reported by Kitchell and Wells(12), Yakaitis and Wells(13) and Coetzee and Wells(14), and has demonstrated clearly a reciprocal functional interrelationship between the foetal pituitary and the adrenal cortex.

The effect of adrenal cortical hormones on lymphatic tissue in adult animals has been studied extensively and the subject has been reviewed by Dougherty(15) and Dougherty *et al*(16). In the adult the adreno-cortical hormones are part of a homeostatic mechanism regulating lymphatic organ mass, and the work reported here indicates that a similar homeostatic mechanism is active during foetal life in the rat and rabbit regulating thymus growth.

Summary. Rat foetuses were subjected to hypophysectomy by decapitation at 18½ days of development. At 21½ days the experimental foetuses showed a hypertrophy of the thymus and a decrease in adrenal size compared with the litter mate controls When depot ACTH was injected into the foetuses at time of decapitation, this hypertrophy of the thymus was inhibited, and the experimental foetuses showed a hypoplasia of the thymus, and a hypertrophy of the adrenals. These results are interpreted as indicating that the foetal pituitary acting through the foetal adrenals is part of a homeostatic mechanism providing an inhibitory regulatory control on thymus growth. These experiments also suggest that the thymus hypertrophy found in human anencephalic foetuses is secondary to the hypoplasia of the foetal adrenals found in this condition.

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Histobacteriology of the Genus *Pasteurella* II. Specificity and Stability Of the Various Species of *Pasteurella*. (31110)

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The use of immunofluorescence for identification of microorganisms has received general acceptance since its introduction by Coons *et al*(1). The refinements of fluorescent antibody technique and its broad application in the fields of microbiology, experimental pathology, and immunology have been reviewed by Cherry and Moody(2), Beutner (3), Nairn(4), Smith *et al*(5), and others.

Study of the interrelationship between invading parasites and their host tissues is dependent upon the specific identification of the organism or its products *in situ*. Immunofluorescence has been used to determine the

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fate of bacterial antigens, Coons and Kaplan (6), Walker(7), and the distribution of invading microorganisms, Coons *et al*(8), Moulton(9), and Goldman(10). The materials used in these studies were either fresh frozen tissue sections or impression smears of unfixed tissues. Tissues obtained from surgical procedures and necropsy, except when used in specialized research, are routinely preserved in 10% formalin and processed for paraffin sectioning. Successful application of fluorescent antibody technique for identification of bacteria in tissues subjected to harsh chemical treatments such as fixation, dehydration, and paraffin impregnation depends upon stability of the bacterial antigen being studied. During the course of investigations of the histobacteriology of *Pasteurella* infections, the antigenic stability of the various species of the genus *Pasteurella* to chemical treatment was studied.

Materials and methods. A catalogue of the *Pasteurella* strains used was presented previously by Marshall, Hansen, and Eveland(11).

Whole cell antisera were prepared against the following representative strains of *Pasteurella* species by a modification of the Ando *et al*(12) method as presented earlier by Marshall, Hansen, and Eveland(11): *Pasteurella anatipestifer* B2, *P. gallinarum* 833, *P. haemolytica* 826, *P. novicida* U112, *P. tularensis* Schu 4. Polyvalent antisera were prepared against *P. multocida* strains 5240, 1305-1, 398, EC and 879, and *P. pseudotuberculosis* strains 1, 16, 25, 32 and 43. Purified Fraction 1 obtained from Dr. K. F. Meyer was used to prepare *P. pestis* Fraction 1 antiserum. All sera were stored at —20°C until used, and were conjugated with a single lot of fluorescein isothiocyanate according to Marshall *et al*(13). Lissamine rhodamine conjugated bovine serum albumin was added as a counter stain to each serum, Smith *et al*(14).

Bacterial strains utilized. The following 199 strains of bacteria were used to determine the specificity of all sera used in these experiments: *Pasteurella anatipestifer* 5, *P. gallinarum* 29, *P. haemolytica* 16, *P. multocida* (A-D) 26, *P. novicida* 3, *P. pestis* 24, *P. pseudotuberculosis* (I-V) 30, *P. tularensis* 10, *Br. abortus* 5, *Br. melitensis* 5, *Br. suis* 5, *Moraxella* sp. 5, *Salmonella* sp. (A-E) 15, *Bordetella* sp. 16, *Ps. pseudomallei* 2, and *H. gallinarum* 3.

Characterization of sera. Smears were prepared of saline suspensions of viable test strains. The smears were air-dried and heat-fixed. Staining titers were determined by layering a series of slides with 2-fold dilutions of homologous antiserum and incubating for 15 minutes at room temperature. The smears were washed 5 minutes each in 3 changes of 0.01 M phosphate-buffered saline pH 7.2 and mounted in buffered glycerol at pH 7.2. The highest dilution giving a 4+ reaction was considered to be the staining titer. Smears of heterologous strains were stained with serum dilution containing 10 staining units, *e.g.*, a serum with a titer of 1:80 was used at a 1:8 dilution.

Antigen stability tests. Air-dried, heat-fixed smears of the various strains were subjected to the following chemical treatments: exposure to 10% formalin for 1 hour and 24 hours; exposure to absolute, 95% and 70% ethanol, absolute methanol, ether alcohol, and chloroform for 2 hours. All smears were air-dried and washed in buffered saline (pH 7.2) before being stained.

Specimens of excised normal liver were inoculated with thick suspensions of each species of *Pasteurella*. The tissues were immediately fixed in 10% neutral formalin for 14 days, cut in 5-mm sections and dehydrated by exposure to 80% and 95% ethanol each for 2 hours and absolute ethanol for 3 hours. The dehydrated sections were cleared in chloroform for 3 hours before they were impregnated with paraffin at 56°C for 4 hours and subsequently embedded in paraffin. Sections of 4-6 μ were cut and mounted on microscope slides. The sections were deparaffinized in xylene 4 minutes, rehydrated by passage through graded ethanol, absolute 2 minutes, 95% 2 minutes, 70% 2 minutes, and washed for 10 minutes each in 3 changes of buffered saline pH 7.2. The sections were then stained with conjugated homologous antiserum or conjugated normal rabbit serum for 15 minutes at room temperature, washed for 5 minutes each in 3 changes of buffered saline pH 7.2 and mounted in buffered glycerol pH 7.2.

Results and discussion. Type specific sera produced against *Pasteurella multocida* and *P. pseudotuberculosis* were unsatisfactory, as a high number of cross reactions were observed between serotypes within each species. Polyvalent sera produced against these 2 species reacted with all strains of the respective species. Homologous staining titers ranged from 1:40 to 1:320.

Pasteurella anatipestifer antiserum reacted with all 5 strains of *P. anatipestifer,* but did not react with any of the 194 heterologous strains tested. The 29 strains of *P. gallinarum* were all stained by the homologous antiserum which was non-reactive for all other strains tested. Sera prepared against both *P. haemolytica* and *P. multocida* failed to stain all homologous strains to titer when the strains had been repeatedly transferred on artificial media. When the weakly reacting strains were passed either through embryonated eggs or mice, the resulting isolates reacted with diluted antisera. Neither sera showed any cross reaction with other species tested. Undiluted *P. tularensis* and *P. novicida* antisera cross reacted; however, when used at a 1:5 dilution, they were specific for the species used to induce their production. Neither antiserum stained any of the 15 strains of *Brucella* when tested at a 1:5 dilution nor did any other of the 171 strains tested react. Specific antiserum to Fraction 1 of *P. pestis* stained 22 of 24 strains of *P. pestis* while 2 strains of *P. pestis* DF 1 and M23 failed to stain with anti-Fraction 1 serum but were stained with polyvalent *P. pseudotuberculosis* antiserum. These results were expected, as both strains are either totally lacking or highly deficient in Fraction 1 antigen. Strain M23 is a modified virulent strain similar to the one described by Burrows and Bacon(15) while DF 1 is a human isolate deficient in F1, Meyer(16). The polyvalent *P. pseudotuberculosis* antiserum stained all 30 strains of *P. pseudotuberculosis* regardless of what serotype they were. In addition, cross reactions were observed with Salmonella species and polyvalent *P. pseudotuberculosis* antiserum. These cross reactions were due to shared antigens. Antigen 5 of *P. pseudotuberculosis* type II is similar to antigen 4 of the group B. *Salmonella* and antigen 9 of *P. pseudotuberculosis* type IV is immunologically related to antigen 9 of the group D *Salmonella* (17).

The stability of antigens of *Pasteurella* species to chemical treatment, as determined by their ability to react with conjugated specific immune serum after treatment, was as follows. No alteration in staining reactions was observed with *P. anatipestifer, P. gallinarum, P. haemolytica, P. multocida, P. novicida,* or *P. tularensis,* and their specific antisera when these species were exposed to any of the chemical treatments alone or in sequence. When inoculated liver sections were stained by the fluorescent antibody technique, the organisms stained a bright apple green while the surrounding tissue was a dull red-orange in color. Detailed comparison of standard histological staining and immunological staining of pathological specimens will be reported later.

Antigens of *P. pestis* and *P. pseudotuberculosis* were adversely affected to various degrees by all the chemical treatments except absolute ethanol and absolute methanol. The detrimental effect of acetone could be overcome provided a temperature of —20° was maintained during fixation. When paraffin embedded tissues containing either of these 2 species were stained, large numbers of organisms were seen which had not reacted with the specific antiserum. They appeared the same non-specific red-orange as the surrounding tissues. Walker(18) has demonstrated the presence of the specific antigen, F1, of *P. pestis* in frozen tissues which were unfixed. Hudson *et al*(19) demonstrated the presence of *P. pestis* antigens in desiccated animal remains which had not been exposed to fixatives. The results of these investigators, when compared with our results, indicate that while the immunological stability of Fraction 1 is unaltered by time and ambient environmental temperature, it is rapidly denatured by organic fixatives and solvents.

Summary. Antisera prepared against *Pasteurella anatipestifer, P. gallinarum, P. haemolytica, P. multocida* (polyvalent) were specific for the species against which they were produced. A cross reaction was noted

between *P. tularensis* and *P. novicida* which could be eliminated by diluting either antiserum 1:5. Polyvalent antiserum produced against *P. pseudotuberculosis* stained strains of *P. pestis* deficient in Fraction 1 and species of Salmonella containing either somatic factors 4 and 9. The antigens of various species of the genus *Pasteurella* differ in their reactions with their antibodies after exposure to chemicals used routinely in histological processing. *P. anatipestifer, P. gallinarum, P. haemolytica, P. multocida, P. novicida,* and *P. tularensis* retain their ability to react with species specific antisera after treatment, while *P. pestis* and *P. pseudotuberculosis* are rendered non-reactive by most chemicals used in tissue embedding techniques.

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Antagonism of Anticonvulsants by Adrenergic Blocking Agents. (31111)

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The anticonvulsant effects of diphenylhydantoin(1), acetazolamide(2,3) and chlordiazepoxide(4) have been found to be antagonized by prior administration of reserpine. The mechanism of the antagonism of diphenylhydantoin and chlordiazepoxide by resperpine was found to be by some mechanism other than catecholamine or serotonin depletion(1,4) while that of acetazolamide was found to be mediated through depletion of catecholamines in the central nervous system (2,3). The present study was undertaken to further study the mechanism of action of these anticonvulsants and to determine if their activity was altered by adrenergic blocking agents.

Methods and materials. Male albino mice (Harlan Industries), weighing 18-26 g, were used in all the tests. Maximal electroshock seizures were produced by the method of Swinyard *et al*(5). A current of 50 ma and 0.2 sec duration was delivered *via* corneal electrodes. The criterion for protection against maximal electroshock was abolition of the hind leg extensor component of the seizure.

The ED_{50} values were calculated and compared for significance of differences by the method of Litchfield and Wilcoxon(6). The 95% confidence limits are included in parenthesis in the respective Tables.

The adrenergic blocking agents used in this investigation were: (1) dibenzyline, (2) tolazoline, (3) MJ-1999 (4(2 isopropylamino-