studies in humans indicate that 8 to 10% of Cr^{51} is Fraction II.

Most of the Cr^{51} is retained in the tissue where sequestration of erythrocytes occurs, so that by external counting it is possible to demonstrate in patients the site of erythrocyte sequestration. This is a routine procedure in clinical hematology. However, if hemolysis occurs within the blood vessels, the liberated hemoglobin will be cleared by the reticulo-endothelial elements of the liver, spleen, etc. Hence, external scanning will show a radioactivity distribution pattern as described here (Fig. 6 a, b, c). When following tagged red cell injection, marked accumulation of Cr^{51} in the spleen is demonstrable by means of external counting, splenectomy may be beneficial(14). Our studies seem to shed light upon the underlying mechanism responsible for the high splenic radioactivity in the case of selective splenic sequestration of erythrocytes. In contrast, intravascular hemolysis will result in hepatic and splenic deposition of Cr^{51} in proportion indicated on Fig. 6.

Summary. (1) Following *in vitro* labeling of erythrocytes with $Na_2Cr^{51}O_4$, a considerable amount of the Cr^{51} was found unattached to hemoglobin. (2) This unattached Cr^{51} lost the ability to label erythrocytes, and when given intravenously, was excreted rapidly by the kidneys. (3) Hemoglobin-bound Cr^{51} was cleared from the blood the same as was previously reported with I^{131}-hemoglobin. After clearance, however, much tissue retention of Cr^{51} was noted. (4) Rats metabolized homologous and canine hemoglobin similarly, with slow excretion of Cr^{51} by the kidneys. (5) The clinical implications of these findings indicating some peculiar metabolic characteristic of Cr^{51}-tagged erythrocytes are discussed.

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Vasopressin Antagonism by Azacyclonol in Hydrated Rats.* (28322)

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An antidiuretic action of azacyclonol (α-4-piperidyl diphenyl carbinol hydrochloride) in the rat has been reported by Sanchez and Davis(1). Release of antidiuretic hormone (ADH) by the drug was investigated and excluded as a possible mechanism of this ac-

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tion(1). A further significant observation was the antagonism of exogenous ADH (vasopressin) by a subeffective dose of azacyclonol. Study of the interaction between these agents seemed indicated as possibly leading to an understanding of the azacyclonol antidiuresis. Since Brown *et al.* found hypotension at times with azacyclonol in dogs(2), it also seemed essential to investigate whether an alteration in renal hemodynamics might be the cause of the antidiuresis. Results of these extensions of the earlier study are reported herein.

Methods. Female Sprague-Dawley rats were used in all experiments. Food was withdrawn 12-16 hours prior to tests, but water was available *ad libitum*. Urine was collected from metal metabolism cages, each housing a group of 3 rats. An initial water load of 25 ml/kg was followed after 2 hours by a further 50 ml/kg dose administered orally *via* a rubber tube. Drugs were injected intraperitoneally in a constant volume of 10 ml/kg at the time of the second water load. Water excretion at selected intervals after that time was expressed as percent of total water load minus the amount excreted between the 2 hydrating doses. Any group of rats failing to excrete 50% of the initial load within 2 hours was rejected from use on that day. The rats were reused at intervals of not less than 10 days and were assigned an equal number of times to the control and treatment groups.

Thirty-six rats weighing 200-280 g were used in studying the effect of azacyclonol on renal hemodynamics. Six control groups received 200 mg/kg creatinine and 50 mg/kg para-aminohippuric acid (PAHA) intraperitoneally in 10 ml/kg of distilled water. Seven test groups were treated identically except that azacyclonol was added to the solution to yield a dosage of 50 mg/kg. Five other test groups received creatinine plus PAHA and 75 mg/kg of azacyclonol. Urine samples were collected every 30 minutes, the volume recorded, and subsequent analysis for creatinine and PAHA was made using a B&L Spectronic 20 according to the methods of Bonsnes and Taussky(3), and Smith *et al.*(4), respectively. Amounts of creatinine and PAHA excreted were expressed as percent of total amount administered. Following Van Arman(5) excretion of creatinine was presumed to be proportional to glomerular filtration rate (GFR), and that of PAHA proportional to renal plasma flow (RPF).

Eighty-one rats weighing 150-280 g were used in studying combinations of azacyclonol and vasopressin. Control groups received either saline, vasopressin (0.1 U/kg), or azacyclonol (12.5 to 100 mg/kg). Interactions between vasopressin and azacyclonol were studied in treatment groups which received this antidiuretic dose of vasopressin (Pitressin, Parke, Davis & Co.) in combination with the varied doses of azacyclonol. Solutions combining vasopressin and azacyclonol were prepared so as to enable simultaneous administration of the 2 drugs in one 10 ml/kg intraperitoneal injection. Urine was collected at hourly intervals for 3 hours.

Results. For 1½ hours after injection of 50 mg/kg of azacyclonol there was no significant decrease in clearance of creatinine and PAHA even though a distinct antidiuresis was occurring (Table I). During this period urinary concentration of both substances was significantly increased. However, after 75 mg/kg of azacyclonol there was a pronounced and concurrent diminution of rates of excretion of water, creatinine, and PAHA.

The results of combined ADH-azacyclonol treatment (Fig. 1) confirm a blocking of

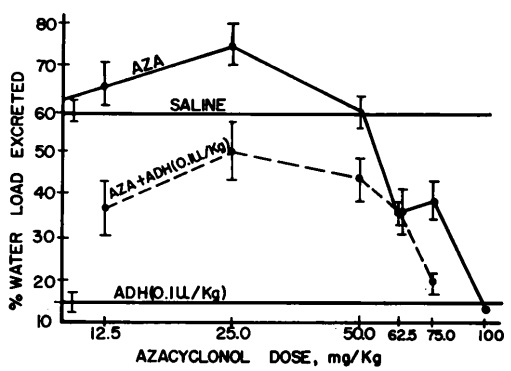

FIG. 1. Effects of azacyclonol-vasopressin combinations on 2-hr cumulative water excretion in comparison to azacyclonol, vasopressin and saline controls. (Vertical bars represent standard error.)

TABLE I. Excretion of Water, Creatinine, and PAHA as Cumulative Percent of Load (%L) and Cumulative Percent of Control Excretion (%C).

Substance excreted and treatments	No. of groups	Periods of collection: 1 hr %L	1 hr %C	1½ hr %L	1½ hr %C	2 hr %L	2 hr %C	2½ hr %L	2½ hr %C
Water									
Control	6	30.7		53.7		68.5		79.2	
Azacyclonol 50 mg/kg	7	*15.3**	51.4	*42.3*	78.8	69.3	101.2	82.5	104.1
Azacyclonol 75 mg/kg	5	*8.3*	27.0	*30.0*	55.8	*43.9*	64.1	62.3	78.6
Creatinine									
Control	6	44.0		59.8		67.5		72.4	
Azacyclonol 50 mg/kg	7	44.3	100.7	60.0	100.5	72.4	107.2	75.2	103.8
Azacyclonol 75 mg/kg	5	*19.1*	43.4	*36.8*	61.5	*47.0*	69.6	*54.5*	75.3
PAHA									
Control	6	72.8		86.6		90.9		92.1	
Azacyclonol 50 mg/kg	7	66.2	90.9	81.4	94.0	84.8	93.3	87.6	95.1
Azacyclonol 75 mg/kg	5	*38.7*	53.1	*69.1*	79.8	*77.5*	85.2	*80.4*	87.3

* Italicized averages are significantly less than appropriate control values ($P < .05$).

ADH-antidiuresis by azacyclonol as was suggested by earlier data(1). Azacyclonol was antagonistic to ADH at doses from 12.5 to 62.5 mg/kg, while at 3.0, 6.25 and 75.0 the antagonism was absent. In addition, a mild but statistically significant diuretic effect was noted for the first time in control groups receiving only azacyclonol at the 12.5 and 25 mg/kg dosages (Fig. 1).

Discussion. The finding that azacyclonol in a dose of 50 mg/kg produced a significant antidiuretic response cannot be attributed to a decrease in glomerular filtration rate or renal plasma flow since total excretion of creatinine and PAHA was not decreased. Moreover, there was an increase in urinary concentration of creatinine and PAHA during the period of antidiuresis. This concentrating effect which must have involved a reabsorption of water suggests an ADH-like action. It cannot be ascertained from these observations whether the distinct reduction of GFR and RPF by 75 mg/kg azacyclonol was totally responsible for the antidiuresis noted with this and higher dosages.

A possibility considered upon beginning the study of vasopressin-azacyclonol combinations was that azacyclonol displays a weak ADH-like action on the kidney whereby the azacyclonol-ADH antagonism would result from a "partial agonist" effect(6). This would be a competitive antagonism in which azacyclonol and ADH share an affinity for the same tissue receptors, but with the intrinsic activity of azacyclonol being much less than that of the ADH molecule. According to this hypothesis the combination of slightly to moderately antidiuretic doses of azacyclonol with an antidiuretic dose of ADH would be expected to yield results intermediate between the responses induced by either drug alone. Our observations are much in accord with this expectation.

Unexpectedly, a mild but significant diuretic effect of azacyclonol was noted at 12.5 and 25 mg/kg. Possibly the antagonism between these doses of azacyclonol and the dose of vasopressin (Fig. 1) could be interpreted as resulting partly or solely from this diuretic tendency. However, such an interpretation would not suffice to explain the interaction between ADH and azacyclonol in doses from 50 to 62.5 mg/kg which alone certainly did not have diuretic action, but rather were antidiuretic for the first hour or two. Since azacyclonol in doses of 75 mg/kg or greater alters the renal hemodynamics significantly, any conclusions regarding the in-

teraction of azacyclonol and ADH at this dose range are unwarranted. Further investigation is required to explain the diphasic pattern of action of azacyclonol upon urine output in the rat.

Summary. Studies of creatinine and PAHA clearance showed a sufficient reduction of GFR and RPF to explain the antidiuretic action of a large dose (75 mg/kg) of azacyclonol. However, a moderate dose (50 mg/kg) produced antidiuresis in the absence of such changes in creatinine and PAHA clearance. A mild diuretic effect was noted in lower doses (12.5 to 25.0 mg/kg). Azacyclonol in doses from 12.5 to 62.5 mg/kg showed antagonism to the antidiuretic action of vasopressin. It is suggested that this antagonism may be the result of weak ADH-like properties of azacyclonol.

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Enzymatic Digestion of Mucoproteins.* (28323)

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Mucoproteins are found in blood, in mucus, in tumors and about sites of injury. They are elevated in the blood after injury, in arthritis, tuberculosis, cancer and obstructive biliary disease(1,2,3). They are increased in amount about wounds and in malignant tumors. To determine what enzymes attack mucoproteins is the subject of this investigation. This information may help us to understand how mucoproteins are concentrated and whether enzymes are involved in the changes. There are no previous publications on the susceptibility of mucoproteins although trypsin and chymotrypsin have been used to degrade mucoproteins to study breakdown products(4). Recently Pigman *et al.* have stated that mucins are poorly digested by trypsin(5). The enzymes to be employed consist of some that should attack the carbohydrate moiety of the mucoprotein, some that should attack the protein fraction and some that should attack both.

Methods. The seromucoproteins employed were extracted from plasmas of rabbit and human blood by an adaptation of the quantitative method of Winzler, Devon, Mehl and Smyth(6). For this isolation a volume of 0.75 M perchloric acid double that of the plasma was added slowly with shaking. After standing for exactly 10 minutes the precipitate of other proteins obtained was filtered off. Longer contact destroys human mucoproteins (Table I). Then a volume of 5% phosphotungstic acid in 2N hydrochloric acid, equal to the original volume of plasma used was added to the filtrate and shaken to bring down a flocculant white precipitate. After being refrigerated overnight this second precipitate was filtered off. Then just enough 0.2 N sodium hydroxide was added to dissolve the precipitate off the filter paper, washing several times with distilled water to remove the last traces. This concentrated solution of mucoproteins, amino acids and

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