oactivity into protein extractable with hot TCA. The lower the initial hydroxyproline content, the less the incorporation. These experiments suggest that the capacity of the liver to synthesize collagen depends on the extent of liver damage produced by the toxic agent employed. This impies that the increase in the total collagen content in the liver of rats treated chronically with CCl_4 is due mainly to biosynthesis of collagen.

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Reaction of a Cobra Venom Factor with Guinea Pig Complement and Generation of an Activity Chemotactic for Polymorphonuclear Leukocytes* (33840)

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Since cobra venom was shown to destroy serum complement in 1903, much work has been done on the biological properties of this interesting substance (1, 2). Recently it has been reported that a purified nontoxic factor from the venom of cobra, *Naja naja*, selectively destroyed C'3 in human serum (3).[1] In guinea pig serum, on the other hand, a purified nontoxic factor from cobra, *Naja haje*, destroyed both C'3 and C'5, and destruction of C'3, C'5, C'6, C'8, and C'9 on administration of venom factor to guinea pigs has been reported (4–6). The generation of anaphylatoxic activity in the guinea pig serum treated with cobra venom has been described (7). In animals treated with cobra venom factor (CVF), the death caused by injection of Forssman antibody was prevented (4), the Arthus reaction was inhibited (6, 8), survival time for xenografts was prolonged (9), and the ability to generate slow-reacting substance of anaphylaxis was impaired (10). Thus, cobra venom factor is a useful experimental tool for studying inflammatory processes initiated by antigen–antibody reactions.

The present paper reports the results of *in*

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[1] Abbreviations: C'1, C'4, C'2, C'3, C'5, C'6, C'7, C'8, C'9: first, fourth, second, third, fifth, sixth, seventh, eighth, and ninth components of the complement system.

TABLE I. Pattern of Complement Component Destruction by Cobra Venom Factor.

No.	Final serum dilution	Final CVF concentration (μg/ml serum)	Incubation temp (°)	Complement consumed (%)[a]								
				C'1	C'4	C'2	C'3	C'5	C'6	C'7	C'8	C'9
1	1: 1.25	4.0	37	<10	<10	<10	>95	92	88	83	43	44
2	1: 1.25	1.3	37	<10	<10	<10	>95	56	63	78	32	32
3	1: 1.25	0.43	37	<10	<10	<10	>95	22	30	77	10	9
4	1: 3.75	4.0	37	<10	<10	<10	>95	81	69	80	39	39
5	1:11.25	4.0	37	<10	<10	<10	>95	58	33	78	19	20
6	1: 1.25	4.0	30	<10	<10	<10	>95	90	95	81	54	44
7	1: 1.25	0	37	<10	<10	<10	59	<10	<10	<10	<10	<10
8	1: 3.75	0	37	<10	<10	<10	69	<10	<10	<10	<10	<10
9	1:11.25	0	37	<10	<10	<10	65	<10	<10	<10	<10	<10
10	1: 1.25	0	30	<10	<10	<10	49	<10	<10	<10	<10	<10
				Titer (× 1000 units/ml)								
Guinea pig serum, diluted 1:1.25, kept 0° for 1 hr				95	5	15	15	80	20	50	300	100

[a] Percentage consumption in relation to the control serum kept at 0° for 60 min.

vitro experiments which show that the purified venom factor inactivates not only C'3 and C'5 in guinea pig serum, but also C'6, C'7, C'8, and C'9. In the course of this process it generates chemotactic activity for rabbit polymorphonuclear leukocytes.

Materials and Methods. Lyophilized *Naja haje* venom (Ross Allen's Reptile Institute, Inc., Silver Spring, Fla.) was fractionated on DEAE-cellulose column and assayed for guinea pig complement destroying activity as described (4). The active fractions from the DEAE-cellulose column were pooled, concentrated by ultrafiltration and further purified by polyacrylamide gel disc electrophoresis at alkaline pH (11). About 3 mg of pooled material were electrophoresed in a glass tube, 1.4 cm i.d. containing small pore gel (7.0% acrylamide and 0.2% bisacrylamide) and large pore gel measuring 5 and 2 cm in height, respectively. The run was carried out between 3 and 6° for twice as long as it took for the tracking dye, bromphenol blue, to pass through the entire length of the small pore gel. Sections cut from the small pore gel were eluted in pyrogen-free 0.15 *M* NaCl.

For the inactivation of complement by the venom factor, pooled guinea pig serum was adjusted to pH 7.3 and diluted 3- and 9-fold in Veronal buffered saline [(VBS^{2+}), pH 7.3, containing 0.1% gelatin, 0.5 m*M* Mg^{2+} and 0.15 m*M* Ca^{2+} as described in Ref. (12)], resulting in final serum dilutions of 1:3.75 and 1:11.25, respectively. One-ml portions of different dilutions of serum were mixed with varying amounts of purified cobra venom factor in 0.25 ml of VBS^{2+}. As a control, serum was mixed with VBS^{2+} in the same proportion. One set of these mixtures was incubated for 60 min at 30° and another at 37°. For purposes of reference, guinea pig serum diluted 1:1.25 in VBS^{++} was kept at 0° for 60 min and titrated for each of the 9 complement components, as shown in Table I. The consumption of each of the complement components in the reaction mixtures with venom factor, as well as in the controls, were expressed, percentagewise, in relation to this 1:1.25 dilution of guinea pig serum. Experiments were also set up to test the effect of venom factor on each of the 4 terminal components. For this purpose, 1-ml portions of semipurified C'6, C'7, C'8, and C'9 preparations (13) in VBS^{++}, 2000 units/ml, were mixed with 0.25 ml portions of VBS^{2+} containing 4 μg of venom factor and incubated for 60 min at 37°. The hemolytic activ-

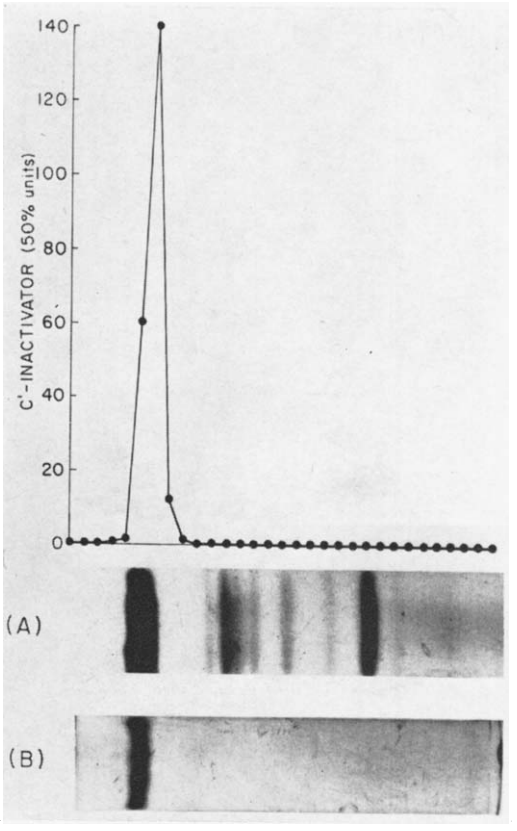

FIG. 1. Analytic polyacrylamide gel disc electrophoresis at alkaline pH (11): the run was terminated when the tracking dye emerged from the anodal end of the small pore gel; (A), about 50 μg of DEAE-cellulose column pool; small pore gel sectioned and each section eluted in 1 ml of pyrogen-free saline; complement inactivating activity assayed as shown; (B), about 15 μg of purified cobra venom factor.

ity of whole complement and of the complement components was determined as described (12, 13).

The method for assay of chemotactic activity for rabbit polymorphonuclear leukocytes has been described elsewhere (14, 15). For the generation of chemotactic activity from guinea pig serum, one part of undiluted serum was mixed with 0.25 part of VBS^{2+} containing 16 μg of venom factor/ml. The mixture was incubated for 60 min at 37° followed by 30 min heating at 57°. To estimate the molecular weight of the chemotactic factor(s), 2.5 ml of serum treated with venom factor was passed through a Sephadex G-100 column equilibrated with 0.02 *M* phosphate buffer, pH 7.2, containing 0.15 *M* NaCl. The fractions were diluted 3-fold in Gey's solution containing 2% bovine serum albumin for assay of chemotactic activity.

Results. A simple two-step purification procedure produced the cobra venom factor in a homogeneous state as judged by disc electrophoresis, as shown in Fig. 1. The recovery of activity from the gel ranged between 60 and 90%.

As shown in Table I, there was complete consumption of C'3 at every one of the serum dilution and venom concentrations tested. In the control tests without vernom, the loss of C'3 ranged between 50 and 70%. The consumption of C'5 through C'9 was extensive at low dilution of serum and high concentration of venom factor. In the controls incubated without venom, there were no losses of C'5 through C'9. The venom factor did not inactivate C'1, C'4, and C'2. Little differences was seen between 30 and 37° treatment. For purposes of comparative evaluation and in order to indicate the range of dilutions used in the titration of complement components, Table I also shows the titer of each of 9 components in diluted (1:1.25) guinea pig serum after 60 min at 0°. It was reported that venom factor did not inactivate purified C'3 or C'5 (3–5). The venom factor also did not inactivate semi-purified C'6, C'7, C'8, and C'9.

In the chemotactic assay, serum treated with venom factor, at dilution of 1:37.5 in Gey's solution containing 2% bovine serum albumin, gave a cell count exceeding 800 neutrophils/high power field compared to the counts of 48 for untreated serum and 6 for venom factor alone. The major peak of chemotactic activity in treated serum eluted from a Sephadex G-100 column slightly before horse heart cytochrome C, as shown in Fig. 2. Accordingly, the molecular weight was estimated to be about 15,000. The 2 minor chemotactic peaks, one corresponding to very high and the other to very low molecular weight, were also observed in untreated serum, unlike the major peak (mol wt. 15,-

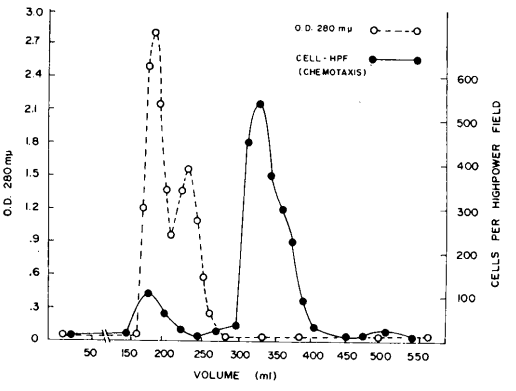

FIG. 2. Elution pattern of chemotactic activity of the serum treated with the venom factor from the Sephadex G-100 column: elution peak for blue dextran, cytochrome C and NaCl were 186, 356, and 500 ml, respectively.

000) which appeared only after venom factor treatment.

Discussion. While earlier investigators of the action of cobra venom on guinea pig complement found destruction of C′3 and C′5 *in vitro,* in the present experiments marked consumption of all of the six terminal components, *i.e.,* C′3 through C′9, was observed. Probably, the difference in results can be attributed to variations in serum dilution and venom factor concentration. As shown in Table I, consumption of C′3 was complete in every one of the present experiments, whereas the consumption of C′5 through C′9 varied depending on the concentrations of the reactants. In all cases, there was no detectable loss of C′1, C′4, and C′2. Thus, the pattern of inactivation of the complement components by the venom factor is essentially similar to that seen on treatment of guinea pig serum with endotoxin (16). However, the action of the venom factor could not be attributed to possible contamination with endotoxin, since tests for pyrogen were negative. It is not known whether the consumption of the six terminal components is mediated through sequential activation of a small proportion of the early-acting components resulting in highly efficient C′4,2a "convertase" formation, or whether the consumption of the six terminal components is mediated through an alternative pathway. The investigations by Müller-Eberhard *et al.* (3) and Nelson (4) suggest the latter possibility. It is also not known whether the venom factor produces changes in serum other than those observed in the complement system.

It is of considerable interest that the cobra venom factor generates substantial chemotactic activity. The molecular weight of this material was estimated at about 15,000 which is the same as that of the C′5a fragment, as well as of the chemotactic factor generated on treatment of guinea pig serum with endotoxin (14, 15). While this information does not suffice to identify the venom-generated chemotactic activity with the C′5a fragment, it is clear from the molecular weight of about 15,000, that the major portion of the chemotactic activity can not be attributed to the complex of activated C′5, C′6, and C′7 (17).

Summary. A purified cobra venom factor consumes C′3 through C′9 in guinea pig serum without detectable effect on C′1, C′4, and C′2. In serum so treated, chemotactic material(s) for rabbit polymorphonuclear leukocytes is generated. This factor(s) has a molecular weight of about 15,000.

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Measurement of Peripheral Renin Activity in the Rat* (33841)

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Measurement of circulating renin activity in the rat is complicated by the need to obtain amounts of blood which are large in comparison with its blood volume. Removal of blood samples of the necessary magnitude required for standard methods (1–3) induces physiological responses which lead to an alteration of the level of peripheral renin.

This difficulty has been approached in several ways. Gross and associates (4) have utilized an isovolemic cross circulation technique to demonstrate an alteration in renin concentration in the rats circulation. This approach lacks specificity and is expensive in its requirement of assay rats. Boucher and co-workers (5) have devised a micromethod, employing exogenous rat renin substrate, which enables assay of renin activity in the plasma of the unanesthetized rat. The small size of the sample taken does not cause any disturbance of the circulating levels of peripheral renin activity. They have established in a quantitative fashion the levels found in a variety of states (6).

We have utilized dialysis of the serum sample against disodium EDTA to inactivate angiotensinase. This dialysis is accomplished without volume alterations when 1 ml of serum is used. Employing bilateral nephrectomy immediately before collection of a large blood sample we clearly separated the renin activity results occurring in several differing states.

Methods. Experimental animals. Sprague-Dawley rats, weighing 250–350 g, of both sexes were used. Blood samples were collected from the right carotid artery after bilateral nephrectomy had been performed under sodium pentobarbital anesthesia, 40 mg/kg ip. The interval between nephrectomy and sacrifice by exsanguination was 15 min. The serum was stored frozen until dialyzed.

Groups of rats were subjected to the following treatments: bilateral adrenalectomy without replacement therapy for 7 days; hemorrhage with the kidneys *in situ*; deoxycorticosterone (DOCA) in peanut oil im, 5 mg administered on alternate days for a total dose of 95 mg with 0.9% saline as drinking water; and 5% dextrose, 10 ml/kg im on one occasion.

Peripheral renin activity determination (PRA). One-ml aliquots of sera were dialyzed in 8/32-in. cellophane tubing sacs against 0.003 *M* disodium EDTA in 0.9% sodium chloride solution for 16 hr in the cold. Two-tenths-ml of serum was added to a siliconized tube containing 0.2 ml of rat renin substrate; 0.1 ml of 0.001 *M* phenylmercuric acetate; 0.1 ml of 0.5 *M* sodium phosphate buffer, pH 5.5; 0.01 ml of 5% diisopropylfluorophosphate in isopropyl alcohol; and 0.4 ml of 0.9% sodium chloride solution. Incu-

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