citatory action of ether on ACTH secretion. The increase in circulating ACTH which occurs during the early phases of ether anesthesia in intact dogs may well be adequate to account for the failure of Monase to inhibit corticosteroid secretion in contrast to the marked corticosteroid suppression observed during barbiturate anesthesia. Whether Monase alters secretion of ACTH by pharmacological effects on peripheral sensory receptors or by acting on some area of the central nervous system remains to be determined.

Summary. Clinical toxicity encountered with 3-(2-aminobutyl)-indole acetate (Monase) has included syncope and hypotension. Hence the effects of this compound on adrenocortical function were studied. A sharp decrease in adrenocortical steroid output of dogs under pentobarbital anesthesia was seen shortly after intravenous injection of this compound. Doses of 5 mg/kg and 10 mg/kg were used. Adrenal venous blood levels of 17-hydroxycorticosteroids returned to normal at 1-2 hours after drug administration. ACTH infusion as well as ether anesthesia prevented this corticosteroid depressant action. After hypophysectomy, Monase did not alter the increased corticosteroid secretion induced by exogenous ACTH. The data suggest that the pharmacologic action of Monase in reducing 17-hydroxycorticosteroid secretion in intact dogs is a result of suppression either of formation or release of ACTH in dogs under pentobarbital anesthesia.

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Identification of Rabbit, Monkey, and Dog Antibodies with PCA Activity for Guinea Pigs.* (29388)

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Allergic reactions in man are known to be caused by reagins, an antibody type distinct from human γ_2 antibodies(1). Recent studies of the antibodies responsible for anaphylactic reactions in experimental animals revealed that guinea pigs(2), rats(3), dogs (4), and mice(5) possess in addition a special antibody type, distinct from γ_2 globulins, responsible for anaphylactic reactions in each of these animal species. The anaphylactic antibodies of these various animal species and of man have faster electrophoretic mobilities than their γ_2 globulins and bind to tissues of their respective species to sensitize them for anaphylaxis. Only anaphylactic antibodies of the guinea pig and the mouse have been well characterized, being the only ones produced in sufficient quantities to allow specific isolation and study. They have been found to belong to a distinct antibody type, which has been called γ_1 globulin, to dis-

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tinguish them from γ_2 and γ_{1A} or β_{2A} globulins(2,5).

Passive anaphylactic reactions of the guinea pig, both systemic(1) and local [passive cutaneous anaphylaxis—PCA(6)] have been very extensively studied as a model of anaphylactic reactions and also, especially in the case of PCA, as a method to detect and measure antibodies(6,7). These techniques proved very useful due to the high sensitivity of PCA and also because, besides guinea pig γ_1 antibodies, the antibodies of many foreign species, rabbits, monkeys, humans, mice, dogs, and cats(7) are capable of transferring anaphylactic sensitivity to the guinea pig.

The demonstration in the sera of some of these species of several antibody types, only one of which is capable of sensitizing its own species(3-5), makes it essential to attempt to characterize the foreign antibody types capable of transferring PCA to guinea pigs and to compare them with guinea pig γ_1 anaphylactic antibodies in this respect. A study was made of the rabbit, monkey, and dog antibody types capable of sensitizing the guinea pig using the electrophoretic techniques which allowed the characterization of guinea pig γ_1 antibodies and their separation from guinea pig γ_2 antibodies(2,8).

Materials and methods. Antigens. Highly derivatized conjugates of the 2,4-dinitrophenyl group with bovine gamma globulin (DNP-BGG), bovine serum albumin (DNP-BSA), and bovine fibrinogen were prepared according to the techniques described(9).

Antibodies. Rabbits, Rhesus monkeys, and dogs of mixed breed were initially immunized with DNP-BGG emulsified in complete Freund's adjuvant (Difco Lab., Inc., Detroit). Each animal received intramuscularly 0.25 ml of the emulsion containing 1 mg DNP-BGG in each of 4 sites. Monkey and dogs received 3 booster injections at 7 to 10 day intervals: 1 mg of DNP-BGG in 1 ml of 0.15 M NaCl was administered intramuscularly and intradermally in divided doses at several sites. Rabbits were boosted intravenously twice a week beginning 2 weeks after the initial injection. All animals were bled one week after the last booster injection.

Specific antibody isolation. Purified anti-DNP antibodies were isolated from the antisera of dogs, rabbits, and monkeys after decomplementation with an immune precipitate of rabbit antiovalbumin and ovalbumin at equivalence using about 10 mg of antiovalbumin per 10 ml of serum to be decomplemented.

The anti-DNP antibodies were specifically precipitated with DNP-bovine fibrinogen, and were specifically eluted with ϵ-DNP-lysine, 2×10^{-3} M. Streptomycin, 35 mg/ml, was then added to precipitate the DNP-fibrinogen. The purified antibody preparations were dialyzed against pH 7.6 phosphate buffered 0.15 M NaCl(8).

Immunoelectrophoresis. Immunoelectrophoretic analysis of the purified monkey, dog, and rabbit antibody preparations was carried out using a modification(8) of Scheidegger's technique(10). They were developed with rabbit antisera prepared against dog or monkey serum, or with a sheep antiserum against rabbit serum.

Zone electrophoretic separations of antibodies. A preparation of purified monkey anti-DNP antibodies containing 30 mg of antibody protein was separated by starch block electrophoresis using the techniques previously described for guinea pig antibodies (8). The fractions were eluted from half-inch cuts of the block, lyophilized and redissolved in 1 ml of 0.15 M NaCl. They were tested for their ability to agglutinate DNP-BSA coated, tanned sheep erythrocytes and to lyse these cells in presence of guinea pig complement. The PCA titers for guinea pigs and the complement fixation titers, using guinea pig complement, of the various fractions were measured. A similar electrophoretic separation of a rabbit anti-DNP-BGG serum (serum 104) was performed on another starch block. The various fractions were assayed for PCA in guinea pigs and for passive hemolysis of DNP-BSA coated, tanned sheep erythrocytes.

A preparation of dog anti-DNP antibodies from an individual dog which produced a sufficient amount of antibodies to allow electrophoretic separation was analyzed by immunoelectrophoresis on agar gel. One milli-

TABLE I. PCA and Passive Hemolysis with Rabbit Anti-DNP-BGG #104, Starch Block Electrophoretic Eluates.

Fractions	Protein content of eluate (μg)	PCA titer*	Passive hemolysis*
0	270	1/100	1/50
1	290	1/100	1/100
2	230	1/100	1/100
3	200	1/100	1/100
4	190	1/100	1/100
5	120	1/50	1/50
6	60	1/10	1/5
7	30	1/2	1/8
8	15	Undiluted	1/4
9	30	0	0

* Last dilution which gave positive result.

gram samples of antibodies were placed into each of 2 wells of an immunoelectrophoresis plate. Following electrophoretic separation, a specific rabbit antiserum was added to the outer trough adjacent to one of the wells. The agar band near the second well was cut into 4 mm strips numbered 1 to 9. The individual strips were broken with a glass rod and extracted with 0.5 ml of 0.15 M NaCl for 24 hours at 4°C. The eluted fractions were tested for their ability to agglutinate and lyse DNP-BSA coated, tanned sheep erythrocytes and for PCA activity in dogs and guinea pigs.

Serological tests. Passive hemagglutination of DNP-BSA coated, tannic acid treated sheep erythrocytes and passive lysis of these cells by anti-DNP antibody in presence of guinea pig complement were performed as described previously(11). Complement fixation by purified anti-DNP antibodies and DNP-BSA was carried out using the techniques already described for guinea pig anti-DNP antibodies(11).

Passive cutaneous anaphylaxis (PCA). PCA with anti-DNP antibodies was performed in guinea pigs, dogs, and monkeys, using the methods described(6). DNP-BSA was used as eliciting antigen.

Results. I. PCA in guinea pigs with rabbit antibody. The biological activities of the various fractions obtained from a starch block electrophoretic separation of a rabbit anti-DNP-BGG serum are presented in Table I. A nearly perfect correlation is found between the PCA and passive hemolysis titers in all fractions, indicating that the same rabbit antibody type is able to bind complement and to sensitize guinea pigs for anaphylaxis. The rabbit antibody type capable of transferring PCA to guinea pigs was identified by immunoelectrophoresis using a preparation of purified rabbit anti-DNP antibodies. Immunoelectrophoretic analysis of this preparation developed by a sheep anti-rabbit serum showed only a single precipitin line corresponding to rabbit γ_2 globulin. Eluates from agar cuts of an identical electrophoretic separation performed on the same plate were assayed for their ability to transfer PCA to guinea pigs. PCA activity was found in all fractions corresponding to the γ_2 precipitin line and only in those fractions. These experiments show that rabbit γ_2 complement-fixing antibodies are able to sensitize guinea pigs for PCA.

II. PCA in guinea pigs with dog antibodies. The results of an immunoelectrophoretic separation of purified dog-anti-DNP antibodies are shown in Fig. 1. The rabbit antiserum used to develop the immunoelectrophoresis identifies the location of one major dog antibody population (presumably $7S\gamma_2$) in the "slow" region. Hemagglutinating antibodies were present in fractions 1-8 but lytic antibodies were confined to fractions 5-7. Similarly, guinea pig skin sensitizing antibodies were found in fractions 5-7; the slight PCA activity of fraction 1 may be attributed to antibody remaining near the point of application to the gel.

A dog was injected in the skin of the back with 0.25 ml of eluted fractions 1-9. After a 24-hour latent period, Evans blue dye was injected intravenously, followed, after 15 minutes, by antigen (DNP-BSA, 1 mg protein/kg of body weight). Slight bluing of the skin, indicating a weakly positive reaction was observed only with fractions 2 and 3. Although these experiments were repeated several times, it was not possible to obtain more definite PCA results in the dog with the quantities of antibody available. However, in all experiments, sensitization of guinea pig skin was obtained only with antibody eluted from the "slow" region of the gel.

The dog antibody type capable of trans-

ferring PCA to guinea pigs appears to be a γ_2 complement-fixing globulin which is not able to transfer anaphylactic sensitivity in its own species.

III. PCA in guinea pigs with monkey antibodies. Rhesus monkeys immunized with DNP-BGG as described above produced large amounts of precipitating anti-hapten antibodies. The results of an electrophoretic separation of a purified monkey anti-DNP antibody preparation on starch block are shown in Fig. 2. The peak of antibody concentration was contained in fractions 6-10 obtained from the "slow" region of the gamma globulin zone. Although there was some trailing of protein in the "fast" region, there did not appear to be significant amounts of fast migrating antibody.

The distribution of hemagglutinating, hemolytic, complement-fixing and guinea pig

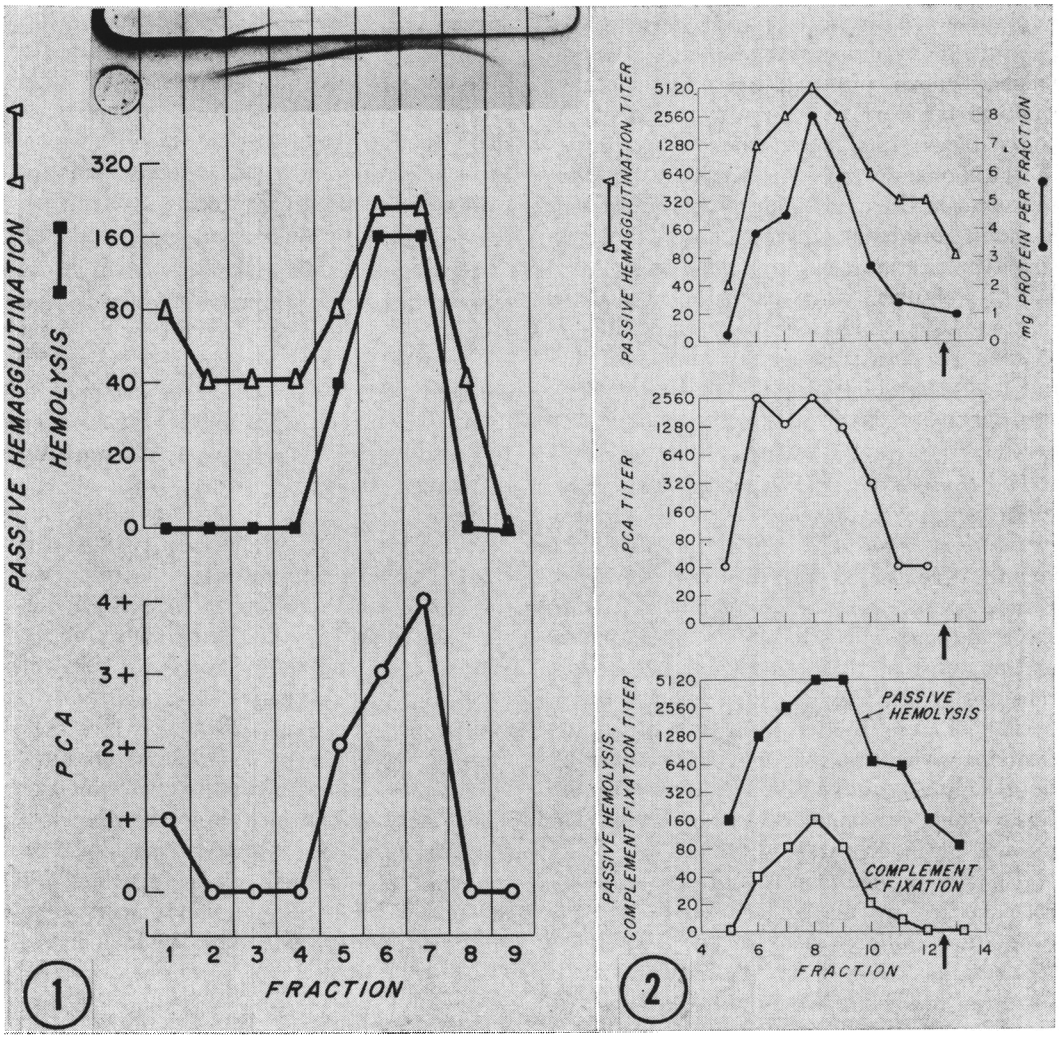

FIG. 1. Passive hemagglutinating, hemolytic and guinea pig skin-sensitizing activity of eluates from agar strips containing electrophoretically separated dog anti-DNP antibodies. Wells were filled with solution containing 1 mg antibody; trough (at top of figure) was filled with 0.1 ml of specific rabbit antiserum. Fractions are numbered from positive to negative.

FIG. 2. Titration of protein concentration, passive hemagglutinating, hemolytic, complement-fixing and guinea pig skin-sensitizing activity of fractions from starch block electrophoresis of a purified monkey anti-DNP antibody preparation. Arrow indicates point of application of serum on starch block. Fractions are numbered from negative to positive.

skin sensitizing antibody activity closely paralleled the distribution curve of protein concentration.

A rhesus monkey was injected intradermally in the skin of the abdomen with 0.1 ml of each eluted fraction (containing 0.01 to 0.5 mg antibody protein depending upon the fraction tested). After a 48-hour latent period Evans blue dye was injected intravenously followed 15 minutes later by the injection of DNP-BSA. No visible reactions were obtained, in several experiments, with any of the fractions tested. In all experiments, guinea pig skin-sensitizing activity was present in the γ_2 globulin region of the starch block. Immunoelectrophoretic analysis of the monkey anti-DNP antibody preparation used in these experiments developed with a rabbit anti-monkey serum showed only a single precipitin line corresponding to monkey γ_2 globulins. Thus, as in the case of the dog and the rabbit, the monkey antibodies capable of sensitizing guinea pigs for PCA are the γ_2 antibodies which are not able to transfer anaphylactic sensitivity in their own species.

IV. Comparison of the sensitizing properties for guinea pigs of guinea pig γ_1 antibodies, and rabbit and monkey γ_2 antibodies. Quantitative studies were made with purified guinea pig γ_1, rabbit and monkey γ_2, anti-DNP antibodies to obtain data on the minimal amounts of these antibodies required to sensitize guinea pigs for PCA. Guinea pig γ_1 antibodies were isolated from guinea pigs immunized without adjuvants which produce almost only γ_1 antibodies(8). Their identity was verified by immunoelectrophoresis. The purified antibodies were dissolved in 0.15 M NaCl, the protein content of the solutions was checked by the Folin reaction and appropriate dilutions were made in 0.15 M NaCl saline. For every experiment freshly dissolved antibodies were used as it was seen that low concentrations of purified antibodies lost their biological activities when stored. All 3 antibody preparations were assayed on the same guinea pigs. The results presented in Table II are the average of 4 experiments in 16 different animals.

TABLE II. Minimal Amount of Purified Anti-DNP-BGG Antibody Giving Threshold PCA Reactions in Guinea Pig.

Antibody	Protein content in μg/.1 ml
Rabbit	.08 to .1
Guinea pig	.07 to .1
Monkey	.28 to .33

Rabbit γ_2 and guinea pig γ_1 antibodies seem to be as efficient on a weight basis in transferring PCA to guinea pigs and about 3 times more efficient in this respect than monkey γ_2 antibodies. The persistence of these purified anti-DNP antibodies in guinea pigs' skin was also investigated. 0.25 μg of rabbit γ_2 and 1.75 μg of monkey γ_2 antibodies sensitized guinea pigs for about one day whereas equivalent amounts of γ_1 guinea pig antibodies persisted actively in the skin for more than 2 but less than 4 days.

Discussion. Anaphylactic reactions in guinea pigs are mediated by a special antibody type, γ_1 globulins, which lack the capacity to bind complement in the presence of antigen(2,8,11). Guinea pig γ_2 complement fixing antibodies cannot sensitize guinea pigs for anaphylaxis but can act as blocking antibodies by competing with γ_1 antibodies for antigen. Other species, man, monkey, mice, dogs, possess also an antibody type, distinct from their γ_2 globulins, which is responsible for anaphylactic sensitivity in each of these species. However, when antibodies from foreign species, rabbit, monkey, or dog, are used to transfer anaphylactic sensitivity to guinea pigs, the results show that it is the γ_2 antibody type which is capable of binding to guinea pigs tissues to sensitize them for PCA. These antibodies which can sensitize guinea pigs are incapable of transferring anaphylactic sensitivity in their original species. Similar results have been obtained with human and with mouse antibodies. Only human γ_2 (12-14) and mouse γ_2(5) antibodies can sensitize guinea pigs for PCA, while human reagin(12) and mouse γ_1 antibodies(5) which are the anaphylactic antibodies of their respective species fail to transfer PCA to guinea pigs. A general rule can therefore be formulated that when guinea pigs are sensitized with antibodies from foreign species, it is not the anaphylactic antibody of the species

which is capable of transferring PCA to guinea pigs, but rather their γ_2 antibody globulin.

Since the capacity to transfer anaphylactic reactions depends upon the presence on the sensitizing antibodies of receptors able to bind to specific tissue sites(15), the observations imply that the anaphylactic antibodies of different species differ in the structure of their tissue receptors. It is therefore a matter of chance, probably due to the fact that all mammalian γ-globulins are built according to similar patterns, that the γ_2 antibodies of some foreign species, man, monkey, dog, and mouse, possess the structure able to bind to guinea pig tissues, which characterizes guinea pig γ_1 antibodies. Such receptors are not found, however, in all γ_2 mammalian globulins since neither horse, cow, or sheep antibodies can sensitize guinea pigs for PCA(16). The fact that γ_2 antibodies of several mammalian species, their largest antibody type, are capable of sensitizing guinea pigs for PCA explains the great usefulness of this technique for detection of antibodies produced by these various species.

Summary. Guinea pigs may be sensitized for PCA reactions by guinea pig 7S gamma 1 antibodies but not by guinea pig 7S gamma 2 antibodies. However, with all other species capable of sensitizing the guinea pig, it is always the 7S gamma 2 antibody type which mediates sensitization of guinea pigs for PCA.

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A Description of Two Newly-Recognized Rhinoviruses of Human Origin. (29389)

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As presently constituted, the human picornaviruses are divided into 3 groups; (a) the enteroviruses, subgrouped as polioviruses, Coxsackie viruses and echoviruses (b) the rhinoviruses and (c) an unclassified group (1,2). Rhinoviruses, like the enteroviruses are small, ether-resistant viruses containing an RNA core. However, they can be distinguished from enteroviruses by their property of acid lability(3,4).

To date, 6 prototype rhinoviruses(5,6) have been sufficiently characterized to meet

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