to those required of streptomycin. Inhibition at such concentrations, however, is fleeting in the case of aureomycin, much larger amounts being required for permanent suppression of growth. The new agent is bacteriostatic rather than bactericidal in its effect. Its antibacterial action is greatly diminished in the presence of serum, *in vitro*, and deterioration at room temperature is marked. Differences in the size of the inoculum have a moderate effect upon the minimal inhibitory concentration.

The authors are grateful to Miss Elizabeth Burr and Miss Jean Fisher of this department for technical assistance, and to Miss Minnie Schreiber and Miss Rosemary Stokes, of the Biological Division, The Johns Hopkins Hospital, for providing many of the test strains.

16759

Further Studies in Experimental Allergic Encephalitis in the Guinea Pig.

HILARY KOPROWSKI AND GEORGE A. JERVIS. (Introduced by Herald R. Cox.)
From the Section for Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., and the Research Department, New York State Department of Mental Hygiene, Letchworth Village, Thiells, N. Y.

It has been indicated[1,2] that an encephalitic process, presumably allergic in nature, results in guinea pigs inoculated with rabbit-brain tissue and "adjuvants". A brief report is herein given of additional experiments with brain-tissue inocula derived from various animal species, as well as on the effect of progressive dilutions of brain-tissue inocula, attempts at purifying the antigen, effect of intracerebral re-injection of the antigen, and on the development of antibrain antibodies in animals inoculated either subcutaneously alone, or subcutaneously and then followed by an intracerebral inoculation of the brain suspension.

1. *Inoculation of brain tissue from various animal species.* The technic followed was the same as reported previously[2] except that brain material from 5 different species was used for the subcutaneous inoculation. Paralysis of inoculated guinea pigs was observed usually 21 or more days after the injection. Death followed from 1 to 15 days after onset of paralysis and occasionally occurred in animals which had shown no neurological manifestations. The results obtained (Table I) do not lend themselves to a satisfactory comparison because of the low and unequal number of animals used in each group. In general, the percentage of animals which succumbed was proportionately similar in each group regardless of the source of the brain tissue used in the inoculum.

Lesions in the central nervous system (CNS), of the type already described,[2] were observed in all animals. In general no conspicuous differences were noted in the lesions which could be ascribed to the use of heterologous antigens, but in some guinea pigs more extensive lesions were obtained when guinea pig brain suspensions were used as inoculum.

2. *Effect of progressive dilutions of brain tissue inoculum.* Phenolized rabies vaccines prepared from calf and rabbit brain tissue, respectively, were used as source material for the inoculum. Starting with the 1:20 dilution of brain emulsion, serial twofold dilutions were made in saline, incorporated into adjuvants and injected into guinea pigs. For the sake of brevity, the results of the experiment (Table II) were grouped irrespective of the brain tissue used as inoculum. For comparison are included data summarizing the results of various experiments in which guinea pigs were inoculated with a suspension of normal rabbit brain tissue in 1:10 dilution. Up to and including the 1:80 dilution, there appeared to be no material difference in the

[1] Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

[2] Jervis, G. A., and Koprowski, H., *J. Neuropath. and Exp. Neurol.*, 1948, **7**, 309.

TABLE I.
Results Obtained in Guinea Pigs Injected with Brain-tissue Inocula Derived from Various Animal Species.

Brain tissue used	Guinea pigs inoculated, No.	Guinea pigs: Paralyzed No.	Paralyzed %	Died No.	Died %
Rabbit	94	45	48	39	41
Guinea pig	20	11	55	10	50
Human	20	7	35	11	55
Calf	19	9	47	7	37
Swine	9	6	66	6	66
Total	162	78	48	73	45

TABLE II.
Results Obtained in Guinea Pigs Inoculated with Various Dilutions of Rabies Vaccines.

Dilution of brain tissue	Guinea pigs inoculated, No.	Guinea pigs: Paralyzed No.	Paralyzed %	Died No.	Died %
1:20	19	9	47	6	32
1:40	15	10	66	10	66
1:80	19	10	52	13	68
1:160	20	4	20	5	25
1:320	19	1	5	2	10
1:10*	162	78	48	73	45

* Normal rabbit-brain tissue used as inoculum.

number of paralyzed or dead animals, and the dilution used. However, a definite decline in the incidence of paralysis or death was observed among the animals inoculated with the 1:160 and 1:320 dilutions.

3. *Attempt at purification of brain antigen.* Precipitation with methanol[3] failed either to enhance or to inhibit the antigenic property of the human brain substance and further fractionation was done by Klenk's technic.[4] The methanol-chloroform extracted fraction, which contains cerebrosides and sphyngomyelin, caused paralysis in 2 animals of 10, while 3 of 10 animals showed paralysis following the injection of the protein fraction remaining after extraction of all lipoids. The pathological picture differed in no way from that obtained with the whole brain tissue.

The results obtained with the lipoid fraction conform to expectation since it has been shown that the substance responsible for the allergic reaction is present in the myelin sheaths[5,6] which have a high cerebroside content. Alvord[6] has recently reported that the phosphatide fraction obtained with Bloor's technic is also effective in producing allergic encephalitis. Apparently the antigenic property is shared by more than one substance present in the white matter of the CNS. It should be noted, however, that the fractions used are not chemically pure substances but only mixtures of cerebrosides and sphyngomyelins, or of lecithins and cephalins, containing many impurities. The purification and identification of the antigen awaits further study and elucidation.

4. *Intracerebral re-inoculation of the antigen.* Five to 7 weeks after the primary subcutaneous injection of brain antigen combined with adjuvants, guinea pigs which had survived were given intracerebrally 0.1 ml of

[3] Koprowski, H., Black, J., and Cox, H. R., *Proc. IVth Internat. Congr. Microbiol.*, Copenhagen, 1947, in press.

[4] Klenk, E., *Hoppe-Seyler Z., f. physiol. Chem.*, 1939, **262**, 128.

[5] Kabat, A. E., Wolf, A., and Bezer, E. A., *J. Exp. Med.*, 1947, **85**, 117.

[6] Alvord, E. C., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 459.

TABLE III.
Results of Intracerebral Inoculation of Guinea Pigs Previously Sensitized by Subcutaneous Injection of Homologous or Heterologous Antigen.

Brain tissue inoculated subcut.	Brain tissue used in subsequent intracerebral inoculation*			
	Rabbit	Guinea pig	Human	Calf
Rabbit	17/19	1/2		
Guinea pig	0/3	1/6		
Human			9/9	
Calf	0/3	0/3		3/3

* Denominator denotes the number of guinea pigs inoculated, numerator the number which died.

10% suspension of brain tissue in saline. With one exception, a high mortality ratio was obtained (Table III) in those groups which had received intracerebral inoculation of the same species-brain tissue suspension to which they had been previously sensitized. Death of the animals, occurring 3 to 15 days after the intracerebral inoculation, was usually preceded by signs of tremors, tonic spasms of the neck, paralysis, and occasionally by convulsions. The exception, mentioned above, occurred in the group of 6 animals which were injected both subcutaneously and intracerebrally with guinea pig brain. In this group only 1 of 6 animals died and its death may have been accidental since no marked pathologic changes were noted on examination of the brain tissues (see below). In the remaining groups of guinea pigs which had received intracerebrally brain tissue suspensions of animals other than from those to which they had been previously sensitized by subcutaneous inoculation, only 1 of 11 died.

Histopathological examinations[2] were made of the brain and spinal cord tissues of all guinea pigs that died, and of those which survived and had been sacrificed approximately 30 days after inoculation. Conspicuous changes were observed at the site of injection. In animals which had died within a few days after the inoculation, the local lesion consisted of a large necrotic hemorrhagic area. Only tissue-debris was seen at the center, while at the periphery large numbers of granular compound corpuscles and rich perivascular infiltrations of hematogenous elements were observed. In animals which died after the first week, hematogenous elements were scanty and infiltrations with compound granular elements predominated so that the local lesion was mainly one of demyelination with central necrosis. Furthermore, throughout the CNS, numerous disseminated areas of demyelination and perivascular infiltration of the type previously described[2] were observed. The fact that the animals had shown no conspicuous neurological signs before the intracerebral injection suggests that at least some of these lesions were of recent appearance and followed the intracerebral administration of antigen, and not older lesions occasioned by the subcutaneous injection. The pathological changes were most striking in those animals which received intracerebrally the same species-antigen to which they had been sensitized, and much less severe in those which had received antigen derived from two different species. It was, however, a difference in degree rather than in quality. The 6 animals (Table III) which were injected both subcutaneously and intracerebrally with guinea pig brain were an exception, since the local pathological lesion was mild.

Ten control animals which received intracerebral injections of brain tissue, without previous sensitization, showed no clinical signs and only minimal pathological lesions.

The focal necrotic lesions of the brain observed in these experiments bore a resemblance to those reported in experimental Arthus-phenomenon in the brain of rabbit,[7] guinea pig,[8] and monkey.[9] That the lesion

[7] Davidoff, L. M., Seegal, B. C., and Seegal, D., *J. Exp. Med.*, 1932, **55**, 163.

[8] Alexander, L., and Campbell, A. C. P., *Am. J. Path.*, 1937, **13**, 229.

was more marked when the intracerebral inoculum was from the same species as that injected subcutaneously, conforms to the hypothesis that the pathological changes are anaphylactic in nature. However, the process seems to be of uncommon intensity in these experiments as evidenced by the high mortality ratio. It is interesting to note that Freund[1] also observed high mortality after reinoculation of the brain substance intracutaneously into a sensitized animal. These findings may offer some indication as to the possible allergic nature of the encephalomyelitic process.

5. *Determination of antibrain antibodies in animals.* The presence of antibrain antibodies in the blood serum of guinea pigs was determined by complement-fixation tests against three antigens: human, normal guinea pig and rabbit brain which were prepared according to the technic of Casals and Palacios.[10] The technic of the test itself followed the one generally employed in this laboratory[11] except that 1.5 units of guinea-pig complement was used. Determinations were performed (a) in animals injected subcutaneously as described under items 1 and 3, and (b) in animals first injected subcutaneously and then intracerebrally as described under item 4.

(a) Complement-fixing antibodies against rabbit brain antigen were first noted on the 7th day after the subcutaneous injection of rabbit-brain suspension combined with adjuvants. On the 11th day, antibodies against brain antigens of all 3 species appeared, and the titer rose rapidly reaching a peak between the 16th and 21st day, followed by a gradual decline. In 2 of the 4 animals examined, antibodies were still present on the 193rd day after injection. With the rabbit-brain inoculum, the titer of antibodies in serum was always higher when tested against rabbit-brain antigen than against guinea pig or human antigen. In further experiments, it was noted than brain-specific antibodies appear in all injected animals irrespective of the species-source of the brain tissue inoculum. The only significant exception was the guinea pig brain antigen with which, while it induced marked incidence of paralysis in the inoculated animals (Table I), no antibrain antibodies were demonstrated. In general, no correlation was observed between the titer of antibrain antibodies and the clinical picture presented. Following the injection of rabbit-brain tissue, incorporated in Bayol F and lanolin but without the addition of acid fast bacilli, complement-fixing antibodies against rabbit brain were first observed on the 20th day after injection, a slight decrease in titer was seen on the 27th, and no antibodies were demonstrable on the 41st day. Against human brain, antibodies were observed at about the same time as antibodies against rabbit brain, but the titer of the former was much lower. No antibodies fixing guinea pig brain were detected at any time after inoculation. In animals injected with two human-brain fractions, as described under item 3, no antibodies against nonfractionated human brain and guinea-pig brain antigens were detected on the 13th day after injection, but 2 weeks later almost all of the guinea-pig sera showed titers ranging from 1:4 to 1:32 against human-brain antigen and slightly lower titers against guinea-pig brain antigen.

From the foregoing it seems reasonable to consider the appearance of complement-fixing antibodies as a constant phenomenon in guinea pigs injected with brain substance incorporated into adjuvants. This is in contrast to some previous studies in which inoculation of adjuvant-brain emulsions into guinea pigs failed to elicit antibodies,[12] or their presence was demonstrable only in 1 of 3 animals.[1] These antibodies are apparently not species-specific. The addition of acid fast bacilli to the emulsion of brain tissue definitely enhances formation of antibodies and broadens

[9] Jervis, G. A., Ferraro, A., Kopeloff, L. M., and Kopeloff, N., *Arch. Neurol. and Psychiatr.*, 1941, **45**, 733.

[10] Casals, J., and Palacios, R., *J. Exp. Med.*, 1941, **74**, 409.

[11] Kolmer, J. A., and Boerner, F., Approved Laboratory Technic, Chap. 29, 3rd edition, Appleton Century Co., New York, 1941.

[12] Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1947, **57**, 229.

their immunological pattern, but apparently it is not an indispensable factor. The formation of antibrain antibodies was described by several groups of investigators[13-16] who used as antigen brain tissue without adjuvants. However, inclusion of adjuvants in the inoculum seemed to facilitate the formation of antibodies.[17] The lack of any significant relation between the clinical status of the animal and the level of antibrain precipitins was noted also by Kopeloff and Kopeloff[12] in rabbits.

(b) Antibrain antibodies were determined in animals first injected subcutaneously and then intracerebrally, as described under item 4. In almost all, the titers of antibrain antibodies obtained after the subcutaneous inoculation showed an increase following the intracerebral inoculation. However, there appeared to be no correlation between the clinical status of the animals and the level of antibodies before and after the intracerebral inoculation. Guinea pigs which had shown no antibrain antibodies after the subcutaneous inoculation with guinea pig brain, did develop antibodies following the intracerebral inoculation of the same antigen. A rise in antibody level after intracerebral injection was noted also in those animals which had been injected subcutaneously with rabbit brain suspension combined with adjuvants but without *Mycobacterium tuberculosis*. In the control experiments, no complement-fixing antibodies were detected in 10 nonsensitized guinea pigs inoculated intracerebrally with 0.1 ml of 10% rabbit-brain suspension.

These serological findings, although not directly related to the clinical status of the animal, may be correlated with the pathological data reported above, which indicate that the difference in the histopathological lesions observed in sensitized guinea pigs that died or survived after the intracerebral inoculation was one of degree rather than quality. Possibly the increase in titer of antibrain antibodies is due to the release in the previously sensitized animal of small amounts of antigen from the brain tissue as a result of some microscopic injury suffered at the time of the intracerebral inoculation. However, this latter hypothesis requires further experimentation before any conclusions are warranted. The intracerebral "challenge" technic of previously sensitized guinea pigs, described above, may prove of value in the investigation of allergenic or paralitogenic properties of normal brain tissue or its fractions. This technic may be further broadened by injecting either non-fractionated homologous brain tissue, or purified fractions of the brain substance into animals previously sensitized by the subcutaneous route. Clinical observations of the animals, coupled with serological study, may throw additional light on the puzzling phenomenon of the allergenic properties of brain tissue.

In the discussions of the various aspects of the experiments reported above, reference to any possible application of the findings to human pathological conditions has been purposely avoided. Further experimental work is essential before one is justified to draw any conclusions applicable to conditions observed in human subjects, which could be attributed to the allergenic properties of brain tissue.

Summary. Allergic encephalitis in guinea pigs was produced by inoculation of brain tissue derived from 5 different mammalian species. Dilutions of phenolized rabies vaccines combined with adjuvants also caused paralysis of the inoculated animals. The majority of the guinea pigs previously sensitized by subcutaneous injection of brain tissue incorporated into adjuvants died after the intracerebral inoculation of homologous brain tissue (but not when brain tissue of cavian origin was used). Pathologic changes observed in the animals were described. Complement-fixing antibrain antibodies were found in sera of guinea pigs inoculated subcutaneously with brain tissue. Intracerebral reinoculation of these animals usually resulted in the rise of titer of the humoral antibrain antibodies.

[13] Witebsky, E., and Steinfeld, J., *Z. f. Immunitätsf. u. Exp. Therap.*, 1928, **58**, 271.

[14] Lewis, J. H., *J. Immunol.*, 1933, **24**, 193.

[15] Schwentker, F. F., and Rivers, T. M., *J. Exp. Med.*, 1934, **60**, 559.

[16] Bailey, G. H., and Gardner, R. E., *J. Exp. Med.*, 1940, **72**, 499.

[17] Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1944, **48**, 297.