

The Diuretic Action of Ruscus Aculeatus

by

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I - INTRODUCTION

For many years the inhabitants of the mountainous districts in certain regions of the Near East have used the infusion of *Ruscus aculeatus*, commonly known as Butcher's Broom, or Petit-Houx, as a diuretic, and local physicians have recognized its efficacy in treatment of some edemas. The rhizome of this plant has long been used by some pharmacists as an adulterant for Senega (1). In 1901, Dubat (2) reported the presence of sacchrose, mannane, dextrane, and pentosane in the seed of the plant. Castoro (3) found a trace of arabane and confirmed the presence of mannane in the seed. Bourquelot (4) estimated the amount of sacchrose in the leaf and stems to be about 3.6 per cent and Greshoff (5) has isolated a saponin from this part of the plant. Jonesco (6) detected the presence of the dyes, anthocyane and anthocyanidine in the coloring matter of the fruit.

Recently a report of certain pharmacological studies by Balansard and Delphaut (7) on the diuretic action of *R. aculeatus* was called to our attention. This in so far as we can determine is the only pharmacological study of this plant which has appeared in the literature prior to 1940. In this laboratory, preliminary experiments with intravenous injections of watery extracts of *R. aculeatus* (8) showed not only a diuresis but also a marked transient fall of blood pressure in the anesthetized dog.

The present studies have been undertaken with a two-fold purpose:

(a) To isolate and identify the principle responsible for the

diuresis and

(b) To determine the efficacy of the principle and its site of action.

II - CHEMICAL INVESTIGATIONS

Preliminary experiments with intravenous injection of the water extract of *R. aculeatus* showed the presence of vasodepressor and diuretic principles (8). The present chemical investigation was undertaken in an attempt to separate these two principles and to isolate and identify the one responsible for the diuresis.

1- Methods: The dried leaves and small stems of *R. aculeatus* are exhaustively extracted with a minimum of hot water and the total volume of the watery extract reduced to 1/4 by evaporation. This concentrated solution is then treated with ethyl acetate in a separatory funnel and allowed to stand for several days during which time a white precipitate is slowly formed. This precipitate, removed by centrifuging, is dissolved in a known quantity of water such that each cubic centimeter of the solution represents one gram of the dried leaf. This precipitate will henceforth be referred to as the Eth.Ac.Ppt. The remaining aqueous solution is separated from the ethyl acetate layer then freed of any dissolved ethyl acetate by distillation, after which it is treated with sufficient ethyl alcohol (95%) to make a final alcoholic solution of 50 %. The precipitate thus formed is removed by centrifuging. It is then washed with 50% alcohol and dried. This precipitate will be referred to as the EtOH Ppt. The filtrate is next freed of alcohol by distillation and this final aqueous solution will be referred to as the Resid. Sol.

2- Results: Pharmacological studies of these fractions revealed that the Eth.Ac.Ppt. produced a diuretic effect whereas EtOH Ppt. and Resid. Sol. had no such action (section III of this report). We have been able to confirm the presence of the vasomotor factor.

Figure 1 shows the blood pressure tracings in the dog under dial-urethane anesthesia. The intravenous injection of the water extract produced a marked, transient fall in blood pressure. The Eth.Ac.Ppt., which has been shown to possess diuretic properties, produces no demonstrable change in the blood pressure. The EtOH Ppt. shows the presence of the vasodepressor factor but studies have shown it to lack the diuretic property. The Resid. Sol. also causes a lowering of blood pressure but does not induce diuresis. Thus we can conclude that, by the procedure outlined above, it has been possible to separate effectively these two principles.

Chemical analysis of the Eth. Ac. Ppt. showed the presence of carbon, hydrogen, oxygen and calcium. The calcium was found to make up about 40 % of the total weight of the dried fraction. Thus far, all attempts made to prove the purity of this substance have failed. It is soluble in water and freely soluble in dilute acids and alkalies. The water solution of this fraction is neutral to litmus. Furthermore, it decomposes when heated to about 120° C and is able to reduce potassium permanganate in the cold. Although this diuretic principle can be extracted from the dried leaf with alcohol, after extraction by infusion or decoction it is precipitated from solution on the addition of alcohol.

We feel that the experimental data now available is too meager to venture any opinion regarding the chemical nature of this substance.

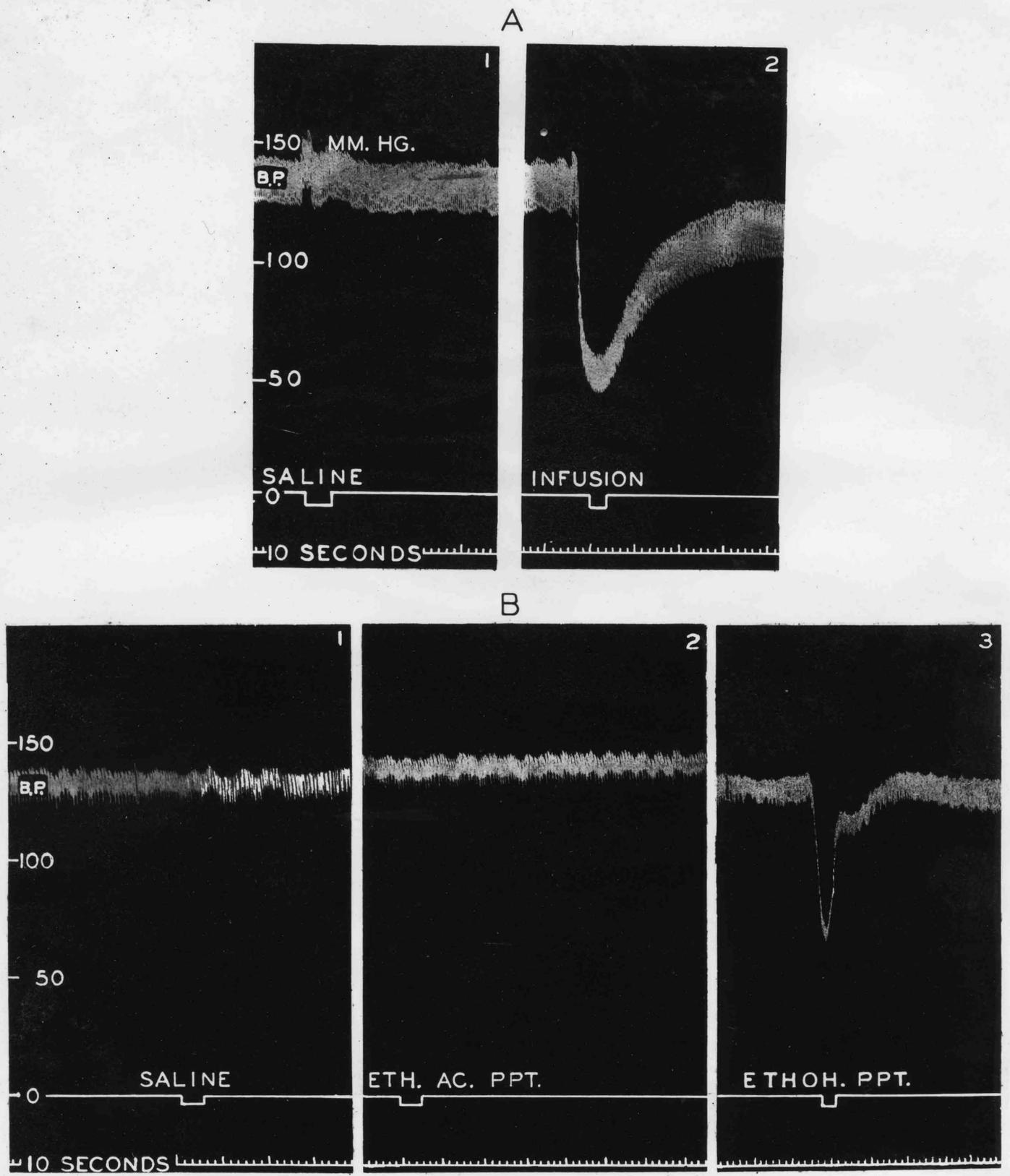


Figure 1: The effects of intravenous injections of saline, infusion of *Ruscus aculeatus*, Eth. Ac. Ppt. and EtOH Ppt. on the arterial blood pressure in dogs under dial-urethane anesthesia. Tracings A are from ~~one~~ animal; tracings B from another.

III - PHARMACOLOGICAL STUDIES

It was necessary to use a biological method in tracing the diuretic principle through the various steps of the chemical procedures employed in the fractionation. To this end the effect of each fraction on the chloride and urine volume output in the anesthetized dog was determined. Studies on the efficacy of these fractions as compared with some of the clinically acceptable diuretic agents were undertaken on the unanesthetized animal.

A - Studies on the Anesthetized Dog

1- Methods: Dogs weighing 7 kg. or more were used. On the eve of the experiment they received the usual amount of food and in addition 300 to 700 cc. of water by stomach tube. The next morning, about 3 hours before the start of the experiment, the animals were given a similar quantity of water. Chloralose (90 mg. per kg of body weight) was the anesthetic employed in 12 experiments, while in 15 others the animals were anesthetized with dial-urethane (Ciba) (0.6-0.8 cc. per kgm.). In 8 other experiments sodium pentobarbital (39 mgm. per kgm. intraperitoneally) or sodium phenobarbital (125 mgm. per kgm. intravenously) was employed as the anesthetic agent. The responses did not appear to be influenced by the anesthetic. It was found that dial-urethane, properly administered, was a satisfactory anesthetic for our purposes as it produces a moderately deep anesthesia and maintains the anesthesia at a constant level for a period of from 4 to 7 hours without depressing the arterial blood pressure below the critical level for renal activity.

The operative procedure was the same in all experiments.

The blood pressure was recorded from one of the carotids by means of the ordinary mercury manometer. The trachea was cannulated for artificial respiration and one of the femoral veins was prepared for injections. The ureters, exposed by a median abdominal incision, were carefully cleaned of connective tissue and fat, and cannulated near the bladder with fine glass cannulae attached to narrow rubber tubes. Calibrated 5 cc. graduated cylinders were arranged for collecting the urine. The animal was then given 150 to 300 cc. of normal saline by vein and 45 to 60 minutes later the observations were begun. Samples of urine were collected continuously throughout the course of the experiment and the chloride content estimated by the Volhard method, as modified by Harvey (9). In 4 experiments blood analyses were performed concurrently with the urinalysis. The non-protein nitrogen of the blood and total nitrogen of the urine were determined by the method of Koch and McMeekin (10). The chloride content of the whole blood was determined by the method of Wilson and Ball (11). The specific gravity of the urine was determined by weighing a measured volume of the urine and comparing the weight to that of an equal volume of redistilled water.

2- Results: Early experiments with the infusion of *R. aculeatus*, performed on dogs under chloralose anesthesia demonstrated the presence of a principle which was able to cause a definite though relatively small increase in the rate of urine flow. The percentage increase in urine flow ranged from 90% to 300% above the normal rate and 50% to 175% above the rate of urine flow after the control injection of normal saline. In addition it was noted that the

specific gravity of the urine, after *R. aculeatus* injections, increased concurrently with the increase in rate of urine flow. The percentage increase being from 2% to 5%. In these same animals the injection of normal saline (10 cc.) caused either no effect or a slight decrease in the specific gravity. However, in either case the total solids excreted was the same as before the injection. Intravenous injection of mercurial diuretics (Salyrgan) (100 mgm.) caused a marked increase in the rate of urine flow but the specific gravity of this urine was so low that there was only a slight increase in the total solids excreted. *Ruscus aculeatus* extracts, on the other hand, caused but a small increase in the rate of urine flow but the specific gravity was so markedly elevated that the calculated excretion of total solids showed a definite increase.

As we soon found the chloride excretion greatly affected by the extracts employed, this urinary constituent was chosen as an indicator of diuresis and the degree of diuresis.

Fifteen consecutive experiments are submitted and the results summarized in figure 2. In these experiments the only anesthetic employed was dial-urethane (Ciba). Four experiments consisted only of collecting and analyzing the urine over a period of 140 minutes to determine the effect of the anesthetic and the experimental conditions described above. In 5 additional control experiments the animals received only normal saline (10 cc.) 80 minutes after the start of the experiment. In the remaining 6 experiments the animals received 10 cc. of saline 40 minutes after the start of the experiment, then 40 minutes later were given the diuretic fraction made up to 10 cc. with saline. In 6 other experiments the effect of this preliminary injection of saline was followed for 100 minutes. The slight effect on urine volume and chloride content usually noted invariably subsided within 20 minutes of the injection. In 4 other

experiments the blood as well as the urine was analyzed.

The succeeding protocols are from typical experiments illustrative of the procedure employed and the results obtained.⁽¹⁾

(1) It may be well to keep in mind the effect of the experimental conditions on the renal activity in animals which did not receive any injection subsequent to the initial saline infusion. There was a brisk flow of urine immediately following the infusion. The peak of this diuresis came about 20 minutes after the infusion was started and soon the flow of urine began to diminish. By the end of the first hour the urine flow had decreased to an almost constant rate. Usually by the end of the third hour the rate of urine flow began to increase very gradually. The chloride content, although very high at first, decreased very rapidly to a low and rather constant level. At the end of the third hour, when the urine flow began to increase gradually, the chloride content began to fall in a rather marked fashion. As all experiments were carried out under very similar procedures and experimental conditions, it seems safe to assume that the functioning kidney tissue of each animal acted in a similar fashion. Therefore the effects produced by the injection of any single substance should be discussed in the light of this phenomenon.

Protocol I, Experiment E-21:

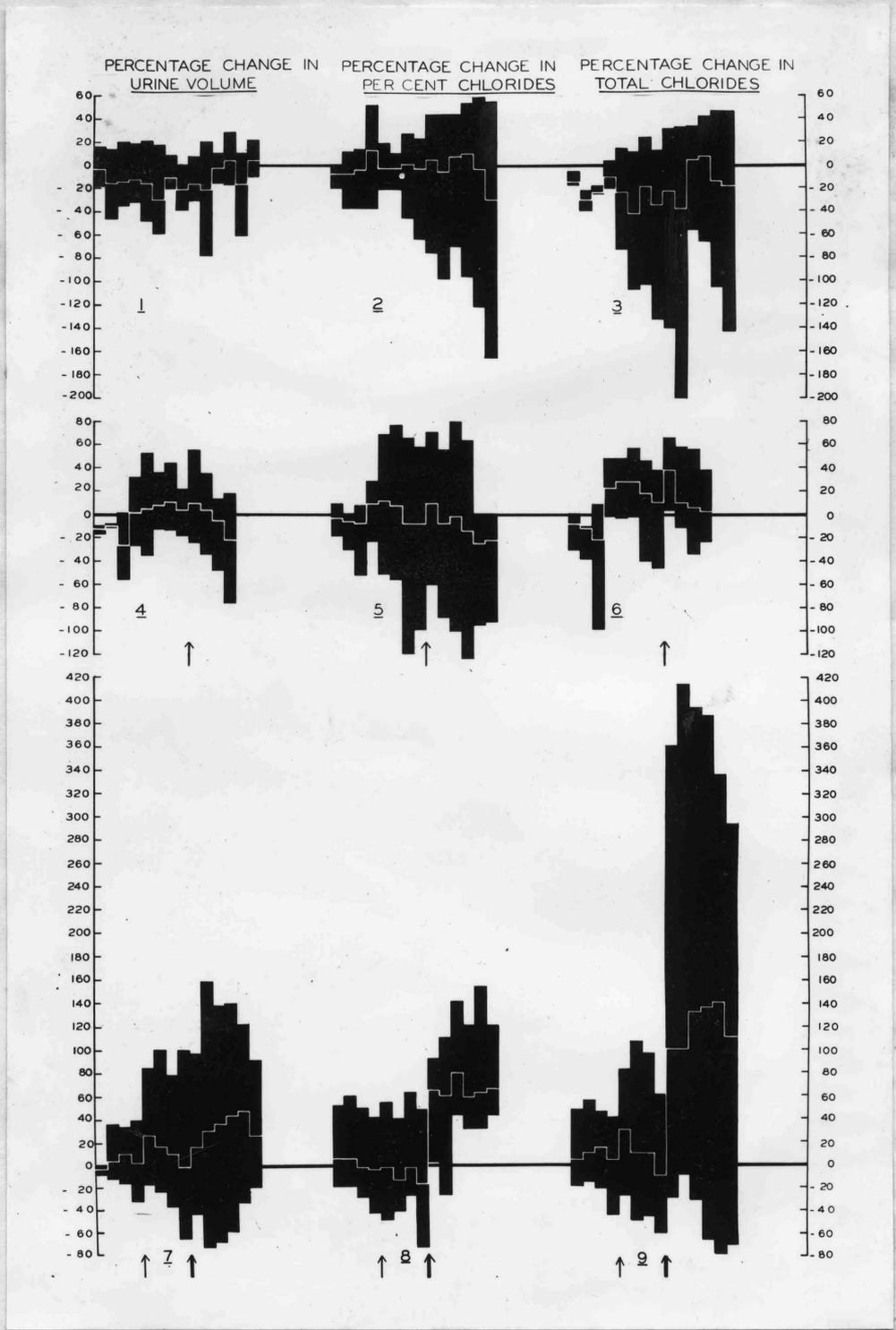
A male dog weighing 19.7 kgm. was anesthetized with dial-urethane (Ciba) (0.85 cc. per kgm.) intraperitoneally. Five hundred cubic centimeters of water had been given by stomach tube the evening preceding the experiment. A similar quantity was given 3 hours prior to the administration of the anesthetic. Forty-five minutes before the observations were begun, normal saline (300 cc.) was given by vein. Urine samples were collected at 10 minutes intervals. All substances were given by vein. The volume of the ureteral cannulae was 0.15 cc each. Table I summarizes the data.

Substance given	Sample No.	Urine Volume (cc.)	Chlorides per cent (mgs.)	Total Chlorides (mgs.)
Saline 10 cc.	1	1.64	178	2.92
	2	1.47	185	2.71
	3	1.39	171	2.37
	4	1.33	192	2.55
	5	1.39	174	2.42
	6	1.42	121	1.72
	7	1.40	163	2.29
	8	1.40	162	2.27
Eth. Ac. Ppt. 5 cc.				
Saline 5 cc.	9	1.64	260	4.25
	10	1.91	185	3.52
	11	1.95	231	4.50
	12	2.29	220	5.05
	13	2.64	242	6.38
	14	2.49	213	5.30

Table I: Tabulation of the data from Experiment E-21, Protocol I.

The effect of the diuretic fraction on urine volume, chloride per cent, and total chloride, from one ten minute period to the next, is shown in figure 2. The range of the distribution of the values is shown by the black bars: The averages are represented by the white line superimposed on each graph.

The increase in the urine volume after the injection of the diuretic fraction was only slightly greater than that after an equal volume of physiological saline, but the chloride excretion was much greater than after saline. In fact, the chloride per cent excreted is 40 times greater after Eth. Ac. Ppt. than with an equal volume of normal saline. Furthermore, the slight diuretic action of the saline ~~disappears~~ in about ten minutes, whereas the Eth. Ac. Ppt. effect is still at its peak 60 minutes after the injection. In the case of total chloride excretion, the increase evoked by the diuretic fraction is ~~eighteen fold~~ greater than that following an equal quantity of physiological saline. The experiment submitted in protocol II shows definitely that EthOH Ppt. and Resid. Sol. alter the renal activity only to the same extent as an equal volume of normal saline.



Figures 2: Percentage change in urine volume, per cent chlorides and total chlorides in dogs under dial-urethane anesthesia. Each column represents a 10-minute period. Graphs 1, 2 and 3, no injections made: Graphs 4, 5 and 6, physiological saline (10 cc.) at arrows: Graphs 7, 8 and 9, physiological saline (10 cc.) at light arrows; Eth. Ac. Ppt. at heavy arrows. Black columns show range of distribution of values: Averages are represented by the superimposed white lines.

Protocol II, Experiment E-23:

A male dog weighing 21.8 kgm. was anesthetized with dial-urethane (Ciba) (0.70 cc. per kgm.) intraperitoneally. Five hundred cubic centimeters of water was given by stomach tube during the early evening of the day preceding the experiment. This was repeated on the morning of the experiment. Physiological saline (300 cc.) was given by vein 45 minutes before the actual observations were begun. Urine samples were collected at 10 minute intervals. All substances were given by vein. The volume of the urteral cannulae was 0.15 cc. each. Table II summarizes the data.

Substance given	Sample No.	Urine Volume (cc.)	Chlorides per cent (mgs.)	Total Chlorides (mgs.)	
Saline	10 cc.	1	1.70	213	3.62
		2	1.68	260	4.36
		3	1.66	209	3.47
		4	1.59	192	3.06
		5	1.50	213	3.20
		6	1.34	184	2.46
		7	1.20	222	2.66
		8	1.17	238	2.78
Resid. Sol.	5 cc.	9	0.50	258	1.29
Saline	5 cc.	10	0.00	-	--
		11	0.00	-	--
		12	0.00	-	--
		13	0.50	222	1.11
		14	0.85	317	2.69
		15	0.60	222	1.33
		16	0.65	222	1.44
EtOH. Ppt. Saline	5 cc.	17	0.81	289	2.34
	5 cc.	18	0.52	209	1.09
		19	0.63	298	1.88
		20	0.68	435	2.96
		21	0.70	398	2.78
		22	0.68	494	3.36
Eth. Ac. Ppt. Saline	5 cc.	23	0.65	795	5.16
	5 cc.	24	1.63	556	9.08
		25	1.37	639	8.75
		26	1.41	476	6.71
		27	1.13	782	8.85
		28	1.41	810	11.41

Table II: Tabulation of the data from Experiment E - 23,

Protocol II.

It may be pointed out that the increase in chloride excretion obtained with the diuretic fraction of *R. aculeatus* is comparable with that obtained with rather large doses of certain mercurialsdiuretics by Melville and Stehle (12) in the anesthetized dog.

Protocol III is one of a series of four experiments in which an attempt was made to study the changes in the blood brought about by Eth. Ac. Ppt.

Protocol III, Experiment E-47:

A male dog weighing 14.2 kgm. was anesthetized with dial-urethane (Ciba) (0.80 cc. per kgm.) intraperitoneally. Five hundred cubic centimeters of water was given by ~~stomach~~ tube during the early evening of the day preceding the experiment and a similar quantity on the morning of the experiment. Normal saline (300cc.) was given by vein 50 minutes before the actual observations were begun. Urine samples were collected at 10 minute intervals and blood taken mid-way between these periods. All substances were given by vein. The volume of the ureteral cannulae was 0.15 cc. each. Table III summarizes the data.

Remarks	Sample No.	U r i n e					B l o o d	
		Volume (cc.)	Chlorides		Total N		Chlorides per cent (mgs.)	N.P.N. per cent (mgs.)
			Total (mgs.)	per cent (mgs.)	Total (mgs.)	per cent (mgs.)		
	1	2.00	6.90	345	22.6	1128	308	81.4
	2	1.55	3.88	249	20.1	1295	314	92.6
	3	1.53	3.12	204	19.1	1250	308	89.1
	4	1.40	2.06	147	18.6	1329	310	81.4
Saline 10 cc.	5	1.31	1.84	91	20.4	1550	308	92.6
	6	1.28	1.01	79	19.4	1515	318	96.2
	7	1.17	0.33	28	20.9	1787	--	--
	8	1.16	0.39	34	21.6	1866	308	82.0
Et.Ac.Ppt. 5 cc.								
Saline 5 cc.	9	1.07	1.15	108	23.5	2195	304	61.8
	10	1.16	0.99	85	25.5	2195	304	70.0
	11	1.12	0.89	79	24.2	2155	304	57.5
	12	0.77	0.96	124	18.9	2450	295	46.5
	13	0.56	0.76	124	12.3	2195	326	70.5
	14	0.42	0.57	136	10.8	2579	320	38.9

Table III: Tabulation of the data from Experiment E-47,

Protocol III.

The response of urinary chlorides and urine volume to Eth. Ac. Ppt., in these experiments, is the same as in the previous experiments (see tables I and II and figure 2). Eth. Ac. Ppt. produced a fall in blood chlorides of from 3% to 8% below the normal level. This was followed, at the end of about 40 minutes, by a sharp rise in blood chloride content to a level about 5% higher than the normal for that animal. The blood chlorides then gradually returned to the normal level over a period of 30 minutes. This effect was seen in all of the four experiments performed. The injection of normal saline produced no effect on the blood chloride level. After Eth. Ac. Ppt., the non-protein nitrogen of the blood showed a marked decrease of from 16% to 53%. At the end of 80 minutes the N.P.N. had not yet returned to the original level. In one experiment this fall was preceded by a transitory rise of 7%. In all but one experiment (Protocol III), the increase in urinary nitrogen excreted was sufficient to account for this marked fall in the N.P.N. of the blood. The per cent nitrogen of the urine was increased by from 40% to 75%. The total nitrogen excreted showed an increase of from 90% to 200%. Here again the injection of normal saline showed no demonstrable changes.

B - Studies on the Unanesthetized Dog

It was deemed advisable to continue the study of the effects of *R. aculeatus* on the unanesthetized dog. Because of the obvious difficulties and sources of error in making studies on urine collected by means of a metabolism cage, we decided to use dogs with fistulae of the urinary bladder. In these studies it was planned to administer the extracts of *R. aculeatus* by stomach tube as well as by injection and to compare these effects with those induced by some of the standard diuretics. We had hoped also to induce edema in these animals by plasmaphoresis and note the effect of *R. aculeatus* on this pathological condition.

1- Methods: Female dogs of 5 kgm. or more were employed. A suprapubic cystotomy was performed under aseptic conditions and the incised mucosa of the urinary bladder was everted over the skin and firmly anchored by a continuous suture of silk. After 2 weeks, allowing time for recovery from the effects of the operation, the animal was trained to remain quietly in a dog stand. During this period of training the kidney function of the animal was tested by means of phenolsulphonphthalein. The main purpose of this procedure was to determine if *R. aculeatus* extracts would affect the renal efficiency if given repeatedly over a protracted period of time. The response of the animal to small quantities of water by stomach tube (50-100 cc.) and physiological saline (10-25 cc.) by injection was determined. Six weeks after the operation we were prepared to start the studies on responses to diuretic agents.

The urine was collected in calibrated graduated cylinders by means of a Pezzer catheter inserted into the bladder through the fistula. Several additional openings were made in the mushroom end of

the catheter to insure a more thorough drainage of the bladder, and the tube of the catheter was cut short so that only a few centimeters remained attached to the mushroom end.

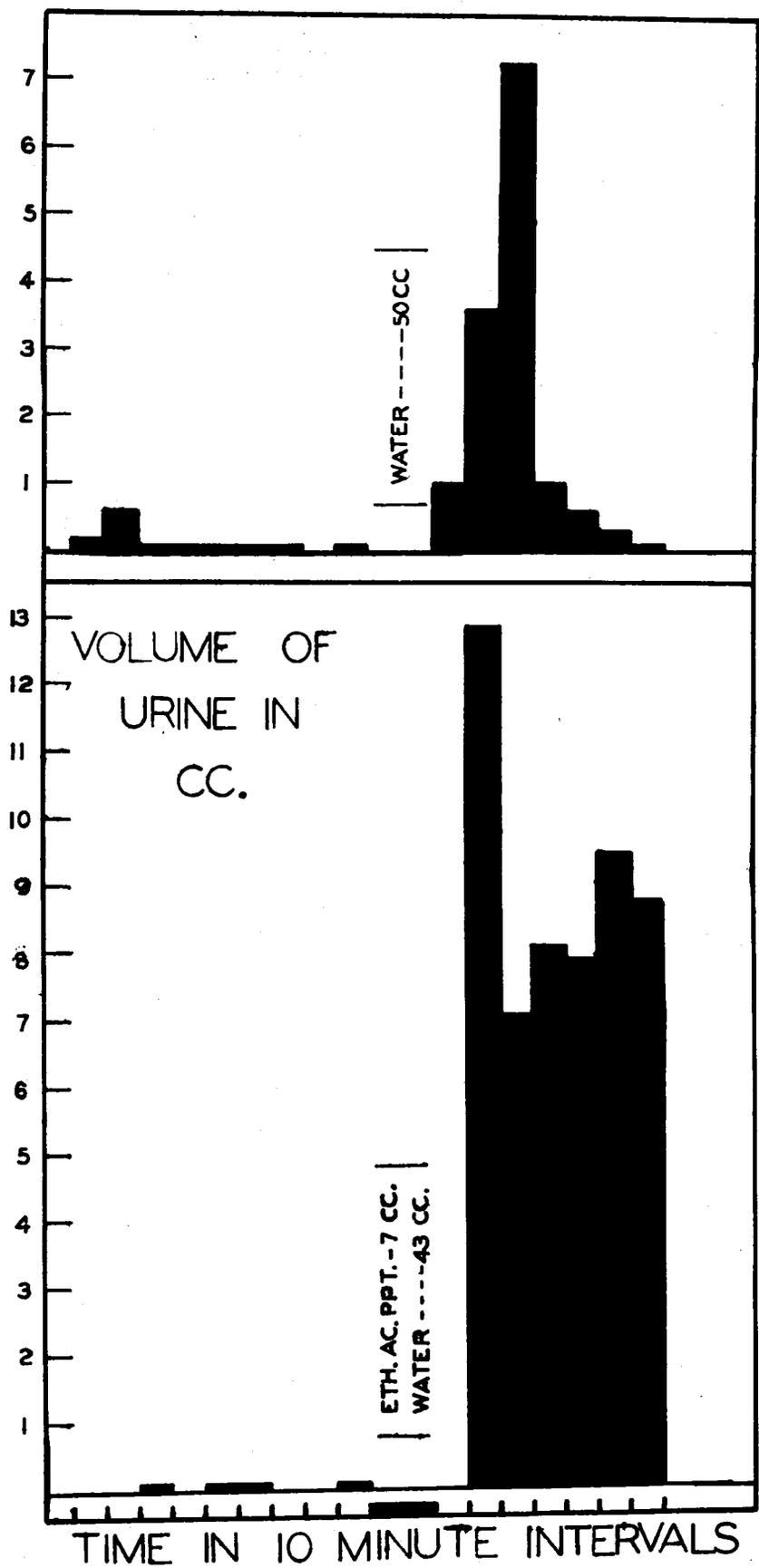
2- Results: Unfortunately, we have had little success with this procedure. Six attempts were made and in each case we were confronted with the same two obstacles. In the first place the wounded surface of the bladder wall failed to heal completely. This resulted in slight but persistent hemorrhage every time the wound was disturbed by even so minor a procedure as the insertion of a Pezzer catheter. As the shed blood contaminated the urine, a study of the composition of the urine is without significance. The second, and more serious obstacle, was that of ascending urinary infection which invariably terminated in a fatal issue. The onset of the infection was about 6-7 weeks after the operation.

In spite of these obstacles we did manage to perform 4 experiments involving the use of *R. aculeatus* fractions. Although we do not possess any significant data on the composition of the urine, protocol IV demonstrates the typical effect of Eth.Aa.Ppt. on the rate of urine flow.

Protocol IV, Experiment C4-7: March 4, 1939

Female bird dog weighing 19.6 kgm: Supra-pubic cystotomy performed on January 24, 1939. The dog was placed in the dog stand and preliminary readings of the rate of urine flow were taken for 90 minutes. Seven cubic centimeters of Eth. Ac. Ppt. were given by stomach tube and washed in with 43 cc. of water. Readings of the urine volume were taken every 10 minutes. Figure 3 summarizes the data.

Figure 3: Rate of urine excretion in an unanesthetized dog with fistula of the urinary bladder (Protocol IV). Each column represents a 10-minute period. The uppermost graph shows the response to water (50 cc.). The lower graph shows the response of the same animal to Eth. Ac. Ppt. (7 cc.) diluted with water (43 cc.).



In our study of the dogs with fistulae of the urinary bladder we have observed that the kidneys were most active during the first 3 to 4 hours after the animals were fed, and soon thereafter returning to what may be termed a "basal level". During this period of basal activity the kidney would produce only from 0.3 to 1.5 cc. of urine per hour. At this time, 50 cc. of water given by stomach tube would cause the rate of urine flow to increase to about 35 to 40 cc. per hour. The increase in flow was first detected about 10 minutes after the water was given and the effect passed off in a period of about 20 minutes. It was further noted that when 50 to 100 cc. of water was given by stomach tube, only $\frac{1}{3}$ to $\frac{1}{4}$ the volume given appeared in the urine. With these facts in mind it is interesting to note, in figure 3, that 7 cc. of Eth. Ac. Ppt. made up to 50 cc. with water caused a marked increase in the rate of urine flow. At the end of 70 minutes, 52 cc. of urine had passed from the bladder and still there were no signs of a return to the normal rate of flow. The animal showed a marked restlessness and thirst. When offered water, the animal drank avidly, the water intake being 103 cc. It is well to remember, in analyzing these results, that dogs normally drink very little water between meals: The maximum intake of water occurs soon after the animals are fed. This has been clearly shown by Kleitman (13) and by Gregersen (14). The restlessness may be explained as being caused by the marked thirst. Figure 3 also shows the effect on this animal of 50 cc. of water.

In the three other experiments, performed on three other dogs, the results were similar. In one dog of 5 kgm., 3 cc of the Eth.Ac.Ppt. made up to 50 cc. with water evoked a diuresis that was equal to that produced by 450 cc. of water alone.

C - Studies on the Rat

The rat was selected as a suitable animal in which to compare the effects of *R. aculeatus* with other diuretic agents. The reason for this choice is that it is very convenient to study these drugs on small groups of rats and to use these groups repeatedly. In this fashion, should a sufficient number of experiments be available, a statistical treatment of the data would be feasible and significant.

1- Methods: The rats were segregated according to sex, divided into groups of four each and placed into roomy circular cages constructed of heavy screening material. These cages were suspended on large glass funnels and calibrated graduated cylinders were placed below the spouts to catch the urine. The inner walls of the funnels were smoothly paraffined. A circular piece of finely meshed, paraffined screening was placed inside the funnel in such a manner as to prevent the feces of the rats from falling into the collecting cylinders below. All substances were administered by stomach tube. A fine rubber catheter, attached to a 50 cc. hypodermic syringe, served as the stomach tube. When the entire system was filled with the fluid to be administered, this method provided an accurate means of giving the fluid and obviated the necessity of using additional water to wash the drug into the stomach. The quantity of substance given each rat was determined on the basis of the body weight: Pregnant rats were not used in any of the experiments. We were not able to detect any differences in the response attributable to ~~sex~~ sex.

The chemical procedures referred to in part III, section

A-1 were used in studying the constituents of the rat's urine. In several instances the uric acid content of the urine was determined by the method of Folin (15). In some cases the specific gravity of the urine was determined by the direct weighing method. This data was used as an indication of the total solids present.

2- Results: In performing comparative studies of this nature, it is advisable and necessary to maintain the rats on a rigidly controlled diet. Four attempts were made and in each case we were forced to abandon the experiment because of the difficulties which arose in the maintenance of the chosen diet. Although these experiments may not be acceptable for a comparative and statistical study, they do show the effects of *R. aculeatus* extracts on the renal function in the unanesthetized rat, in comparison with an accepted diuretic. In the series submitted, 4 groups of 4 rats each were used and 7 experiments were performed. Diuretin in doses ranging from 20 to 40 mgms. per 100 gms. of body weight, was the diuretic chosen. Eth.Ac.Ppt. was administered in concentrations representing 1.3 to 5.0 gms. of the dried leaf per 100 gms. of body weight. All solutions were diluted so that 5 cc. of the fluid was given for each 100 gms. of body weight. The individual rats ranged in weight from 130 to 180 gms. but the rats were grouped so that the four groups were of approximately equal weight. In each experiment the groups of rats were placed in their cages and the urine collected over a period of 3 hours. At the end of this period the rats were given either Diuretin, Eth.Ac.Ppt. or water by stomach tube and the urine was again collected over a period of 3 hours. The percentage changes in

urine volume, chlorides per cent, total chlorides and specific gravity between these two periods were determined and are plotted in figure 4. The black areas represent the distribution of the values while the superimposed white line represents the average. In 8 experiments the rats were stomach tubed but no substance was given. These experiments demonstrated that the procedure employed in handling and stomach tubing the rats had no effect on renal function that could be detected by the methods at our disposal.

Figure 4: The effect of water (Column A), Diuretin (Column B) and Eth. Ac. Ppt. (Column C) on the urine volume, specific gravity, per cent chlorides and total chlorides in unanesthetized rats. The values plotted are the percentage change induced by the substance given as compared with the normal for those rats. The black areas show the range of distribution of these values: averages are represented by the superimposed white lines.

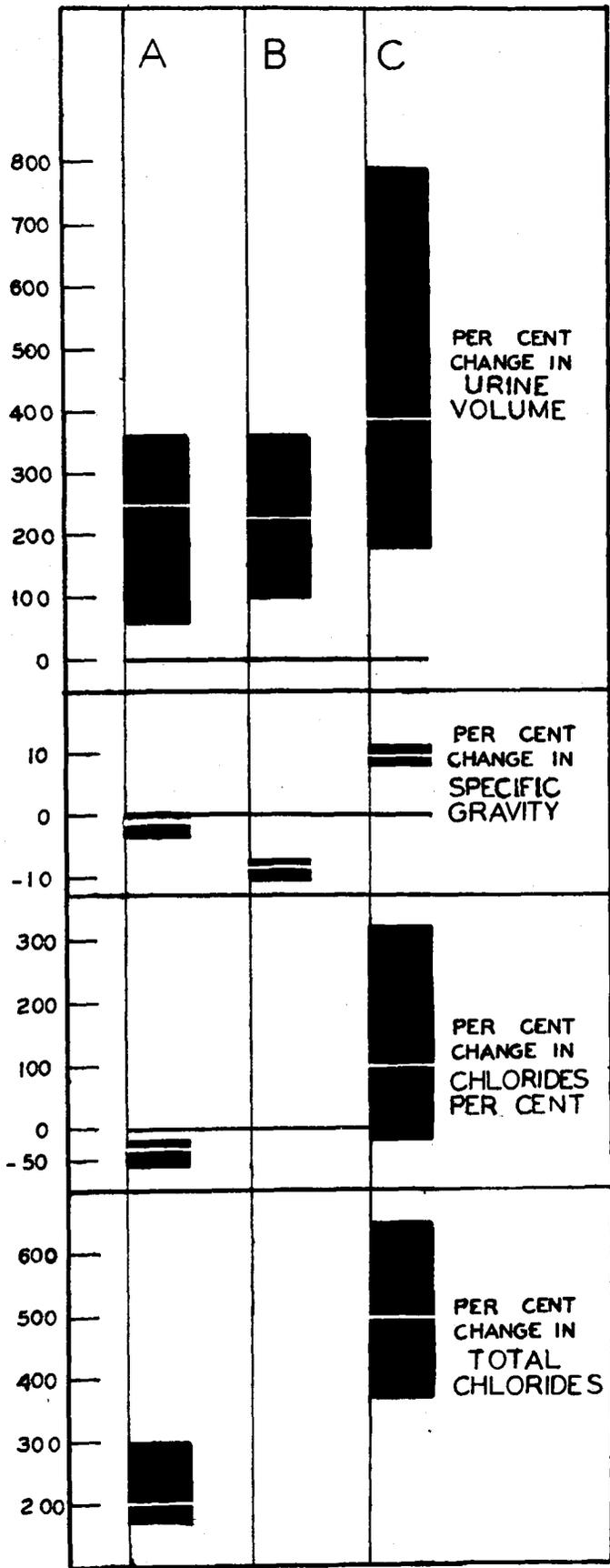


Figure 4

Figure 4 shows beyond doubt that Eth.Ac.Ppt. causes not only an increase in the rate of urine elaboration but also an increase in the total solids excreted. The latter can be very readily seen from the percentage changes in the specific gravity. This fact is further born out, in the case of Eth.Ac.Ppt., by the percentage increases in the chlorides per cent and total chlorides. We would like to point out that in two cases of this series, the percentage increase in total chlorides, caused by Eth.Ac.Ppt., ~~was~~ 2215% and 1653%. These two figures were not included in figure 4.

Although the total uric acid excreted was increased markedly (300% to 800%), the excretion per cent was increased by only a few tenths of one per cent. Robertson (16) maintains that during diuresis the total uric acid excretion is increased but the per cent excretion is decreased. This can be interpreted as a "washing out" effect of the uric acid produced by the increase in the rate of urine elaboration. In that event we are lead to conclude that the Eth.Ac.Ppt. has, in some way, stimulated the uric acid excretion.

IV - DISCUSSION

As stated in the introduction, it was hoped that this study would lead to the isolation of the diuretic principle of *R. aculeatus* and to satisfactory information concerning the nature of its effects. It is quite evident from the data submitted in section III of this report that this principle has diuretic properties. In the anesthetized dog the effect on the rate of urine flow is not very pronounced, but we must keep in mind the artificial conditions which this type of procedure imposes. It is a well known fact that anesthetics, barbiturates in particular, have a depressant action on renal function. The procedure used also necessitated keeping the animal in a supine position throughout the course of the experiment. The intra-abdominal pressure also was altered when the peritoneal cavity was opened. There is no doubt that all these factors combined to depress and hinder the renal activity to some extent. It is of interest to note that despite this depressed activity, Eth.Ac.Ppt. was able to evoke not only an increase in the flow of urine but also a marked increase in the total solids excreted. It is true that under the same experimental conditions organic mercurial diuretics (Salyrgan) produced a more marked increase in the rate of urine flow but at the same time the total solids, as indicated by the specific gravity of the urine, fell to such a low concentration that the total amount excreted during the diuresis was less than that excreted during the Eth.Ac.Ppt. diuresis. Furthermore, in the un-anesthetized animals, Eth.Ac.Ppt. has been shown to cause a marked increase in the rate of urine flow as well as an increase in the concentration of the urinary constituents.

The composition of the urine during diuresis has been characterized as having a more abundant chloride content, a less marked acidity, and a lower specific gravity than the normal (17). Although the total amount of chlorides excreted during the diuresis is increased, the concentration is decreased. These facts have been utilized by Cushny, and other proponents of the "modern" theory, in support of the thesis of filtration and reabsorption. The theory seems adequate to explain the alterations of urinary constituents observed in most types of diuresis. In the case of the diuresis induced by extracts of *R. aculeatus*, on the other hand, the chlorides per cent and the total chlorides are increased. To us it appears inconceivable that this can be explained on the basis of the "modern" theory without accrediting the kidney with a new group of "vitalistic" functions. ^{See footnote (2), Page 31} This increase in the chlorides per cent is not an isolated observation. It has been observed in 43 consecutive experiments with dogs anesthetized with a variety of anesthetics including barbiturates (dial-urethane, sodium phenobarbital, and sodium pentobarbital), chloralose, paraldehyde, chloral hydrate and ether. This fact has also been observed in 28 experiments with the unanesthetized rat. The total nitrogen responds in the same way- the per cent excretion is increased. With uric acid, the increase in the per cent excreted does not rise by very much but it certainly does not decrease. It appears to us that it may be necessary to search for an explanation of renal function other than the "modern" theory in order to satisfactorily explain the diuresis caused by *R. aculeatus*.

The first important step would be to locate the site of action and the mechanism involved in this diuresis. It is obvious that the data presented in this report are too incomplete even to attempt to point out the probable mechanism involved. We have but a vague idea of the condition of the circulatory system during the diuresis.

It can only be stated that the diuretic fraction of *R. aculeatus* does not appear to alter the blood pressure or heart action. As is pointed out by Cushny and others, the mere fact that the general circulation is not appreciably altered is no guarantee that the renal portion of the circulation has remained unaltered. On the other hand, it should also be pointed out that Barcroft, Brodie and Straub and Richards and Plant have demonstrated that diuresis is not necessarily accompanied by an increase in the blood flow through the kidney. A more satisfactory knowledge of the changes in blood composition, than is already at our disposal, must be acquired also before a significant discussion of the mechanism can be undertaken. A direct method of obtaining this information would be through the use of a modified perfusion scheme. In this scheme, two dogs are used. The aorta of dog A is anastomosed to that of dog B in such a manner that the blood of dog A flows through the kidneys of dog B to the exclusion of the blood of dog B. In a similar fashion, the blood of dog B is also detoured to flow through the kidneys of dog A. The blood supply to the head of dog B is then cut off and the heart denervated. In this manner we are perfusing intact kidneys. Another advantage of this modified heart-lung preparation is that the blood is not altered in any way, as defibrination is unnecessary. Still another advantage is that the whole blood is passed through living tissue in the upper part of the dog's body and that the amount and kind of tissue included in this circuit can be controlled by ligating appropriate vessels. Using this perfusion method it should be possible to determine the renal and extra-renal effects of *R. aculeatus* and so give us ample data for a discussion of the mechanism involved in the diuresis. It is quite obvious that such a scheme for perfusion has application in many fields of pharmacological research.

Another, and more practical point, which has yet to be determined is the toxicity of this diuretic fraction and possibilities of its clinical use. In section III of this report we have mentioned several types of experiment by which it was hoped to find a satisfactory answer to these important questions. For reasons already mentioned, these attempts were unsuccessful.

(2) During diuresis, the composition of the urine approaches that of a proteinless plasma. With *R. aculeatus*, however, the increased chloride elimination is out of all proportion to the increase in the total volume of the urine excreted. This might be explained as due to a mobilization of the chlorides from the tissues but the rapidity of onset of the diuretic effect and the failure to find an increased blood chloride level seems to indicate that this is not an acceptable explanation for this diuresis.

SUMMARY

- 1- The aqueous extract of *Ruscus aculeatus* contains diuretic and vasodepressor principles. These principles can be readily separated from each other by chemical means.
- 2- On the anesthetized dog, the diuretic fraction causes a small increase in the rate of urine flow and a marked increase in the specific gravity of the urine. A series of 15 experiments on dogs under dial-urethane (Ciba) anesthesia, is submitted in which it is shown that this diuretic fraction causes marked increases in the relative and absolute excretion of chlorides.
- 3- Preliminary studies, on unanesthetized dogs with fistulae of the urinary bladder, show that this diuretic fraction can induce a marked increase in the rate of urine flow.
- 4- On the unanesthetized rat, this diuretic fraction produced marked increases in urine volume, specific gravity, and chlorides and uric acid excreted. The effect on urine volume and specific gravity appears to be greater than even sub-toxic doses of theobromine sodium salicylate (Diuretin). The effect of Diuretin on chlorides and uric acid excretion was not determined.
- 5- Preliminary chemical studies indicate that this diuretic fraction is made up of carbon, hydrogen, oxygen and calcium.

BIBLIOGRAPHY

- (1) Hager's Handbuch der Pharmaceutischen Praxis, II, pages 761 and 882 (1907)
- (2) Dubat: Comp. Rend. 133: 942 (1901)
- (3) Castaro: Z. Physiol. Chem. 49: 96 (1906)
- (4) Bourquelot: J. Pharm. Chim. 19: 241 (1903)
- (5) Greshoff: Kew. Bull. 1909, page 397
- (6) Jonesco: Comp. Rend. 173: 168 (1921)
- (7) Balansard and Delphaut: Comp. Rend. 129: 308 (1938)
- (8) Pinkston and Kanan; Unpublished experiments (1938)
- (9) Harvey: Arch. Int. Med. 6: 12 (1910)
- (10) Koch and McMeekin: J. Am. Chem. Soc. 56: 2066 (1924)
- (11) Wilson and Ball: J. Biol. Chem. 79: 221 (1928)
- (12) Melville and Stehle: J. Exper. Pharmac. and Therap. 34: 209 (1928)
- (13) Kleitman: Am. J. Physiol. 81: 336 (1927)
- (14) Gregersen: Ibid 102: 344 (1932)
- (15) Folin: J. Biol. Chem. 101: 111 (1933)
- (16) Robertson: Am. J. Physiol. 33: 324
- (17) Cushny: Secretion of Urine, 1926 (London) page 14

ADDENDUM

In studying the diuretic action of *Ruscus aculeatus* extracts it was noted that not only was the total amount of such urinary constituents as chlorides, total nitrogen, and uric acid increased but the concentration of these substances was also increased even at the height of the diuresis. The effect on the urine volume, in many cases, were not very great. It seemed that this diuresis could not be explained on the basis of the "modern" theory of urine secretion without endowing the kidney with still more "vitalistic" functions. ^{See footnote (2), page 31} The diuretic action of *R. aculeatus* has led us to ~~examine~~ examine the present theories of kidney function, in an attempt to arrive at a satisfactory explanation for this diuresis.

This addendum is submitted only for the sake of record and is not supported, as yet, by adequate experimental evidence.

experimental support until the important investigation of Heidenhain. Probably the most important objection ever raised against Ludwig's theory of glomerular filtration and tubular reabsorption is found in Heidenhain's experiments with the dye, indigo-carmin. He found that after the injection of this dye into an animal, examination of the kidney showed the presence of colored granules in the lumen and cells of the tubules but no discoloration in the glomerulus. Therefore he concluded that the tubules have a secretory power. Von Sobieranski repeated Heidenhain's work and got the same results. He found, however, that if a large amount of the dye was injected, or if a dehydrated animal was used, the glomerulus contained the dye also. His next discovery was that fresh sections of kidney tissue stained very

slowly when immersed in serum from an animal injected with the dye. It is obvious, he concluded, that in order to be stained during life, the renal epithelium must be exposed to a strong solution of the dye. This strong solution could be attained if one assumes glomerular filtration followed by concentration due to tubular reabsorption. This appears to be rather convincing evidence in favor of Ludwig's theory. It is rather difficult, however, to decide whether intra-vital staining of renal epithelium really represents the passage of the dye through the cell and if it does, the direction of the passage. If the cells have a secretory power would not that concentrate the dye? It may be of interest to recall, in this connection, the experiments performed by Richards and his collaborators in which the excretion of indigo-carmin was directly observed in the living frog kidney. The indigo-carmin solution was injected into the anterior abdominal vein of the frog and an exposed kidney was observed. To paraphrase the authors' description of the events that followed: First the arteries became blue, then the glomerular tuft and soon thereafter the glomerular fluid became tinged with blue. The veins then became blue and soon the kidney became so darkly colored as to be opaque. In about 3 to 5 minutes the color was seen to fade, 10 to 30 minutes later the cells of the tubules, which normally are barely visible, become more easily seen as their walls were being lined with blue granules and presently thread-like collections of the dye were found in the lumen. The bladder urine made a darker stain on filter paper than did the glomerular

urine. The authors offered these observations as a demonstration that dyes are filtered in the glomerulus and concentrated in the tubules.

Cushny's "modern" compromise, a hybrid conception of glomerular filtration, tubular reabsorption and active secretion - the latter "to be invoked only if the simpler view----proves inadequate", has received the support of many physiologists. There have been several outstanding attempts to prove tubular reabsorption. That of von Sobieranski is mentioned above. The experiment of Ribbert was probably among the earliest attempts to prove tubular reabsorption. A unilateral nephrectomy was performed on a rabbit and the medulla extirpated from the remaining kidney. These animals showed a marked increase in urine volume as compared to control rabbits on which the same operative procedure was carried out with the exception of removing the medulla. This experiment was successfully repeated by Hausmann. It was been concluded that a dilute fluid was formed in the cortex which is normally concentrated by the reabsorption of water. Bradford found, however, that the removal of a section from any part of the kidney was always followed by diuresis regardless of whether the medulla was left intact or not.

In the perfusion of the frog's kidney, as described by Cullis and subsequently used by many other workers, the perfusion of the tubules with a urea-Ringer's solution via the portal vein had no marked effect on urine flow while perfusing the glomerulus with the same solution via the renal artery caused a marked diuresis. This observation is decidedly in favor of Cushny's theory. However, it should be pointed out that most, if not all, of the results of Cullis and many other workers have been brought into question because they did not fully appreciate the fact that unequal pressures in their systems would cause back-flows. It should also be

remembered that the tubules receive perfusion fluid whether the kidney is perfused through the artery or the renal-portal vein. This method, under carefully controlled conditions, only permits the exclusion of the glomerulus. Bainbridge and Beddard found that frogs with glomeruli occluded showed a diuresis after the injection of urea solution into a lymph sac. In these attempts at a direct demonstration of the role of the glomerulus in filtration, and of the tubule in reabsorption, each experiment, considered by itself and in the light of the author's interpretation, is convincing. When, as Richards has remarked, they are viewed as a whole, the most one could conclude, in favor of the "modern" theory, is that there is no proof as yet, either direct or indirect.

Probably the most famous and most direct attempt to prove glomerular filtration and tubular reabsorption has been the experiments of Richards and his students. In brief, his method is to insert a fine glass capillary pipette into a glomerulus of a frog's kidney and withdraw the fluid found there for analysis. The method employed deserves some discussion. The kidney is exposed, stretched across a small window on the microscope and transilluminated. An open glomerulus is then punctured and collection of the fluid begun. These workers found that a glass pipette was usually not strong enough to pierce the glomerular wall so they suggest the use of quartz capillary pipettes filled with mercury. It is also suggested that a little ragged edge be left on the point of the capillary to assist in piercing the glomerular wall. Usually 2 or 3 vigorous efforts were made before the glomerulus was entered. It was noted that the blood flow through the glomerular tuft usually ceased for a few seconds to several minutes after entrance was effected. The glomerular fluid was removed by sucking back the column of mercury in the capillary pipette. These workers found that by drying the

surface of the kidney with a fine stream of air from a hypodermic needle, entrance into the glomerulus is made much easier- the dried tissue not being "so tough and elastic". They claim that this drying process has the further advantage of removing the tissue fluid from the operative field and so preventing contamination of the glomerular fluid. In their later experiments, Richards and his students blocked the neck of the tubule issuing from the punctured glomerulus by pressing a fine glass rod on the tissue overlying the tubule. In some cases the glass rod was accurately applied while in other cases it was just pressed down over the region where the neck of the tubule was thought to be. The "urine" thus obtained was subjected to analysis.

Inasmuch as these experiments are considered by many to be of great importance in giving support to the "modern" theory, it may be of interest to consider the methods used. In order to enter Bowman's capsule, the pipette has to push before it, and lacerate, the overlying tissue. To reduce the amount of force needed to puncture the glomerular wall, and to clear the ~~area of~~ tissue fluid, the operative field is dried by a current of air. It is possible that some of the tissue fluid was blown off by the fine stream of air and it is highly probable that much of the tissue fluid was dried in the field of operation. In addition, it is beyond the most optimistic of dreams to even hope that by playing a stream of air on the surface one could remove the tissue fluid found in the tissue between the surface of the kidney and the wall of the glomerulus. All that could be accomplished by this method would be to dry the tissue fluid; and incidentally the tissue substance, in situ. In the ordinary microanalysis such a source of contamination would not result in a serious error but these men were analyzing a milligram of glomerular fluid

and the concentrations found were of the order of 2 to 4 gamma of sodium chloride. In such determinations the entire sodium chloride content found may well have been contamination from this source. Richards and Wearn were apparently aware of this fact to some extent. They washed the operative area of the kidney with chloride-free water before drying the surface. This may have helped to some extent but probably did not improve the situation very much. Ten years ~~after~~ after the work was started Richards and his co-workers realized that the drying of the kidney surface could be a source of error. It is a matter of record that they definitely proved that the drying increased the chloride content of the glomerular filtrate. This they attributed to a concentration of the capsular fluid by evaporation. In some of their experiments they reported that the fluid in the capillary pipettes clotted. This, it was claimed, was due to effusion. There is no guarantee that this effusion did not occur, to some extent, in all the experiments. How much weight can one put on an experiment, designed to show ultra-filtration in the glomeruli and reabsorption in the tubuli, when it is of such nature as to disturb the glomerulus to such an extent that effusion occurs? What can be proved by ultraprecise analysis of such "filtrate-effusion" fluids?

The nephelometric method of T. E. Richards was used to estimate the sodium chloride content. Practice determinations on pure sodium chloride solutions resulted in mean errors of from 2% to 6% with the error increasing as the concentration was decreased from 11 to 6 gamma (the usual concentration of sodium chloride in the actual experiments was about 1 1/4 to 3 gamma). Assuming for the moment that the fluid in the glomerulus is an

ultra-filtrate of the blood, what would the effect of such constituents as uric acid, sulfates, phosphates, etc., have on the nephelometric determinations? It was only within the past ten years that this source of error was pointed out to Dr. Richards. This objection must have had some foundation because soon it was announced that a more sensitive colorimetric method has been modified for ultra-microanalysis. This was based on the method described by Isaacs. In this method they found that the zinc hydroxide precipitation must be applied to the glomerular urine "when it contains significant quantities of proteins or uric acid". Regardless of whether the amount of protein present was significant or not, its mere presence should be sufficient grounds for invalidating the results.

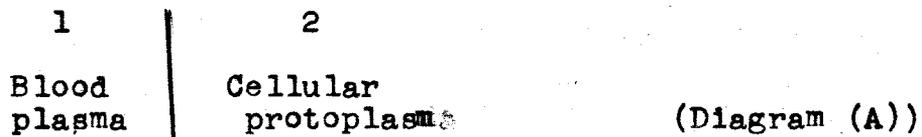
In the nephelometric method the chloride content of the bladder urine and blood plasma was determined by the method of Whitehorn. In the experiments with the colorimetric method, the bladder urine was not analyzed for chlorides.

It is obvious that these criticisms alone would suffice to render highly questionable the results claimed by these workers. Many more auxiliary criticisms could be offered.

In considering the kidney, it seems that physiologists have tended always to go to extremes. Either the organ was endowed with discriminating powers to secrete a urine of ideal composition or else the process was described as a mere filtration with a selective reabsorption. It already has been pointed out how both schools of renal function have attempted to prove their particular theses with questionable success. In all these researches it seems that the kidney as a living organ, composed of living cells, has been generally forgotten. It has been remembered only when a proponent of a thesis found need to invoke a special power in order to

make his observations fit the theory.

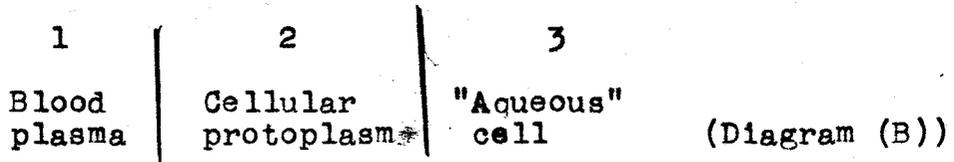
The metabolic processes of a cell are carried on in what may be considered a closed system. This system can be represented in the following manner:



All metabolic processes of the cell are carried on either in or between the two "cells" of this system. It should be remembered that the blood plasma is in reality "open" - inasmuch as it is able to derive substances from the outside. In the vicinity of the cell, however, it behaves as if it were a closed chemical system. In this 2-cell system take place all the functions concomitant with the life of the cell. It should also be kept in mind that this system is by no means a simple one. It is composed of two colloid cells, each fundamentally different from the other and separated by a colloidal membrane. It must be admitted that very little is known at present about the chemistry of such colloidal systems but there can be no doubt that it is of a very complex nature. Biochemists have made some advances in this study and these known facts may well be applied to the study of renal function.

Let us for the moment disregard the anatomical structure of the kidney and concentrate on its "chemical" structure. The kidney, in common with the exocrine glands, is not only composed of the basic 2-cell closed system but has, in addition, a third cell. This cell may be considered as an aqueous cell which opens to the exterior. This structure can be represented in the follo-

wing way:



"Cells" 1 and 2 are part of a closed system. "Cell" 3, which communicates with "cell" 2 through a colloidal membrane, is open. Therefore, by applying the laws of mass action, the interactions in a system of the "cell 1- cell 2" type may reach an equilibrium. Those in the system of the "cell 2- cell 3" type, under normal conditions, can continue to operate in one direction indefinitely.

Another fact which should be recalled is that the kidney is not the only organ able to rid the body of unnecessary substances. The salivary glands, for example, secrete a fluid which contains, besides the ferments and proteins peculiar to it, urea, uric acid, sodium, potassium, calcium, magnesium, ammonia as the chloride, carbonate, phosphate and sulfate. In chronic nephritis the uric acid and urea content is found to have increased. In diabetes, some of the patients are found to secrete glucose in the saliva and almost invariably the saliva, which is normally alkaline, is found to be acid. It is interesting to note further that the salivation caused by mercury results in a saliva which contains an increased amount of proteins and inorganic salts. This seems to show a marked resemblance to the diuresis caused by mercury. In the bile, urea and ammonia are normally found and glucose has been detected in pathological conditions. The sweat glands secrete traces of albumen, urea, creatinine, cholesterol and inorganic salts. Glucose is found in the sweat of diabetic patients and during uremia the urea content is in-

creased. The sweat normally has an acid reaction but the acidity decreases during profuse sweating. (1)

The striking resemblance between these secretions and urine cannot be mistaken. So far as we can ascertain, physiologists do not oppose the view that these secretions are the result of the metabolism of the glands concerned and that blood pressure, as such, plays a minor role in the elaboration of these secretions. Yet, these secretions contain constituents found in the urine even though the glands which elaborated them do not contain so remarkable a structure, the renal glomerulus- which many workers in the field of renal physiology take as an a priori argument in favour of filtration.

It seems possible to explain urine elaboration as the result of metabolic processes of the renal parenchyma. These processes may not be of necessity peculiar only to the renal epithelium. It appears feasible, after comparing diagrams (A) and (B), that the arrangement of the chemical system found in the kidney could result in the formation of urine. It cannot be denied that blood pressure plays a role in renal activity. In so far as we are able to determine, physiologists are in agreement that this is also true of other parts of the body. In this laboratory it has been observed on anesthetized dogs that a carotid

(1) The constituents of secretions given in this paragraph are quoted from the citations in Hoppe-Seyler-Thierfelder Physiologisch u. Pathologisch Chemische Analyse, Berlin (1924), Pages 893-899, 908-920.

artery pressure of about 80 mm. mercury is a critical level below which the kidney will not function. Between 80 mm. and 110 mm. mercury the blood pressure seems to have the effect of improving the renal activity. Beyond 110 mm. mercury pressure, no further effect could be detected. No vasomotor drugs were given these dogs: These various blood pressure levels were attained solely through the vasomotor mechanisms already present in the animal.

Returning, for the moment, to an anatomical consideration of the nephron, one might consider the glomerulus as a structure specifically designed for filtering the blood. It also could be considered as a wide surface area of contact between the blood plasma, cell protoplasm, and "aqueous" cell. As such it could be compared to structures like the lung. What is remarkable of the glomerulus is that so large a surface area has been squeezed into so small a space. Thin though it may be, the glomerular wall must still be regarded as composed of living protoplasm capable of carrying on the metabolism necessary for life. . Mollendorf (2) has pointed out the resemblance between the cells of the convoluted tubules and those of the intestinal villi- the inference being that they are absorbent in function. However, it may be pointed out that in the intestine the food-stuffs are presented in "cell" 3 (Diagram (B)) while in the kidney the substances are presented by the blood plasma in "cell" 1. It would appear possible, therefore, to conclude that the "absorbing"

(2) Ergebnis der Physiol. 18: 141 (1920)

activities of these two systems are in opposite directions.

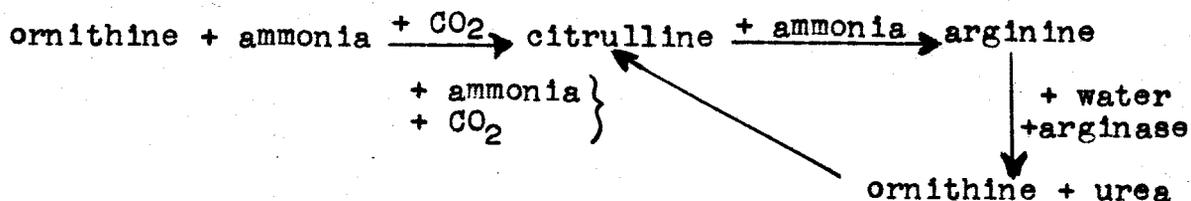
To illustrate this possible explanation of renal function, let us consider a normal constituent of the urine and follow it from the blood stream into the urine. Urea is an admirable substance, with which to illustrate our point, for 3 reasons:

1) The recent work of Krebs has thrown some light on the probable mechanism of urea metabolism: (3)

2) Cughny has classified this compound as a "threshold" substance:

3) It appears highly probable that the ammonia formed by the kidney is derived from urea: (4)

In the liver, the ammonia formed by the deaminization of amino acids, is probably converted to urea by the following mechanism: (3)



In the liver this cycle can run continuously in one direction because there is a continuous excess of ammonia present to propagate the cycle. The urea thus formed in the liver-cord cell diffuses into the blood plasma and is subsequently presented to all the cells of the body. Practically all the tissues of the

(3) Krebs and Heuseleit Hoppe-Seyler's Z. Physiol.Chemie. 210: 33 (1932)

(4) Bollman and Mann Am. J. Physiol. 92: 92 (1930)

body contain arginine. Arginine can be regarded as ureasaturated ornithine. Urea not being very soluble in the cell colloids, the blood soon saturates the tissues with all the urea they can hold. It has already been pointed out that the plasma-cell relationship is one of a closed system. When the blood reaches the kidney, however, an entirely different situation arises. The blood starts to saturate the renal cell with urea. This excess of urea may cause the reaction, described above, to be reversed, forming arginine from ornithine. Such a reversal is possible according to the law of mass action and is similar to the situation in the liver where excess of ammonia forces the reaction to go in one direction. The cytoplasm of the cell is in a constant state of flux and soon the arginine reaches the lumen side of the cell. Here the relative lack of urea permits the arginine to try to reach an equilibrium with ornithine resulting in the liberation of urea:



The liberated urea may reach the "aqueous" cell by one of 3 mechanisms:

- (1) The lumen end of the renal epithelium may extrude the molecule across the membrane into the lumen. This would involve a special vital activity on the part of the kidney and should not be resorted to until all other possibilities have been exploited and found wanting:
- (2) The urea molecules may just diffuse across the membrane into the "aqueous" cell. Although this may appear to be sufficient to explain the appearance of some substances in the urine, it is not sufficient in the case of urea at any rate:

(3) The urea may be more soluble in water than in the cell colloids. This would result in an "extraction" of the urea from the cell. Such a phenomenon, in conjunction with diffusion, may be sufficient to explain the concentration of the various constituents in the urine.

The liberated ornithine, on its return to the basal end of the cell, can combine with more urea and so continue the cycle.

It is obvious that this reaction, like all other chemical reactions, proceeds at a certain rate. This would account for the "threshold" value for urea. This explanation is able also to explain the formation of ammonia from urea. When the pH of the cell protoplasm becomes slightly lower, it may be that the arginine, instead of breaking down into urea and ornithine, breaks down into citrulline and ammonia, and citrulline, in turn, becomes converted to ornithine with the formation of an additional molecule of ammonia. Therefore, it is possible that with certain small changes in the pH of the cell, the reaction, which forms urea in the liver, may be reserved to form ammonia.

It may be of interest to note that the kidney is not unique in its ability to form ammonia. Other glands which secrete urea also secrete ammonia.

In a similar way, the other urinary constituents may be traced from the blood to the urine by considering the mechanism of their metabolism only. It is unfortunate that at the present time so little is known of the metabolic mechanism and a better understanding of these mechanisms will anticipate a more thorough knowledge of renal activity and glandular activity in general.