trol mechanism caused by endotoxin acting on the carotid sinus also has been noted(10). Histamine release, particularly during the early phase of endotoxin shock, has been demonstrated(11) and would tend to antagonize the constrictor action of released vasoconstrictors. These factors, plus a local buildup of vasodilator metabolites, apparently exactly balance constrictor forces until the animal deteriorates beyond some critical point.

Finally, it is of interest that up to 90 to 120 minutes following a lethal, overwhelming dose of endotoxin, except during the very early phase, renal blood flow does not appear to be reduced as much as would be expected from the precarious state of the animal. And it is only near death that flow is actually reduced out of proportion to renal perfusion pressure in a majority of kidneys while other kidneys maintain a low resistance even when the dogs's arterial pressure is extremely low.

Summary. Experiments were completed in anesthetized, mongrel dogs. Kidneys were exposed retroperitoneally through a flank incision to facilitate direct blood flow measurement from a cannulated renal vein. Perfusion was through the intact renal artery of the dog. Renal vascular resistance increased markedly as perfusion pressure and renal flow decreased to low levels by 2 minutes after intravenous endotoxin injection. By 30 minutes, perfusion pressure and flow were still low but renal resistance was at pre-endotoxin level where it remained until 90-120 minutes after endotoxin at which time resistance either increased or remained near control level. Renal perfusion pressure and blood flow were both very low at this point. These data indicate that during irreversible endotoxin shock, except for the very early phase, renal blood flow is not decreased out of proportion to renal perfusion pressure. During the latter period of shock, at extremely low arterial pressure, renal resistance may or may not be elevated.

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Fractionation of H-2 Antigenic Specificities in A/Sn Mice.* (31137)

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The different techniques for subcellular fractionation which have been used for the study of H-2 antigens in mice have shown these to be associated with cellular membranes (1,2,3,4,5). Extraction of these antigens from the membranes presents some difficulties and the "molecular" characteristics of the fractions obtained depend greatly upon the method used for preparation of the antigen. Kandutsch and Stimpfling(6) are the only authors to report a method of extraction and purification of H-2 antigens using detergents (Triton X 100) which has resulted in maintenance

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of antigenic activity. This molecular preparation had all tested antigens except E(5).[†] The absence of some H-2 specificities coming from sarcoma I was discussed by Hilgert *et al*(8).

While the studies of subcellular distribution and chemical characterization of H-2 antigens "in block" are advanced, more information is necessary about the different specificities determined by the same or different H-2 alleles. Previous reports from our laboratory (9,10) suggested the possibility that H-2 antigenic specificities from the same organ can be separated. The present paper deals with experiments designed to test this hypothesis.

Material and methods. Animal strains. A/Sn ($H\text{-}2^a$) mice were used for extraction of antigens. A/Sn, B10.D2($H\text{-}2^d$), A.CA ($H\text{-}2^f$), C3H Di Sn ($H\text{-}2^k$) and A.SW ($H\text{-}2^s$) strains were used as source of red blood cells for hemagglutination tests. Strains used for immunogenicity tests are mentioned when each experiment is described.

Antigenic preparations. Microsomic fractions: the antigens were obtained from the spleens of 3 to 4 months old A/Sn males or females. The animals were killed by cervical dislocation and the spleens immediately extracted and homogenized in cold isotonic sucrose buffered at pH 7.2 with 0.01 M glycine (100 mg fresh spleen/ml). The homogenization was done during one minute at 4°C in a Potter-Elvehjem homogenizer with teflon pestle. The suspension was centrifuged twice at 10,000 × *g* for 15 minutes in a refrigerated centrifuge. Sediments were discarded and the supernatant was centrifuged for one hour at 105,000 × *g*, 0°C in a preparatory Spinco Ultracentrifuge. The pellet was then resuspended in the same buffer. Both residue and supernatant were tested for H-2 antigenic content.

Deoxycholate treated material. A/Sn spleens were homogenized in saline with a manual glass homogenizer (100 mg of fresh spleen/ml) in the cold until only the connective tissue remained undispersed (this takes about 2 minutes). Saline was added to bring fresh spleen concentration to 20 mg/ml. The suspension was passed through a stainless steel sieve and then centrifuged for one hour at 25,000 × *g*, 0°C. The supernatant, devoid of any antigenic activity, was discarded.

The residue was resuspended in 1.0% deoxycholate (pH 8.0) up to 1 ml of solution/200 mg of original fresh spleen, and then rehomogenized in a manual glass homogenizer. The highly viscous material that resulted was dialyzed against saline buffered with 0.01 M glycine (pH 7.4). The dialysis was performed at 4°C for 20 hours with agitation. Usually dialysis was performed using 1 liter of saline/ml homogenized material, with 5 changes of liquid.

After dialysis the material was centrifuged at 105,000 × *g*, 0°C for 3 hours. The supernatant was carefully separated from the pellet and this was resuspended in saline with aid of an homogenizer. Both residue and supernatant were used to measure the antigenic contents.

Antisera. Animals were immunized by weekly intraperitoneal injection of 1:1 mixture of liver and spleen. Usually 3 injections were enough to obtain a good titer.

The pooled antisera were freeze-dried and stored at —20°C. The following antisera were used:

1. Anti A(1), K(11), Y(25), A′(27) serum prepared by immunizing B10.D2/B10.-LP hybrids against A/Sn tissues. The activity of this serum is mainly, if not entirely, anti K(11), considered the strongest antibody.
2. Anti D(4), H(8), K(11), Y(25), A′-(27), C′(29) serum, prepared by immunizing A.*SW* mice against A/Sn tissues. This serum will be considered as anti D(4), H(8), K(11) which are the strongest specificities.
3. Anti H(8), I(9) serum, prepared by immunizing A.Sw mice against A.CA tissues. In this serum H(8) is the only pertinent activity since A/Sn mice are I(9) negative.
4. Anti D^b(2), E(5), V(22) serum was obtained by immunizing B10.D2 animals against B10 tissues. This will be considered only as anti E(5) since A/Sn mice are D^b(2) and V(22) negative.

[†] According to the revised nomenclature for H-2 antigens(7), the specificities will be designated using both old (letters) and new (numbers) systems.

TABLE I. Antigenic Content of A/Sn Spleen Microsomes and Its Supernatant.

	Anti	Anti	Anti
Absorbed antiserum	D^b *E* V* 2- *5*-22	DHK Y A′ C′ *4-8-11* 26-27-29	DHK Y A′ C′ *4-8-11* 25-27-29
Red blood cells	A/Sn($H\text{-}2^a$)	C_3HDiSn($H\text{-}2^k$)	B10.D2($H\text{-}2^d$)
Antigens tested	*E* *5*	*H K* Y A′ C′ *8-11* 25-27-29	*D-H* A′C′ *4-8* 27-29
Microsomic fraction	20.2†	19.6	7.3
Supernatant	5.1	.0	.0

* Italicized specificities are those of major importance. † Units/mg prot.

Antigenic estimation. Gorer and Mikulska's hemagglutinating technique was used(11). The amounts of antigens were estimated by inhibition of antisera hemagglutinating capacity. A quantitative expression of antigens was used according to the empiric formula:

$$\frac{A \times B}{C} \quad \frac{\text{units of antigen}}{\text{units of material}}$$

$A = -{}_2\log$ antiserum titer

B = number of tubes showing complete absorption of antibodies after neutralization with antigenic preparation

C = an expression of concentration of antigenic material used for neutralization.

Immunogenicity tests were done injecting the antigen intraperitoneally into adequate receptor mice. Injections were given weekly. Antisera of injected animals were collected and their hemagglutinating capacity investigated.

Sephadex elution. Samples between 1.5 and 2.0 ml were passed through a 140 × 20 Sephadex G-100 column.‡ The elution was made with saline.

Nitrogen determination was done by micro-Kjeldahl procedure(12).

Protein determination was made according to the method of Lowry(13).

Results. The results obtained, measuring the antigenic content of different preparations, may vary from one experiment to another because the well known lability of H-2 antigens. So it is more important to compare different antigens from the same preparation than to analyze the mean values obtained from several experiments. However, it must be pointed out that the results of the given experiments are illustrative of many experiments.

The antigenic content of spleen microsomic fraction of A/Sn mice and the corresponding supernatant fraction are illustrated in Table I. It can be seen that there are clear differences in the antigenic pattern of distribution if E(5), H(8) and K(11) or D(4) and H(8) are considered separately. While the H(8) and K(11) antigens are present only in the microsomic fractions, E(5) remains in the supernatant. This does not indicate that antigen E(5) is in a completely soluble state. On the contrary, there is evidence to support the assumption that antigen E(5) in the supernatant is associated with a particulate system because:

1. All neutralizing activity of the supernatant falls after 3 hours of centrifugation at 105,000 × *g* and,
2. After passing the supernatant through a Sephadex G-100 column, E(5) activity appears with the first protein fraction which elutes from the column (Fig. 1).

The immunogenic property of this microsomal fraction was also tested. Five B10.D2 females were inoculated i.p. twice with this fraction (2.5 mg protein/animal, each time). The anti E(5) serum titer was measured 21 days after first injection. Three animals gave low but significant hemagglutinating titers (1:8, 1:32, 1:64) with Gorer's dextran technique.

Treatment with deoxycholate. This treatment again gave a separation of specificities (Table II). After detergent disruption, dialysis and ultracentrifugation, E(5) is present only in the residue while H(8) and K(11) are present in both residue and supernatant.

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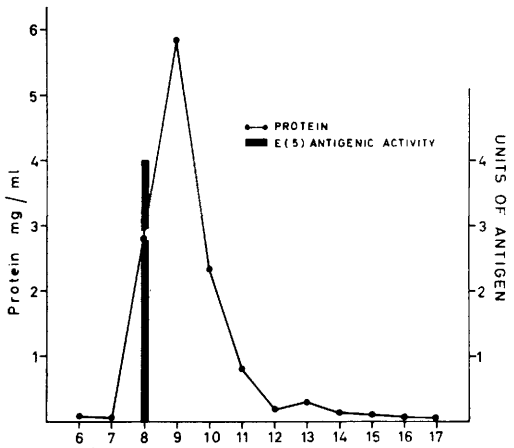
‡ Sephadex, G-100 fine. Lot N° T 02946. Pharmacia Uppsala.



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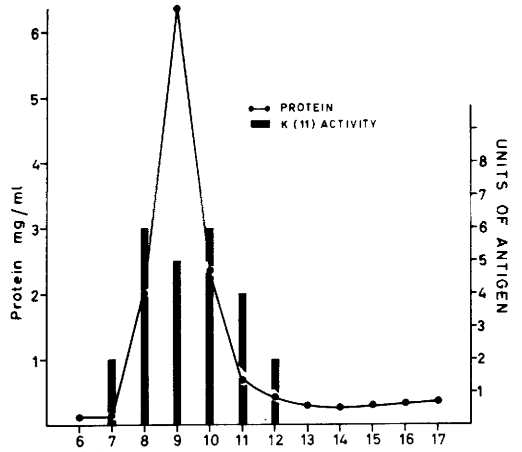

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FIG. 1. E(5) antigenic activity in post microsomal spleen supernatant elution in sephadex G-100 column.

FIG. 2. Sephadex G-100 elution pattern of A/Sn spleen supernatant after treatment with deoxycholate. Antigenic activity detected with anti A(1), K(11), Y(25), A′(27) serum. E(5) activity was absent.

The concentration of the 3 antigens seems not to vary significantly in the residue compared with the original concentration of antigens in the spleen.

As the possibility exists that residual deoxycholate (after dialysis) affects the measurement of antigens, the agglutinating activity of antibodies was measured in presence of variable concentrations of deoxycholate. Concentration up to 0.12% of deoxycholate does not affect significantly the agglutination capacity of antisera.

Passage through Sephadex G-100 column was also done (Fig. 2). In contrast with post microsomal E(5) activity which elutes in a sharp peak, K(11) activity elutes in a wider zone in and outside the void volume of the column. No E(5) activity was detected in this eluted material.

TABLE II. Antigenic Concentration* of Supernatant and Residue of A/Sn Spleen After Deoxycholate Treatment.

Antiserum anti	A *K* Y A′ 1-*11*-25-27	D^b *E* V 2- *5*-22	*H* I *8* 9
Antigens tested	A *K* Y A′ 1-*11*-25-27	*E* *5*	*H* *8*
Whole spleen homogenate (in saline)	30.6	17.5	25.2
Residue	31.0	14.7	25.5
Supernatant	24.1	0.0	14.8

* Units/mg nitrogen.

The immunogenicity of supernatant and residues was also tested. Two strains of mice were used: B10.D2 and B10.*H-2*^{b}. Groups of females (4 animals per group) were injected i.p. either with residue (0.34 mg N per mouse) or supernatant (0.25 mg N per mouse), one injection per week for 2 weeks. Blood samples were collected at the 21st day and antibody titer was measured both separately for each animal and pooling the group samples.

Table III shows that anti H(8) and anti K(11) serum activity was obtained after inoculation of residue or the supernatant but anti E(5) was only obtained by injecting residue.

Discussion. The methods used in the present experiments show the differences in distribution of H-2 antigenic specificities within cell fractions from the same organ of A/Sn mice. Fractions containing E(5) antigens devoid of D(4), K(11), H(8) activities were easily obtained from post microsomal supernatant (Table I), and H(8), K(11) antigenic fractions lacking E(5) activity were obtained after deoxycholate treatment (Table II).

TABLE III. Immunogenicity of Residual and Supernatant Fractions of A/Sn Spleen After Deoxycholate Treatment. Inoculations in B10.D2($H\text{-}2^d$) and B10($H\text{-}2^b$) 8-mo-old females.*

Strain of inoculated mice	No. of animals/group	r.b.c. for test	H-2 antigens tested	Residue inoculation	Supernatant inoculation
B10.D2($H\text{-}2^d$)	4	A.SW($H\text{-}2^s$)	*E* *5*	1/64	traces
		B10.D2($H\text{-}2^d$)	None	Neg.	Neg.
B10($H\text{-}2^b$)	4	C_3HDiSn($H\text{-}2^k$)	A C *H K* Y	1/32	1/32
		B10($H\text{-}2^b$)	None	traces	Neg.

* Inoculations were done intraperitoneally. Two injections separated by 7 days: blood was collected 21 days after first injection. The supernatant was injected in quantities of 0.25 mg total nitrogen per mouse, each time. Residue: 0.34 mg total nitrogen under the same conditions.

The presence of minor quantities of the non-detected antigens in the preparations is of course possible. These experiments show the possibility of a differential fractionation of the H-2 specificities resulting in very different patterns of distribution of antigens.

There is no method for detection of these antigens other than those based on their immunological specificity. Thus, it cannot be concluded either that different molecules carry E(5), and H(8), K(11) or that both belong into the same molecule but under different conditions of fractionation some specificities are inactivated while others are not.

The absorption tests used for quantitative estimations of antigens agreed qualitatively with tests of immunogenicity made by inducing corresponding antibodies into appropriate hosts.

H-2 material obtained after treatment with deoxycholate confirms the results of Kandutsch and Stimpfling using Triton(6). Our results seem also to explain the lack of E(5) activity in their preparation.

Davies(14) and Al-Askari *et al*(15) failed to obtain antigenic activity after detergent treatment. The latter authors explain these differences by differences in techniques used for detection of the antigens. Another possibility could be that the antigens present in the experiments of Al-Askari *et al*(15) are of "E(5) type," disappearing after treatment with detergents. It is also possible that the behavior of antigens in fractionation depends on the source from which they are extracted.

The behavior of both supernatants in respect to Sephadex elution is different (Fig. 1 and 2). The E(5) active material from post microsomal supernatants seems to be constituted by large particles, since all the activity appears in a very narrow zone together with the first fraction of proteic material which elutes from the column. On the other hand, the K(11) activity of deoxycholate supernatant appears in the column in a very wide zone in and outside the void volume. The distribution of K(11) antigens could be explained if deoxycholate provokes disruption of membranes into a variety of molecular associations of different sizes.

The presence of membranous material with H-2 antigenic activity obtained from microsomal supernatant was reported by Manson *et al*(16). Experiments presented here show that E(5) specificity is expressed in material obtained under similar conditions.

In short, evidence is presented as to the possibility of obtaining fractions in which some of the H-2 specificities (antigens) determined by $H\text{-}2^a$ allele in A/Sn mice can be separated from others. No good explanation can be advanced as the meaning of this immunological classification in specific terms is not clear.

Summary. Separation of some H-2 specificities can be seen by subcellular fractionation and deoxycholate disruption of spleens of A/Sn mice. Antigens D(4), E(5) and K(11) show different patterns of distribution when they are examined in microsomal and post microsomal spleen fractions. A similar situation is observed when fractions are examined after deoxycholate fractionation. The antigens were detected by absorption of hemagglutinating capacity of antisera and induction of hemagglutinins on injecting anti-

gen preparations in adequate receptors.

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Inotropic and Chronotropic Effects of a Series of β-Adrenergic Blocking Drugs: Some Structure-Activity Relationships.* (31138)

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Since the discovery in 1958 by Powell and Slater(1) that dichloroisoproterenol (DCI) had significant *beta*-adrenergic receptor blocking[‡] actions, many compounds have become available which have even more specific and potent *beta*-adrenolytic properties. This report deals with a study of the inotropic and chronotropic effects of six of these *beta*-adrenolytic drugs and their isomers on the isolated heart.

While all the compounds to be discussed have some degree of *beta*-adrenergic blocking action, we were impressed with the fact that there were wide differences in the effects of these compounds on the contractile and electrical properties of the heart. Therefore, the material to be presented is an attempt to correlate the chemical structural features of these compounds with (a) *beta*-blocking actions and (b) influence on cardiac contraction, rate, and electrical properties.

Methods. Inotropic studies. The effects of the compounds on the isometric force of contraction of electrically driven rabbit left atrial preparations were studied according to the methods previously described(2). Basically, isolated left atria were suspended in a muscle bath containing 50 ml Krebs-Ringer solution, and stimulated at a rate of 120 beats/min using a square wave stimulus of 5 msec pulse duration at a strength 3 times threshold voltage. The atria were equilibrated for 100 minutes under these conditions before any drugs were added to the bath. Changes in force of contraction are expressed in terms of per cent of the post equilibration force (0 time in the Figures).

Chronotropic studies. The effects of the drugs on heart rate were determined on iso-

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[‡] The terms *beta*-adrenergic receptor blockade, *beta*-adrenolytic and *beta*-blockade are used interchangeably in this report.