sumably enzymatic property of the virus that leads in time to destruction of the receptor, and to elution. Thus the dynamics of hemagglutination by the *H. pertussis* factor, and by mildly inactivated influenza-type virus particles, may differ less markedly than would at first appear to be the case.

The observation that the bond between *H. pertussis* factor and red cells can apparently be dissociated in the competitive presence of red-cell extract, and that the cells thus released can again be agglutinated by addition of fresh *H. pertussis* factor, indicates that attachment of this factor to the red cell does not result in appreciable destruction of the receptor. The dissociation and reassociation reported here bears a closer resemblance to a familiar type of interaction which has been observed between many cellular antigens and their antibodies. Morgenroth, for example(14), noted that if sheep cells were saturated with hemolysin in the absence of complement, and then mixed and incubated with fresh cells for a certain minimum period, the addition of complement would then result in hemolysis of all the cells in the mixture. The influence of time, of amount of antibody added, and of the interaction of 2 red-cell species with an antibody active against both, have been studied in detail by Philosophow(15); and Mayer *et al.* (16) have recently confirmed and extended these observations. Another recent observation, parallel in principle, is that when Rh-positive red cells (presumably saturated with the corresponding maternally produced antibody) from an erythroblastotic infant are mixed with homologous Rh-positive adult blood, the cells of both infant and adult may hemolyze (17). Thus hemagglutination of the type induced by *H. pertussis* factor bears a limited resemblance not only to conventional virus hemagglutination, but also to conventional antigen-antibody interaction.

Summary. The hemagglutinating factor of *Hemophilus pertussis* culture supernatants can apparently be eluted from human erythrocytes by suspending such erythrocytes in a distilled-water extract of frozen and thawed red cells. Following the removal of the *H. pertussis* factor from agglutinated cells, these cells can be re-agglutinated by addition of fresh *H. pertussis* culture supernatant. The factor cannot be eluted by incubation of agglutinated cells at various temperatures, or by suspending the cells in salt-free isotonic glucose solution.

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Effect of 3-Hydroxy-2-phenylcinchoninic Acid on Renal Secretion of Phenyl Red and Penicillin.* (18011)

C. GORDON ZUBROD, EARL H. DEARBORN AND E. K. MARSHALL, JR.

From the Department of Pharmacology and Experimental Therapeutics and Department of Medicine, The Johns Hopkins University.

Certain derivatives of cinchoninic acid have been found to be effective antidiuretics when administered to dogs with induced water diuresis(1,2), to cause an increased rate of excretion of uric acid when administered to man(3,4,5), to stimulate the anterior pituitary causing a decrease in the ascorbic acid content of the adrenal glands of rats(6), and to suppress the secretion of phenol red by the kidney due to blocking the tubular secretory mechanism(7,8). The present communication is concerned with this last pharmacological action, the blocking of renal tubular

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secretion. The effect on the secretion of phenol red is apparently similar to that described for carinamide(9).† Carinamide blocks the tubular secretion of phenol red and compounds such as p-aminohippuric acid and penicillin which are eliminated by the same tubular mechanism but does not interfere with the tubular secretion of N^1-methylnicotinamide(10). One should therefore expect that the derivatives of cinchoninic acid which suppress the secretion of phenol red would also suppress the secretion of penicillin. The present experiments confirm this expectation for 3-hydroxy-2-phenylcinchoninic acid (HPC).

All experiments were performed on female dogs. Suppression of the secretion of penicillin was estimated by determinations of its concentration in the plasma with and without previous injection of drug. The rate of excretion of phenol red was also measured with and without previous injection of drug. Crystalline potassium benzylpenicillin was given intravenously in a dosage of 2.4 mg per kg. Blood was drawn at one, 2, 3 and 4 hours after injection. Citrate was used as an anticoagulant, and the plasma taken for penicillin assay. Phenol red was injected intravenously in a dosage of 6 mg in 5 cc of saline. Urine was taken by catheter at one-half, one and 2 hours after injection, and its phenol red content determined. The drug was injected as the sodium salt intravenously 2 minutes before injection of the penicillin, or phenol red.

The concentration of penicillin in the plasma was determined by the method of Tompsett, Shultz and McDermott(11), using reconstituted lyophilized human plasma‡ as the diluent instead of pooled human serum. The organism used in the test was *Streptococcus dysgalactiae*§(12). The inoculum was made from a 10^{-2} dilution of a 24-hour subculture grown in trypticase soy phosphate broth. This inoculum was inhibited in the presence of 25% plasma by a penicillin concentration of 0.012 μg per ml. The end point in this titration was obtained by observing turbidity after 24 hours incubation at 37.5°C. The penicillin used for standards was crystalline potassium benzyl penicillin. A new standard was made up once weekly in a concentration of 300 μg per ml in distilled water and kept at 4°C. Fresh working standards were made daily from the concentrated standard, the last two dilutions being made up in 25% plasma. Recoveries from human plasma of added amounts (6.0 to 0.06 μg per ml) ranged from 80 to 100%.

The results of the experiments with penicillin injected alone, after carinamide, or after

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† In dogs, under conditions in which the extraction ratio of creatinine was around 20%, our method was not delicate enough to detect any difference between the concentrations of HPC in arterial and renal venous blood. The clearance of HPC in the dog is about 0.05 ml per minute; correcting for about 96% binding to plasma protein gives a clearance of unbound drug around 1 ml per minute. HPC does not appear to be secreted by the renal tubule.

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‡ The human plasma used in these studies was obtained through the kindness of Dr. W. A. Feirer, Sharp and Dohme, Inc.

§ Dr. J. C. Kakavas of the University of Delaware kindly furnished us with the strain of *Str. dysgalactiae*.

TABLE I.
Effects of 3-Hydroxy-2-phenylcinchoninic Acid and Carinamide on Plasma Concentration of Penicillin in the Dog.

Dog	Hr after penicillin	Conc. of penicillin in plasma, μg per ml			
			Carinamide		HPC
		Control	20 mg/kg	40 mg/kg	20 mg/kg
1	1	2.8	3.0	4.8	5.6
	2	.8	.8	2.2	2.0
	3	.1	.2	.8	.8
	4	.0	.0	.3	.3
11	1	1.3	2.4	3.3	3.4
	2	.4	.6	.9	1.4
	3	.1	.3	.2	.6
	4	.0	.1	.0	.8
13	1	2.4		3.6	3.3
	2	.6		1.2	1.8
	3	.2		.7	.7
	4	.1		.3	.3

TABLE II.
Effects of 3-Hydroxy-2-phenylcinchoninic Acid and Carinamide on Urinary Excretion of Phenol Red in the Dog.

Dog	Hrs after phenol red	Mg of phenol red in the urine (cumulative)		
		Control	Carinamide 20 mg/kg	HPC 20 mg/kg
1	0.5	2.52	.71	.91
	1	3.52	1.17	1.58
	2	4.18	2.08	2.34
13	0.5	2.93	.86	.68
	1	3.82	1.36	1.69
	2	4.42	2.08	2.59

HPC are given in Table I. With the exception of the data on 20 mg per kg of carinamide where single experiments were done, the figures are averages of 2 or 3 experiments. It is evident from these results that the injection of 20 mg per kg of carinamide has no effect on the concentration of penicillin in the plasma, but that both 40 mg per kg of carinamide and 20 mg per kg of HPC give definitely higher concentrations of penicillin in the plasma. In Table II, the results of the experiments on phenol red excretion are given. The control values are the mean of 5 or 6 determinations which agree very closely. It can be seen from these data that 20 mg per kg of either carinamide or HPC markedly reduced the excretion of phenol red.

The effectiveness of carinamide and penicillin in elevating the concentration of penicillin in the plasma and in decreasing the excretion of phenol red is of the same order of magnitude. The data seem to indicate that HPC is more effective in raising the concentration of penicillin in the plasma than carinamide but this difference may not be significant.

We have interpreted our data to mean that HPC blocks the renal tubular secretion of both phenol red and penicillin. It has been shown that HPC decreases the renal clearance of phenol red without decreasing glomerular filtrate(8). This interpretation in the case of penicillin is based upon the assumption that the increase in the concentration of penicillin in the plasma is due to a blocking of the secretion of this substance by the renal tubular mechanism. However, there is every reason to believe that this assumption is correct. Carinamide has been found to

decrease the tubular secretion of penicillin and raise its concentration in the plasma after injection. The results reported indicate that cinchoninic acid derivatives which decrease the rate of excretion of phenol red will also block the renal tubular secretion of penicillin.

Summary. Similar to carinamide, 3-hydroxy-2-phenylcinchoninic acid causes higher concentrations of penicillin in the plasma when injected before the administration of the penicillin. Both substances also decrease the rate of excretion of phenol red. The activities of the two drugs are roughly of the same order of magnitude. This increase in concentration of penicillin in the plasma and decrease in rate of excretion of phenol red is attributed to a blocking of the renal tubular secretory mechanism.

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Desoxycorticosterone Acetate and Adenohypophyseal Content of Adrenocorticotrophic Hormone.* (18012)

CHI-PING CHENG AND GEORGE SAYERS

From the Department of Pharmacology, University of Utah, College of Medicine, Salt Lake City.

Twenty-four hours after adrenalectomy the content of adrenocorticotrophic hormone (ACTH) in the rat adenohypophysis is reduced to one-fifth of normal(1). Sayers and Cheng(1) favor the view that this marked depletion of pituitary ACTH is a consequence of rapid and uninhibited discharge of trophin in response to the absence of circulating cortical hormone. The data of the present paper are compatible with this interpretation. They demonstrate the desoxycorticosterone acetate (DCA) prevents the marked depletion of ACTH which normally occurs after adrenalectomy, and that DCA increases the concentration of ACTH in the adenohypophysis of the intact rat.

Methods. The donor rats were male animals obtained from the Sprague-Dawley farm. The body weights of these animals are presented in Table I. DCA pellets (six-15 mg pellets per rat) were implanted 72 hours before sacrifice and adrenalectomy was performed 24 hours before sacrifice. The animals were given free access to food and water at all times except those in Experiments 3 and 4, from whom food was withdrawn 24 hours previous to sacrifice. The animals were anesthetized with sodium pentobarbital, exsanguinated by severing the abdominal aorta and the pituitary dissected away from the sella. The anterior lobes were carefully freed of posterior pituitary tissue, frozen and lyophilized. The concentration of ACTH in the dried tissue was determined in hypophysectomized recipient rats by the method of Burns *et al.*(2).

A previous study(2) has demonstrated that each mg of dried adenohypophysis from untreated intact rats contains the biological equivalent of 80 μg of an ACTH standard (La-1-A†). This value was employed in the calculation of pituitary ACTH content. The weight of the pituitary tissue multiplied by ACTH concentration gives total hormone content per pituitary. For example, in Experiment 1 the content of ACTH in each pituitary of the intact untreated controls was obtained by multiplying 1.35 mg, the average weight of each pituitary, by 80 μg per mg, the concentration of ACTH in the tissue.

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