cortisone administration or in response to non-specific stress(13,14). Twenty-four hours after the injection of epinephrine the blood picture of both pyridoxine-deficient and control rats had returned to pre-injection levels in respect to all cellular constituents with the exception of the eosinophils which were still reduced by over 50%. These findings indicate that pyridoxine deficiency does not impair the ability of the pituitary-adrenal system to respond to acute stress. The activation of the adrenals which occurs in response to epinephrine administration, however, is based primarily if not entirely on the release of preformed ACTH. It is possible that the pyridoxine-deficient rat may have sufficient amounts of this hormone stored in its pituitary to effect a normal response to acute stress but that such rats may have an impaired ability to produce the increased amounts of ACTH required in chronic stress. The role of pyridoxine in protein metabolism suggests the possibility that animals deficient in this vitamin may have an impaired ability to synthesize proteins including adrenocorticotropin, thyrotropin, and others. In view of the increased requirement for thyrotropin(15) and adrenocorticotropin(3) during prolonged exposure to cold, the failure of pyridoxine-deficient rats to adjust to low environmental temperature may result not from pyridoxine deficiency *per se* but rather from a deficient secretion of pituitary hormones. Further experiments are indicated to determine the effects of thyroid, ACTH and cortisone administration on the survival of pyridoxine-deficient rats under conditions of low environmental temperature.

Summary. Immature rats were fed a purified ration containing all the known B vitamins in synthetic form and a similar diet with pyridoxine omitted. Tests were conducted in which rats fed the above diets were exposed to cold ($2 \pm 1.5°C$) from the first day of feeding or after an experimental period of 80 days. 68.8% of the pyridoxine-deficient rats in the latter group succumbed during a 20 day period of cold exposure in contrast to a 0% mortality for animals fed a similar diet supplemented with pyridoxine hydrochloride. In the group exposed to cold from the first day of feeding mortality was 25.0% and 18.7% respectively for rats fed the pyridoxine-deficient and control ration for a period of 100 days.

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Preparation of Specific Complement-Fixing Antigen with Lansing Poliomyelitis Virus.* (19082)

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The complement fixation (CF) technic has been successfully applied to the study of many of the human viral encephalitides with a notable exception of poliomyelitis. Recently Casals and Olitsky(1) described the preparation and use of a specific CF antigen prepared from MEF_1 virus adapted to new-born mice. Pollard(2) described the preparation of a CF antigen from Lansing infected cotton rat (CR) tissues by precipitation of the virus with methanol and use of the eluate as anti-

* Supported by a grant from the National Foundation for Infantile Paralysis, Inc. Part of this problem was performed while at the Hooper Foundation for Medical Research, University of California Medical Center, San Francisco, Calif.

1. Casals, J. and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1951, v75, 315.

2. Pollard, M., *J. Immunol.*, 1951, in press.

gen. A relatively rapid rise and fall in CF antibodies in immunized cotton rats was described with this antigen.

Loring, *et al.*(3) outlined a method of preparing a CF antigen by high speed centrifugation of the virus with the resuspended purified viral sediment being used as CF antigen. The technic recommended involved repeated saline extractions of the tissue and repeated cycles of centrifugation. For some reason the antigens thus prepared appeared to be somewhat nonspecific at low dilution and antigens from normal CR tissues also fixed complement with poliomyelitis serum.

By omitting many of the details advocated by Loring *et al.*, CF antigens were prepared by us in the ultracentrifuge which were highly specific. CF antigens were thus prepared from CR tissues infected with Lansing poliomyelitis, rabies (CVS strain), and St. Louis encephalitis viruses. Each CF antigen thus prepared acted as a control for the specificity of the others.

Methods. 1. *Preparation of Antigen.* Antigens were prepared from cotton rat nerve tissues infected with Lansing poliomyelitis (LCR), St. Louis encephalitis (SLE), and rabies (CVS strain) viruses, as follows:

a. Ultracentrifugation technic. The nerve tissue was emulsified in a Waring blendor to a final concentration in physiological salt solution of 20%. After clarifying the suspension at 5,000 rpm for 30 minutes and 18,000 rpm for 10 minutes, the clear liquid was thoroughly mixed with 1/3 volume of ether and stored at 4°C for 1 hour. After centrifugation at 5,000 rpm for 20 minutes, the aqueous portion was stored at —20°C overnight. The thawed preparation was then centrifuged at 5,000 rpm for 20 minutes and at 144,000 x G for 2 hours (Spinco Preparative Centrifuge†). The viral sediment was resuspended to 1/10 volume with neutral M/5 phosphate buffer, stored at 4°C overnight and then centrifuged at 5,000 rpm for 20 minutes. The clear supernatant fluid represented the antigen. Decimal dilutions thereof were inoculated into mature cotton rats (0.05 ml intracerebrally) in order to determine the quantity of viable virus present in the antigen. The antigen was stored at —20°C.

b. Lyophilization technic. 20% emulsions of nerve tissues in physiological saline were shell frozen in a dry ice-alcohol bath and immediately desiccated in a freeze-drying Cryochem apparatus.‡ The dried tissue suspensions were then extracted 3 times with 3 volumes of C.P. benzene at hourly intervals. After removal of residual benzene with a vacuum pump, the dried material was resuspended to 50% concentration with distilled water. After hydration overnight at 4°C, the suspensions were centrifuged at 10,000 rpm for 15 minutes. The clear supernatant CF antigen was tested for virus by inoculation of decimal dilutions thereof into cotton rats and for complement fixing properties as described below.

2. *Antiserum.* Adult cotton rats were given 3 intraperitoneal inoculations of 10,000 cotton rat LD_{50} of Lansing and of MEF_1 viruses at weekly intervals. They were bled one week after the third inoculation and their pooled serum was stored at —20°C. The monkey serum employed was obtained from hyperimmunized *Macaccus mulatta* monkeys. Each monkey was bled and inoculated at weekly intervals into the gastrocnemius muscle with 1,000 monkey LD_{50} of virus mixed with pendil.§ The clear serums were stored at —20°C.

3. *The CF test.* 0.2 ml quantities of antigen, inactivated antiserum, and complement (2 units) were mixed and after incubation overnight at 4°C, amboceptor and 2% sheep erythrocytes were added. The results were read after incubation at 37°C for 30 minutes. Cotton rat serums were diluted 1:5 and 1:10 in salt solution and inactivated at 61°C for 30 minutes; monkey serums, diluted 1:5, at 61°C for 20 minutes. Antigen, serum and

3. Loring, H. S., Raffel, S., and Anderson, J. C., PROC. SOC. EXP. BIOL. AND MED., 1946, v66, 385.

† Specialized Instruments Co., Belmont, Calif.

‡ Purchased from Stokes Machine Co., Philadelphia, Pa.

§ "Pendil" contains oxycholesterol-derivative, peanut oil, and beeswax and is manufactured by Endo Products, Inc., Richmond Hill, N. Y.

TABLE I. "Box-Titration" of Lansing Poliomyelitis Complement Fixing Antigen Prepared by Ultracentrifugation of the Virus.

Serum dilution	Antigen dilution 1–0	1–1	1–2	1–4	1–8	1–16
1–5	4+	4+	4+	2+	—	
1–10	4+	4+	—	—	—	
1–20	4+	4+	—	—	—	
1–40	4+	2+	—	—	—	
1–80	4+	—	—	—	—	
1–160	—	—	—	—	—	

TABLE II. The Specificity of Complement Fixing Antigens Prepared by Ultracentrifugation of the Virus.

Cotton rat antiserums	Cotton rat antigens LCR	Rabies	SLE
LCR	4+ 1:80	—	—
Rabies	—	4+ 1:40	—
SLE	—	—	4+ 1:80

TABLE III. Complement Fixation Reactions with Ultracentrifuged Antigens and Antiserum Prepared from Monkeys and Cotton Rats.

Serum	Cotton rat antigens Lansing	Rabies	SLE
Lansing	4+ 1:40	Neg	Neg
Brunhilde	Neg	Neg	Neg
Leon	Neg	Neg	Neg
SLE*	Neg	Neg	4+ 1:80
Rabies*	Neg	4+ 1:40	Neg
LCR*	4+ 1:80	Neg	Neg

* Antiserum prepared in cotton rats. SLE = St. Louis encephalitis; LCR = Lansing cotton rat.

complement controls accompanied each test.

Results. Tables I and II indicate that specific CF antigens were prepared by the centrifugation technic described above. The Lansing antigens were not stronger than those prepared by other methods(1,2), rarely exceeding 1:2 dilution for one unit of antigen, but occasionally some antigens were used at 1:8 dilution. Most of the tests contained 2 units of Lansing antigen at 1:1 dilution. Rabies and SLE antigens contained 2 units of CF antigen in dilutions as high as 1:20 and 1:40; however, they were diluted 1:1 and 1:2 when used for control purposes. Serums from immunized cotton rats fixed complement only with the antigen against which they had been immunized. It appeared that the titer of CF antibodies for Lansing virus in cotton rats exhibited a chronological curve similar to that demonstrated previously(2): a sharp rise and rapid decline of CF antibodies. Lansing CF antibody appeared within 10 days after initial inoculation and became undetectable 3 months later. Serum from MEF_1-immune cotton rats fixed complement specifically with the Lansing antigen. The chronological rise and fall of cotton rat CF antibodies against rabies and St. Louis encephalitis viruses were not studied.

Serum specimens from 6 Lansing-immune monkeys fixed complement with Lansing antigen specifically (Table III); while with the same antigen, 3 serums from Brunhilde-immune monkeys and one from a Leon-immune monkey¶ were negative. Three monkeys which were inoculated intramuscularly with Lansing virus in "Pendil" developed CF antibodies in 10 days which persisted for 3 months thereafter. The serums of these monkeys were negative with rabies and SLE antigens.

Of 8 Lansing antigens prepared by the above technic, 7 were specific CF antigens. 10^{-4} dilutions of the latter 7 were infective for cotton rats by the intracerebral route; the other one was not. When ether treatment was omitted, the resulting CF antigen was nonspecific. Three CF antigens were prepared each with rabies and St. Louis encephalitis viruses and all were specific. The average yield of Lansing CF antigen per g of tissue was 0.5 ml. The Lansing antigen retained its CF properties for at least one month at —20°C.

When infected tissues were processed by the lyophilization technic described above, CF antigen could not be obtained. Consistently, the virus content of the antigens was 2-3 logs lower than that of the unprocessed emulsion. Six Lansing CF antigens were processed by the lyophilization technic and none was satisfactory. Specific potent CF antigens were consistently processed from SLE and rabies infected tissues by this technic.

Discussion. The Lansing CF antigens prepared in the ultracentrifuge were as potent as any previously described. The technic was considerably simplified and the results were

¶ Kindly supplied by Dr. Jonas Salk, University of Pittsburgh, Pittsburgh, Pa.

uniformly satisfactory. Considering the cost of equipment and the yield of antigen per g of tissue (0.5 ml/g tissue), this antigen is no less expensive to prepare than that produced by methanol precipitation of the virus. Its advantage lies in consistent results and in the specific nature of its reaction.

It is now felt that the preparation of specific Lansing antigen is predicated on a high level of virus in the tissues employed. The one antigen which was unsuitable was infective only at 10^{-2} dilution.

Antigens prepared by procedures involving lyophilization have been neither antigenic nor infectious. The desiccation technics are very useful for the preparation of rabies and SLE antigens as has previously been demonstrated(4-6), but the technics here successful, failed with poliomyelitis virus. If the MEF_1 virus adapted to newborn mice by Olitsky and Casals(1) has developed a tolerance for desiccation, then these technics may be very useful for this strain of virus.

The Lansing CF antigen fixed complement with serum from Lansing-immune monkeys and failed to do so with serum from 3 Brunhilde-immune and one Leon-immune monkeys. The maximum titer of the Lansing serum was 1:40. Lansing antigens previously prepared with methanol(2) were negative with serum from immunized monkeys. This may be due to the more potent CF antibody response to the Lansing-"Pendil" inoculum or, perhaps, to the production of a more potent or complete CF antigen by the ultracentrifugation technic. The antibody enhancing response of monkeys inoculated with virus-adjuvant mixtures has been demonstrated by others(7,8). The persistent CF response in the Lansing-immune monkeys (3 months) might be attributed to the effect of the depot plus virus inoculum.

There is some evidence of strain specificity with the ultracentrifuged antigens: Lansing CF antigens fixed only with Lansing and MEF_1 antiserum. Since we have not yet demonstrated specific CF antibodies with the Brunhilde- and Leon-immune serums, the specific response must be regarded with some reservation. Specific CF antigens can be prepared for rabies and SLE viruses by technics involving the extraction of desiccated tissue suspensions with ether and benzene (4-6). It appears, however, that lyophilization is so destructive to poliomyelitis virus that the above technic is inapplicable to the conventional Lansing virus in cotton rat tissues(9,10).

The rise and fall of Lansing CF antibody in immunized cotton rats is not inconsistent with that previously encountered with other viral agents in humans and in animals(11,12). The persistent response in monkeys may be abnormal, since monkeys inoculated with virus in saline have manifested a very transitory CF response. If a transient serological pattern can be demonstrated in human infections, in specific manner for all types of poliomyelitis virus, it would be of much help in deciding whether the infection was current or past.

Summary. Specific complement-fixing antigens were prepared from cotton rat tissues infected with Lansing poliomyelitis, rabies, and St. Louis encephalitis viruses. The antigens were prepared by concentration of the viruses through high speed centrifugation. Lansing antigens prepared from lyophilized tissues were of little value as CF antigens.

A relatively rapid rise and fall in poliomyelitis complement-fixing antibody levels in cotton rats is described. The Lansing antigen fixed complement with serum from Lansing-immune monkeys, but not with serum from

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monkeys similarly immunized against Brunhilde and Leon viruses.

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Action of Adrenal Hormones on Lethal Toxicities of Certain Organic Compounds. (19083)

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It has been reported(1) that mice bearing Sarcoma 37 will tolerate larger doses of a number of tumor-damaging trivalent arsenical compounds than will non-tumor-bearing mice. The report by Dalton(2) that tumor growth in mice is accompanied by histologic changes in the adrenals suggested that the observed increased resistance of mice to trivalent arsenical compounds with growth of Sarcoma 37 might be related to a change in adrenal activity. This paper reports results of experiments performed to test for effects of adrenal hormones on the resistance of mice to trivalent arsenical compounds and to certain other organic compounds.

Materials and methods. Injections into mice. Compounds under test for lethal toxicity or tumor-damaging potency were injected in aqueous solution either subcutaneously or intravenously. Hormone preparations under test for ability to counteract toxic or tumor-damaging effects of these compounds were usually injected intraperitoneally. Doses for adrenal extract and for 10% alcohol have been stated as cc per mouse or cc per gram. All other substances employed were dissolved or suspended at concentrations such that the desired doses, in mg per kg, were secured by injecting the test solutions or suspensions into mice in the amount of 0.01 cc of solution or suspension per gram of body weight. The amounts of substances injected, the routes of injection, and the strain, sex and numbers of mice used are indicated in the Tables and Figures.

Optimum time for injecting hormone to secure protection. In any one experiment, each mouse in a control group of at least 25 mice received an LD60-100 amount of the toxic compound, but no hormone preparation. Each experimental group consisted of 10 mice, each of which received the selected dose of a hormone preparation, and the same dose of the toxic compound as that administered to mice in the control group. For each experimental group, a different time interval between administration of hormone preparation and administration of toxic compound was employed (Fig. 1). *Lethal dose determinations.* Experiments and calculations were done in accordance with a simplification of Karber's method described by Cornfield and Mantel(3). Generally a given dose of toxic compound was administered to 10 mice, and the doses were varied in 0.1 $\log_{10}$ steps. The time interval between injection of hormone preparation and of toxic compound was in the range indicated by previous experiments as optimal for securing protection of mice against an LD60-100 amount of the toxic compound. *Estimation of gross and histologic damage induced in mouse sarcoma 37 by administration of compounds to mice:* Tumor mash was

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