tested. Δ^4-pregnenol-21-trione-3,12,20 acetate has been found to cause significant sodium retention at 1340 μg and a negative effect at 400 μg. Thus the introduction of the 12 keto group results in a compound possessing about 2 to 5% of desoxycorticosterone. 17-Hydroxycorticosterone produced an increased sodium excretion at 50 μg but the effect was either minimized or obliterated at 200 μg. Increased urinary excretion of sodium has been observed previously for 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone in normal dogs and rats[2] and in partially depancreatized rats.[3] Ingle and coworkers[4] have found that relatively large doses of ether 17-hydroxycorticosterone or 17-hydroxy-11-dehydrocorticosterone caused an immediate increase in sodium excretion during the first 24 hours but that the sodium excretion returned to the control levels in spite of continued treatment.

Summary. Eleven steroid compounds have been studied as to their influence on sodium excretion in adrenalectomized rats. Desoxycorticosterone caused significant retention at one microgram, and the acetate was effective at 25 micrograms, the lowest concentration studied. Δ^4-pregnenol-21-trione-3,12,20-21-acetate gave a significant retention at 1340 micrograms. 17-Hydroxycorticosterone caused a significant excretion of sodium.

The following steroids were found to be negative: 11-dehydrocorticosterone acetate (100 μg), pregnanetrione-3,11,20 (200 μg), corticosterone (400 μg), allopregnanepentol-3 (β), 11(β), 17(α), 20, 21-3(β), 20, 21-triacetate (800 μg), allopregnanetriol-3(β), 17 (α), 21-one-20 (800 μg), testosterone (2000 μg), estradiol (2000 μg), and pregnanetrione-3,12,20 (2000 μg).

Received October 13, 1949. P.S.E.B.M., 1949, **72.**

2 Thorn, G. W., Engel, L. L., and Lewis, R. A., *Science*, 1941, **94**, 348.

3 Ingle, D. J., and Thorn, G. W., *Am. J. Physiol.*, 1941, **132**, 670.

4 Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H., *Endocrinology*, 1945, **37**, 341.

Evaluation of Autolyzed Mouse Brain Tissue Method for Isolation and Adaptation of Poliomyelitis Virus. (17447)

SERGE G. LENSEN, MAX R. STEBBINS AND LENORE JONES (Introduced by C. W. Meuhlberger)

From the Division of Laboratories, Michigan Department of Health, Lansing, Mich.

A method for isolation and adaptation of poliomyelitis virus by direct passage into mice was described by Milzer and Byrd.[1] The method consists in mixing suspensions of infected feces or central nervous system (CNS) material with an equal amount of a 10% suspension of autolyzed normal mouse brain tissue (ABT) and injecting the mixture intracerebrally into white mice. It is obvious that a usable method for primary isolation of poliomyelitis virus without the use of expensive monkeys would present a great advantage. The authors undertook, therefore, to verify this method and carried out a total of 19 experiments with mice and 4 with cotton rats.

Materials and Methods. Feces. Five specimens were taken from poliomyelitis patients, but not tested for virus content. The presence of poliomyelitis virus in 7 other stool specimens was demonstrated by monkey inoculation prior to adaptation experiments. (The stool specimens were obtained through courtesy of Dr. Franklin H. Top, Herman Kiefer Hospital, Detroit, and Dr. Joseph L. Melnick, Yale University).

Monkey adapted strains. Spinal cords of monkeys infected with poliomyelitis virus strains Buffalo, Mahoney and Tennessee were obtained through courtesy of Dr. Thomas Francis, Jr., University of Michigan. The

1 Milzer, A., and Byrd, C. L., *Science*, 1947, **105**, 70.

virus content was confirmed by monkey inoculation prior to adaptation experiments.

Lansing strain. In view of the claim[1,2] that the use of the autolyzed brain tissue technic results in a shortening of the incubation period in mice inoculated with the Lansing strain virus, this strain was also included in some of the experiments.

Mice and Cotton Rats. White mice bred at the Michigan Department of Health (Rockland Swiss mice) were used in 9 experiments, and the CFW (Carworth Farms Webster) strain of mice in 10 experiments. Eastern cotton rats bred at the Michigan Department of Health were used.

Preparation of the Autolyzed Normal Mouse Brain Tissue Suspensions. The technic described by Milzer and Byrd was used. The autolysis was achieved by keeping the sacrificed mice at room temperature for 16-17 hours prior to the removal of the brains. In most cases, the temperatures were between 22°C and 25°C. However, in some experiments, temperatures lower or higher than those indicated were recorded.

In view of the high rate of bacterial contaminations, the mouse brains were emulsified in pairs and the sterility of each pool tested separately by aerobic and anaerobic culture (blood agar plates and thioglycolate broth). The suspensions were kept in the refrigerator for not more than 24 hours, and the sterile suspensions pooled. They were filtered through several thicknesses of gauze before use.

Diluents. In most of the experiments, either nutrient broth of the Baltimore Biological Laboratory (BBL, pH 6.83 - 7.25), or Tyrode's solution (pH 7.3 - 7.9), or both, were used as diluents. In 2 experiments, Bacto nutrient broth (pH 6.8 - 6.85), and in 2 other experiments thioglycolate sterility broth (pH 7.2 - 7.35), were used.

Organization of the experiments. Groups of 10 to 20 mice were inoculated with: (1) stool suspension plus ABT in nutrient broth, (2) stool suspension plus ABT in Tyrode's solution, (3) stool suspension plus nutrient broth (no ABT), (4) stool suspension plus Tyrode's solution (no ABT), (5) ABT plus nutrient broth (no stool), (6) ABT plus Tyrode's solution (no stool). The same setup was used for infected monkey cords and the Lansing strain (mouse cords), and for other diluents. Two or, more frequently, 3 specimens were used in each experiment. The controls mentioned under (3) and (4) were included for each specimen, while those under (5) and (6) served as general controls. The same specimens were used for the experiments repeatedly up to 10 times. In the first 5 experiments, 2 groups of mice were used for each preparation, one inoculated i.c., the other i.c. and i.p. In all the other experiments, only the i.c. route was used.

The experiments with cotton rats were set up in the same way. However, smaller groups of animals (3 to 5) were used. The observation period for both mice and cotton rats was 40 days.

Single and repeated injections, blind passages. There were experiments with a single inoculation, and others with 2, 3, or 4 inoculations (suggestion of Dr. Milzer). In 2 experiments, there was 1 inoculation of virus-ABT mixture, followed by a series of 5 blind passages (no ABT was used for the blind passages).

Experimental. Experiments with mice. The Lansing strain was tested 10 times in Rockland strain mice and once in CFW mice. Nutrient broth (Bacto and BBL) and Tyrode's solution were used as diluents. In no case was any shortening of the incubation period observed.

In 12 experiments in which the stools with previously demonstrated virus content as well as the 3 monkey adapted strains were tested repeatedly, none of the mice developed any symptoms. In 2 of these experiments, the 3 monkey adapted strains were concentrated 7 times by ultracentrifugation. The final dilution of the concentrated virus (after addition of ABT) was 35%. BBL nutrient broth, Tyrode's solution and thioglycolate broth were used as diluents in these experiments.

In 4 experiments some of the mice inocu-

[2] Milzer, A., Byrd, C. L., and Levinson, S. D., Abstracts of Papers Presented at the 47th General Meeting of the Soc. Am. Bacteriologists, 1947, 74.

lated with mixtures of ABT and either stool suspensions or suspensions of the strains Mahoney and Tennessee, developed partial paralysis. Other symptoms such as ruffled fur, humped back, and tremors were present in some cases and absent in others. In all these cases, the character of paralysis was quite different from that observed in mice inoculated with the Lansing strain. Bacteriological examinations of the CNS revealed bacterial infection in all these cases. Histopathological examinations revealed fibrinopurulent exudate covering brain and medulla, or diffuse infiltration of the meninges, or multiple cortical abscesses, but no lesions characteristic for poliomyelitis.*

In one of the experiments, a control mouse inoculated with a suspension of ABT in nutrient broth (no poliomyelitis virus containing material) developed paralysis similar to that observed with the Lansing strain or with some strains of mouse encephalomyelitis virus. There was no bacterial contamination. Pathological examinations did not show any poliomyelitic lesions. The CNS tissue of this mouse was passed into other mice which, however, did not develop any symptoms.

In 2 other experiments, 2 different virus strains producing paralysis in mice were isolated. One of the strains (referred to as ABC) was isolated from the CNS of one of the control mice inoculated i.c. with a suspension of ABT in nutrient broth. The mouse developed paralysis on the 14th day after inoculation. On further mouse passages the shortest incubation period was 2 to 3 days. Several of the mice inoculated i.p. developed paralysis. The shortest incubation period by the i.p. route was 6 days. Since no material was used which could possibly contain human poliomyelitis virus, no monkey was inoculated with this virus.

Another neurotropic virus (referred to as N-C strain) was isolated under the following circumstances. A stool suspension (received through courtesy of Dr. Melnick) was mixed with a suspension of ABT in Tyrode's solution and inoculated i.c. into mice. None of the mice developed symptoms. A few mice were sacrificed at random and serial blind passages were carried out with thioglycollate broth as diluent and without addition of ABT. At the 4th blind passage one mouse developed paralysis after an incubation period of 19 days. On repeated passages the incubation period became shorter (10 days or more). There was no difference whether or not ABT was added to the inoculum. None of the mice inoculated with this strain by the i.p. route developed symptoms. Since poliomyelitis virus containing stool material was used for the very first inoculation of mice, a Rhesus monkey was inoculated with the N-C strain. A 10% suspension of virus was used, and the monkey received 1.5 ml i.c., a total of 4 ml intranasally and a total of 16 ml i.p. The monkey did not develop paralysis, tremors, or any other symptoms. There was no rise of temperature at any time.

Experiments with cotton rats. All 4 experiments with cotton rats were entirely negative. Three monkey adapted strains and several of the stools with previously demonstrated virus content were used for these experiments.

Discussion. From the data presented in this report, based on experiments carried out with a total of over 5,000 mice and 340 cotton rats, it is clear that the authors were not able to confirm the effectiveness of the autolyzed normal mouse brain tissue method either in the primary isolation of poliomyelitis virus by direct passage into rodents, or in the adaptation of monkey adapted strains to mice or cotton rats, or in the shortening of the incubation period in mice inoculated with the Lansing strain.

In a series of 19 experiments with mice and 4 with cotton rats, 2 neurotropic viruses were isolated. It seems clear, however, that the ABC strain is of mouse origin, since no poliomyelitis virus containing material was injected in this case. The circumstances of isolation of the N-C strain were different and do not exclude the possibility of it being a strain of poliomyelitis virus. The subsequent test in a monkey, however, inoculated with a large

* The histopathological examinations were carried out by H. E. Cope, M.D., Clinical Pathologist, Division of Laboratories, Michigan Department of Health.

dose of virus, makes this highly improbable.

Summary and conclusions. The effectiveness of the autolyzed normal mouse brain method for isolation and adaptation of poliomyelitis virus was investigated in 19 experiments with mice and 4 experiments with cotton rats. Three monkey adapted strains (Tennessee, Mahoney and Buffalo) and 12 stool specimens from poliomyelitis patients were used. The Lansing strain was also included in the experiments.

In the experiments with mice, 2 strains of neurotropic viruses were isolated. However, the evidence presented suggests that the strains isolated were of mouse origin. The cotton rat experiments were entirely negative. A shortening of the incubation period in mice inoculated with the Lansing strain was not observed.

The data presented do not confirm the effectiveness of this method for the isolation or adaptation of poliomyelitis virus.

Received October 4, 1949. P.S.E.B.M., 1949, **72**.

Actions of 2,6-Diaminopurine in Mice, Rats, and Dogs.* (17448)

FREDERICK S. PHILIPS AND J. B. THIERSCH† (Introduced by C. P. Rhoads)

From the Division of Experimental Chemotherapy, Sloan-Kettering Institute, New York City.

Recent studies have shown 4-aminopteroylglutamic acid (4-amino-PGA) to cause changes in hematopoietic tissues and intestinal mucosa which closely resemble lesions found in folic acid-deficient manmals.[1,2] These findings and the fact that 4-amino-PGA and its congeners act as competitive antagonists of folic acid (PGA) in certain microorganisms[3-6] have led to the conception that the agents damage tissues in mammals by interference with metabolic functions of PGA.[1,2,5,6]

Studies of derivatives of naturally occurring purines and pyrimidines have uncovered another antagonist of PGA in microorganisms,[7] namely, 2,6-diaminopurine, which also appears active in higher organisms. Thus 2,6-diaminopurine delays development of an experimental leukemia in mice[8] and inhibits estrogen-induced growth in chick oviduct.[9] The purine is related structurally to 4-amino-PGA insofar as both compounds are condensed ring-systems containing 2,4-diamino-pyrimidine. These observations suggested that the purine might act as an antagonist of PGA in mammals. The following studies were undertaken to determine in detail, sites of action of 2,6-diaminopurine in mice, rats and dogs and to compare the lesions obtained with those seen after administration of 4-amino-PGA.

Procedure. Mice, rats, and dogs of the same strains and corresponding in sex, weight, age and maintenance to animals used in previous studies with 4-amino-PGA[1,2] were sub-

* The authors wish to acknowledge support of this study by the National Institute of Health, U. S. Public Health Service.

† Post-doctorate Fellow of the National Cancer Institute; Neale Research Pathologist, University of Adelaide, S. Australia.

1 Philips, F. S., Thiersch, J. B., *J. Pharm. Exp. Therap.*, 1949, **95**, 303.

2 Thiersch, J. B., and Philips, F. S., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 484.

3 Seeger, D. R., Smith, J. M., Jr., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1947, **69**, 2567.

4 Seeger, D. R., Cosulich, D. B., Smith, J. M., Jr., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1949, **71**, 1753.

5 Oleson, J. J., Hutchings, B. L., and SubbaRow, Y., *J. Biol. Chem.*, 1948, **175**, 359.

6 Franklin, A. L., Belt, M., Stokstad, E. L. R., and Jukes, T. H., *J. Biol. Chem.*, 1949, **177**, 621.

7 Hitchings, G. H., Elion, G. B., Vanderherff, H., and Falco, E. A., *J. Biol. Chem.*, 1948, **174**, 765.

8 Burchenal, J. H., Johnston, S. F., Burchenal, J. R., Kushida, M. N., Robinson, E., and Stock, C. C., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 381.

9 Hertz, R., and Tullner, W. M., *Science*, 1949, **109**, 539.