Two experiments were performed to determine whether streptomycin was rapidly inactivated in the chick embryo and thus might appear to have little effect on rickettsial growth when compared with PABA (which is known to remain at adequate levels in the egg for the period of time covered by these experiments.)[9] Streptomycin does not appear to be inactivated, since allantoic fluids and yolks harvested 4 days and 7 days after the inoculation of eggs with 1.0 mg by the yolk sac route were shown to contain the expected amounts, that is, between 5 and 25 μg per ml, by assay with 3 strains of organisms susceptible to streptomycin, *E. coli* (Waksman) and *Bacillus friedländeri* (Yarsik) and *B. friedländeri* (Lundgren).

Discussion. The concentrations of streptomycin in these tests were selected to represent approximately the concentrations attainable in man. With therapeutic effects in eggs of such a slight degree, it seems unlikely that streptomycin would have any very striking effect on human infections caused by *R. prowazeki, R. mooseri,* or *R. orientalis.* The effects are sufficient, however, to caution against the use of streptomycin in the attempt to isolate *R. prowazeki* or *R. mooseri* from material contaminated with streptomycin-sensitive bacteria, particularly if the numbers of rickettsiae in the material are small.

Summary. Streptomycin in amounts of from 0.5 to 2.0 mg injected into infected embryonated eggs has a definite slight inhibitory action on the growth of *R. prowazeki.* In the case of *R. mooseri,* 2.0 mg of streptomycin had a small but significant effect. With *R. orientalis,* a dose of 2.0 mg per egg appeared to have no significant effect on the multiplication of the rickettsiae. By contrast, appropriate doses of PABA (5.5 mg per egg for *R. prowazeki,* and 11.0 mg per egg for *R. mooseri* and *R. orientalis*) produced a striking inhibition of growth, reducing the numbers of viable rickettsiae by 10,000 to over 10,000,000 times as compared with untreated controls.

Addendum. As this manuscript was in preparation, Dr. J. E. Smadel informed the authors that his experiments showed a very definite therapeutic effect of streptomycin against infection of embryonated hens' eggs with *R. prowazeki* when doses of 10 mg (10,000 units) per egg were employed, 5 times the maximum dose administered in the experiments herein reported.

† The minimum concentration of PABA required to produce a detectable inhibition of the growth of *R. orientalis* is considerably greater than that required for inhibition of *R. prowazeki.*[9]

9 Snyder, J. C., and Stevens, D. A., unpublished observations.

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Action of Curare on Temperature Changes in the Brain in Combination With Pentobarbital Narcosis.

SERGEI FEITELBERG AND ERNEST P. PICK.*

From the Laboratories of the Mount Sinai Hospital, New York.

It has been demonstrated that curare-alkaloids (Merck strychnos curare, crystallized tubocurarine hydrochloride) and dihydroerythroidine bromide have a central action, besides the peripheral effect on the neuromuscular junction.[1,2] The central action of these alkaloids was first demonstrated on the electroencephalogram of frogs. At first the amplitude and the frequency of the action

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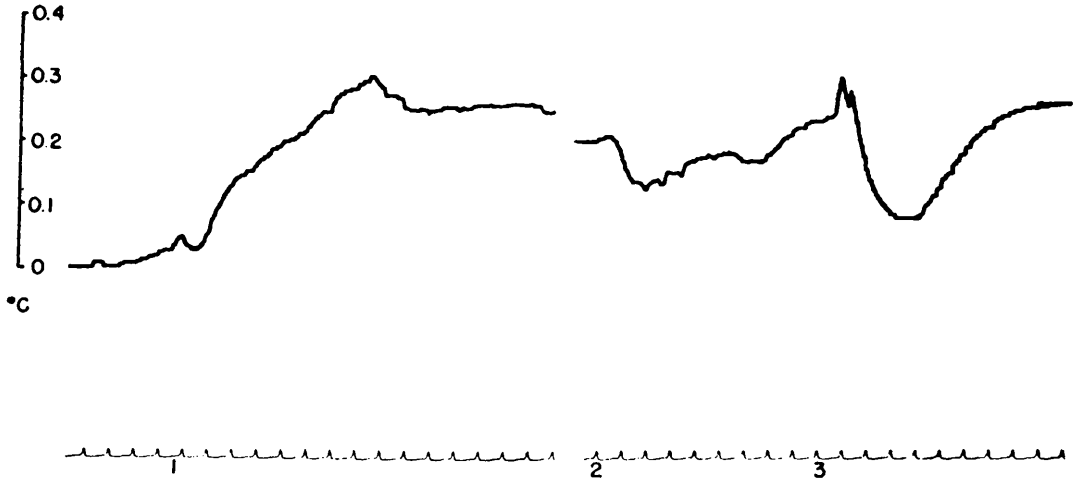

FIG. 1.

Effect of strychnos curare on temperature in the brain during light pentobarbital narcosis. Cat, 3.0 kg, narcotized with pentobarbital 15 mg/kg intraperitoneally and given artificial respiration. Time interval: 1 minute; temperature interval: 0.1°C.

(1) 2 mg strychnos *curare* intravenously causes a *rise* of 0.3°C in the brain temperature, which lasts about 8 minutes.

(2) 30 mg per kg *pentobarbital* intraperitoneally causes a slight drop of the brain temperature which returns to original level in 4 minutes.

(3) 2 mg strychnos *curare* causes a sudden drop in temperature of the brain (0.2°C) lasting about 4 minutes, which returns to normal after about 6 more minutes.

potentials are diminished. Gradually, they disappear completely. The same behavior of the electrical activity in the brain is observed in narcosis. This central action is independent of the peripheral paralysis. It seems desirable to investigate the effect of curare on the brain of mammals with the method of measurement of temperature changes in the brain, developed by Feitelberg and Lampl.[3] Since it has been shown by Pick and Richards[4] that pentobarbital and curare (and curare-like alkaloids) have a synergistic hypnotic action it is interesting to examine to what extent the temperature changes in the brain is influenced by curare in pentobarbital narcosis of varying depth.

The experiments were carried out on cats (average weight 2.5 to 3.5 kg), in pentobarbital narcosis of varying depth, under artificial respiration. Curare (Merck strychnos curare, 1.5 mg per cc of saline solution) was administered intravenously. We began the experiments with light pentobarbital narcosis (15-20 mg/kg); in other experiments we used larger doses of pentobarbital (30 mg/kg) to produce a deeper narcosis. The temperature changes were measured by a method described in detail elsewhere.[3] This method consists essentially in the following: one junction of a thermocouple is introduced into the internal carotid artery, and the other junction into the brain; the difference of temperature between the 2 junctions (between the brain and the carotid blood) is measured by a galvanometer of adequate sensitivity; this difference of temperature is interpreted as a measure of oxidation processes in the brain. The obvious objection that the difference in temperature is influenced by change in blood flow and not by temperature changes in the brain has been discussed elsewhere.[3] It suffices to mention here that the experimental findings on the behavior of temperature changes in the brain cannot be explained only by circulatory processes eventually produced by curare.

1. *Combined action of curare and light pentobarbital narcosis.* Most of our experiments on animals in light pentobarbital an-

[3] Feitelberg, S., and Lampl, H., *Arch. f. exp. Path. und Pharm.*, 1935, **177**, 600, 725.

[4] Pick, E. P., and Richards, G. V., in print, *J. Pharm and Exp. Ther.*, 1947.

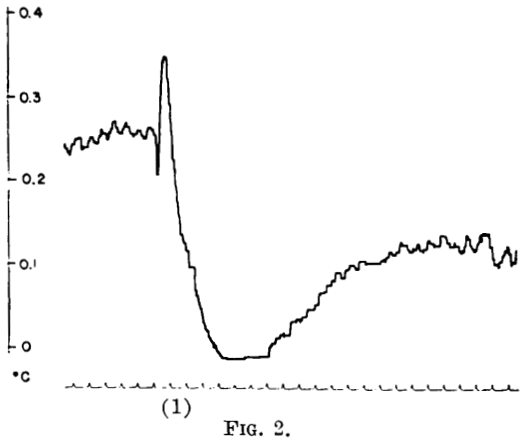

FIG. 2.

Effect of strychnos curare on temperature in the brain during deep pentobarbital narcosis and artificial respiration. Cat, 3.5 kg, narcotized with pentobarbital 30 mg/kg intraperitoneally. Time interval: 1 minute; temperature interval: 0.1°C. At (1) 3 mg strychnos *curare* intravenously causes a *sudden drop* in the brain temperature corresponding to 0.25°C, lasting about 7 minutes.

esthesia show that administration of curare causes a rise in temperature of the brain (Fig. 1). This indicates that curare, given in light narcosis without complete peripheral paralysis, has a stimulating effect on the brain. The increased temperature lasts for 10 to 15 minutes. This effect can be elicited several times on the same narcotised animal. It is independent of the peripheral body temperature. It may well be the result of actual increase in brain metabolism.

2. *Combined action of curare and deep pentobarbital narcosis.* The effect of curare is reversed if the pentobarbital narcosis is deeper, when the peripheral muscle movements are abolished by the narcosis. Under those conditions curare causes a marked decrease in temperature of the brain, lasting 5 to 15 minutes, which may be interpreted as a depression of the oxidation processes in the brain (Fig. 2). It appears therefore that a certain depth of pentobarbital narcosis is necessary for the occurrence of synergism between curare and pentobarital narcosis. No synergism seems to exist when the pentobarbital narcosis is light.

3. *Discussion.* The stimulating and depressing action of curare which we found in experiments on cerebral temperature is in agreement with the experiments reported by McIntyre *et al.*,[5] on the electroencephalogram of dogs in light pentobarbital narcosis after intravenous injection of *d*-tubocurarine. The initial effect of curare was an increase of electrical brain activity in the occipital, parietal and frontal region with a 3-fold increase in amplitude and occasional bursts of high frequency. This stage was followed shortly by a depression of electrical activity. With larger doses the depression occurred earlier and the stage of initial stimulation was brief. The latter findings agree with the earlier statements of Feitelberg and Pick[1] and Pick and Unna[2] about the depressing action of curare and curare-like alkaloids on the electroencephalogram of frogs.

The central stimulating action of curare producing hyperexcitability, accelerated respiration, central vagus stimulation and clonic convulsions was also found recently in experiments with potcurare and purified curarine chloride in cats by v. Euler and Wahlund[6] and in experiments with nonfatal doses of intocostrin and crystalline *d*-tubocurarine chloride on various mammalian species by Cohnberg;[7] central nervous depressants, *i.e.*, sodium amytal or cyclopropane decrease, abolish or prevent the stimulating central effect.

It is further interesting to note that in earlier investigations of the action of *d*-tubocurarine on frogs, Tillie[8] and Jacabhazy[9] have described a stimulation of the brain followed by a general reflex inhibition and paralysis of the spinal cord. According to Fuehner[10] guanidine, which has curare-like properties, produces also an initial central

[5] McIntyre, A. R., Dunn, A. L., and Tullar, P. E., *Fed. Proc.*, Part II, **67**, February, 1946.

[6] v. Euler, U. S., and Wahlund, H., *Acta physiol. Scandinav.*, 1941, **2**, 327.

[7] Cohnberg, R. E., *J. Lab. and Clin. Med.*, 1946, **31**, 866.

[8] Tillie, J., *Arch. f. exp. Path. und Pharm.*, 1890, **27**, 1.

[9] Jacabhazy, S., *Arch. f. exp. Path. und Pharm.*, 1899, **42**, 10.

[10] Fuehner, H., *Arch. f. exp. Path. und Pharm.*, 1908, **58**, 1.

excitation in frogs, followed later by a central paralysis, which occurs 2 hours before peripheral paralysis appears.

4. *Summary.* Curare, which affects the electrical activity of the brain has also a distinct effect on the temperature in the brain in combination with pentobarbital narcosis. These temperature changes in the brain indicating changes of oxidation processes are increased by curare under light pentobarbital narcosis, and decreased under deep pentobarbital narcosis. This latter central action may be involved in the synergistic narcotic effect.

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Growth of Chicks on Purified and Synthetic Diets Containing Amino Acids.*

T. D. LUCKEY, P. R. MOORE, C. A. ELVEHJEM, AND E. B. HART.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Although amino acids have been used in recent investigations[1-3] as the principal nitrogen source in chick diets in order to determine the amino acid requirements of the chick, such diets have various other applications in nutritional investigations. We have fed amino acid diets in an attempt to determine (1) if the amino acids present in casein can be replaced by an equivalent amount of free amino acids; (2) if chicks will grow as well on a mixture of essential amino acids as when additional nonessential amino acids are added; (3) if chicks require strepogenin;[4,5] (4) if chicks will survive and develop normally on a purely synthetic diet (a chemically-defined diet consisting of C.P. chemicals); and (5) if a chemically-defined diet can be used effectively in the elucidation of unknown factors such as the monkey antianemia factor of Cooperman *et al.*[6]

Experimental. Day-old White Leghorn chicks weighing 40-42 g were divided into groups of 3 chicks each and maintained in electrically-heated cages with raised screen bottoms. In Series 1 and 2 (Table II) the chicks were fed a protein and amino acid-free diet for 3 to 4 days (to deplete them of any possible nitrogen stores carried in the yolk sac) prior to feeding the experimental diets. Since this preliminary depletion period appeared to inhibit later growth responses it was discontinued and the animals were placed directly on the experimental diets in subsequent experiments.

The composition of the diets used is given in Table I. Diet I is a typical experimental diet with 18% casein supplemented with arginine, glycine, and cystine and adequate amounts of the vitamins known to be involved in chick nutrition. Diet II is similar to Diet I but contains free amino acids equivalent to the amount present in casein with arginine, glycine, and cystine additions as in Diet I. Diet III contains the amino acids essential to the chick in amounts adequate to equal the nitrogen contents of Diets I and II. Diets IV, V and VI represent

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2 Hegsted, D. M., *J. Biol. Chem.*, 1944, **156**, 247.

3 Grau, C. R., and Peterson, D. W., *J. Nutr.*, 1946, **32**, 181.

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5 Patton, A. R., Marvel, J. P., Petering, H. G., and Waddell, J., *J. Nutr.*, 1946, **31**, 485.

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