level as compared to that obtained with lower doses in adrenal demedullated rats.

These results also indicate that when intact animals are used to test hypoglycemic agents a wide range of dosages should be employed to be sure that the phenomenon of reversal of effects does not occur. Adrenalectomized rats are better test animals since they are extremely sensitive to hypoglycemic drugs and also the likelihood of masking the hypoglycemic effect by adrenal secretory activity induced by high doses is eliminated.

Summary. In the intact rat low doses of *Orinase* produced a significant depression of blood sugar and deposition of liver glycogen while higher doses were ineffective. The adrenalectomized rat was found to be approximately 10 times as sensitive and the adrenal demedullated rat 3-6 times more sensitive to *Orinase* than the intact rat. Hydrocortisone partially and epinephrine completely counteracted the hypoglycemic effects of *Orinase*. Failure of high doses of this sulfonylurea to produce hypoglycemia and liver glycogen deposition in the intact rats may be due to a stimulation of epinephrine secretion by the adrenal medulla. It is also suggested that glucocorticoid secretion is also stimulated by high doses of *Orinase* in the intact and adrenal demedullated animals.

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Received July 24, 1956. P.S.E.B.M., 1956, v93.

Tubular Reabsorption of Protein in Experimentally Produced Proteinuria in Rats. (22687)

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It has long been recognized that cells lining the proximal convoluted tubule of the kidney of various animal species are capable of absorbing colloid substances from the tubule lumen(1-4); the process has been named *athrocytosis*. Dock(5) and Addis(6) were the first to suggest that under *normal* circumstances plasma proteins are to some extent filtered through the glomerulus of the kidney and reabsorbed by cells of the tubules. Because of the enormous volume of glomerular filtrate, a concentration of protein in the glomerular filtrate of only 15 mg 100 ml would result in excretion in the human of about 27 g of protein in 24 hours if the protein were not reabsorbed by the tubule cells. The work of many investigators has contributed to the concept of glomerular filtration and tubular reabsorption of plasma proteins in normal and abnormal conditions. These reports have recently been reviewed by Rather(7). Since this review, the quantitative aspects of tubular reabsorption of protein in the normal rat has been studied by Sellers, *et al.*(8) and the mechanism of tubular handling of reabsorbed protein has been investigated by Oliver and co-workers(9). Theoretically, a decrease in tubular reabsorption of plasma proteins filtered through normal glomerular basement membrane could account for the protein found in urine in most instances of proteinuria. Actually, however, in glomerulonephritis there is evidence of glomerular damage, and even in cases of idiopathic nephrotic syndrome, where only basement membrane thickening is found to suggest changes in glomerular permeability, calculations based on clearance studies in patients with massive proteinuria lead to the conclusion that glomerular permeability to plasma proteins is increased (10). This still leaves open the question of whether proteinuria occurs solely because filtration through abnormally permeable glomeruli exceeds the normal ability of tubules to reabsorb protein, or whether a decrease in tubular reabsorption of protein is an added factor in production of proteinuria.

The dye T-1824 (Evans Blue) binds firmly to serum proteins and has been used to measure renal clearance of albumin in humans(11) as well as tubular reabsorption of protein in rats(5,8,12). The amount of dye accumulating in droplet form in cells of the proximal convoluted tubule is assumed to be a measure of reabsorption from the tubule lumen of dye-labeled serum proteins. In the present report, dye-labeling of serum proteins has been used to study the role of tubular reabsorption of protein in 2 types of experimentally produced proteinuria.

Materials and methods. Female Osborne-Mendel rats of about 150 g body weight were used. Proteinuria due to uranium administration was produced by intravenous administration of 0.174 mg uranyl acetate containing 0.1 mg uranium, in 1.0 ml of 0.15 M saline. Proteinuria due to anti-kidney serum was induced by a single intravenous injection of rabbit anti-rat kidney serum. The nephrotoxic serum was obtained from rabbits immunized by repeated intraperitoneal injections of glomerular fraction of perfused rat kidney homogenate. To obtain urine for pro-