macodynamic action. These groups are essential for the activity of the lactogenic hormone.

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THE EFFECT OF COCARBOXYLASE UPON METABOLISM AND NEURO-PSYCHIATRIC PHENOMENA IN PELLAGRINS WITH BERIBERI[1]

THE present communication is a report of observations on eight selected cases of pellagra with beriberi which were studied from chemical, neurological and psychiatric standpoints. Preceding and following the administration of cocarboxylase, each patient was studied repeatedly by the following methods: (1) Analysis of blood samples for metabolites, including sulfite combining substances. (2) Neurological examination, including chronaximetric measurement. (3) Estimation of the psychiatric status.

The intravenous administration of 50 milligrams of cocarboxylase (Merck) to persons who exhibited signs of an "active process" was followed, in every instance, by dramatic and rapid improvement. The bisulfite-binding substances decreased in quantity. The pathological signs of peripheral and cranial nerves, which had become increased in patients who were being treated with nicotinic acid and riboflavin, became less conspicuous or disappeared. The irritability of a number of muscles, measured in terms of their strength-duration curves, returned from underexcitability before treatment to normal values and in some cases even progressed to overexcitability. Correspondingly, the increased threshold of sensibility to touch and prick in arms and legs was reduced to normal, and the depressed pupillary and corneal reflexes improved quantitatively, often becoming normal.

Following the administration of cocarboxylase, some beneficial effect occurred, in some persons within an hour and in all persons within four hours. Improvement continued for from one to four days thereafter. Since the unbalanced diets of these persons remained essentially unchanged, in the absence of further therapy the patients tended to regress rapidly to their condition preceding treatment. A psychoneurotic syndrome which was recognized in these persons and which responded promptly to the administration of cocarboxylase will be described separately. In contrast, in the persons selected for control, there was no decrease of bisulfite-binding substances in the blood and no improvement in the neurological and psychoneurotic symptoms following cocarboxylase therapy.

The present study shows that the neuropathy accompanying pellagra represents a clinical entity (beriberi), distinct from a deficiency of nicotinic acid or of riboflavin. It shows also that cocarboxylase (pyrophosphate of thiamin) has a striking effect upon certain intermediate products of carbohydrate metabolism and induces improvement in the affected peripheral and cranial nerves. Furthermore, the decrease of bisulphite-binding substances in the blood is accompanied by a decrease or disappearance of certain neurological signs and of psychoneurotic symptoms.

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EVIDENCE FOR THE EXISTENCE OF A RESPIRATORY NEUROHORMONE

IN the course of studies on the pressor effects of acetylcholine, it was observed that amounts of acetylcholine which produce pressor effects and contraction of the nictitating membrane also increase the rate and depth of respiration for brief intervals. It was also observed that the larger the dose of acetylcholine the longer the duration of respiratory stimulation and the greater the depth of respirations. The pressor effect always outlasts the respiratory stimulation.

The minimum amounts of acetylcholine producing respiratory stimulation in atropinized dogs and cats are 0.15 to 0.2 mgm or more per kilogram, but in the presence of optimum amounts of eserine (about 1.5 mgm per kilogram) as little as 0.005 mgm of acetylcholine may produce respiratory stimulation. If 0.05 mgm of acetylcholine is used throughout in atropinized animals, the smallest dose of eserine which is required to produce respiratory stimulation from this amount of acetylcholine is 0.04 to 0.05 mgm per kilogram. In the same animals doses of epinephrine which produced blood pressure elevation and withdrawal of the nictitating membrane also caused a decrease but never an increase in the rate of respiration.

In five cats and eight dogs the carotid sinuses were removed and in some of these animals the vagi above the ganglia nodosa as well as the sympathetics above the superior cervical ganglia were sectioned. In these animals, which were treated with atropine and eserine,

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0.05 to 0.1 mgm of acetylcholine did not produce respiratory stimulation.

In another group of animals treated with atropine and eserine, 3 to 6 mgm of nicotine per kilogram abolished not only the pressor effects of acetylcholine but also diminished or abolished its respiratory stimulant action.

The respiratory effect of intravenously administered acetylcholine appears after a delay of seven to twenty seconds, and slow or rapid injection of comparable doses into the common carotid artery does not accelerate the onset of the respiratory effect. In several dogs acetylcholine in doses effective as respiratory stimulants when administered by the femoral vein produced no or feeble respiratory stimulation when given by the carotid artery.

It is concluded that the respiratory stimulation following acetylcholine injections depends upon the presence of the carotid body and that the active principle is not acetylcholine but a sympathin liberated at the nerve terminations following stimulation of sympathetic ganglia by acetylcholine. Nicotine prevents this stimulation by producing ganglionic paralysis or depression. Examination of the tracings published by Magoun, Ranson and Hetherington[1] and by Harrison, Wang and Berry[2] reveals that sympathins liberated following hypothalamic stimulation in adrenalectomized animals produce not only pressor effects and withdrawal of the nictitating membrane but also marked and brief stimulation of respiration.

It is tentatively suggested that a sympathin, not identical with epinephrine, possesses (via the carotid bodies) properties of a respiratory stimulant.

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THE REDUCING GROUPS OF EGG ALBUMIN

IT has long been known that SH reducing groups can be detected by the nitroprusside test in denatured but not in native egg albumin. In some other proteins SH groups can be detected with nitroprusside even when the protein is native.[1] Various methods have been developed for the quantitative study of protein-reducing groups.[2, 3, 4]

In the present experiments measurements are made of the amount of ferricyanide reduced by denatured egg albumin in the presence of the synthetic detergent Duponol PC and of the effects on the amount of ferricyanide reduced of previous reactions of the protein with iodine and iodoaceticacidamide. These experiments show the kinds of complications which may be involved in the study of protein groups generally. They also illustrate some of the remarkable effects of synthetic detergents on proteins, which I shall describe more completely elsewhere.

At pH 9 ferricyanide oxidizes not only the SH groups of denatured egg albumin but weaker reducing groups as well. The amount of ferrocyanide formed is greater the longer the time of reaction and the higher the concentration of ferricyanide.[5] This also is true at pH 6.8. In addition, the results at pH 6.8 are variable because they depend on the physical state of the denatured protein. Other things being equal, the more the denatured protein is aggregated,[6] the less ferrocyanide is formed.

If, however, denatured egg albumin is oxidized by ferricyanide at pH 6.8 in the presence of Duponol PC, the oxidation takes place at a much lower concentration of ferricyanide than in the absence of Duponol, and the amount of ferrocyanide formed is independent, within wide limits, of the time and temperature of the reaction and of the concentrations of ferricyanide and Duponol. No reduction of ferricyanide takes place if the SH groups of denatured egg albumin are first abolished with formaldehyde or iodoaceticacidamide.

The proper conditions for the reaction between ferricyanide and denatured protein vary from protein to protein. In the case of egg albumin, the reaction is carried out for 10 minutes at 37° C. in 3 cc of a pH 6.8 solution containing 0.002 mM of ferricyanide and 10 mg of Duponol PC (du Pont), a mixture of the C_{10}—C_{18} compounds of $CH_3(CH_2)_nCH_2OSO_3Na$. 0.001 mM of ferrocyanide are formed from 10 mg of denatured egg albumin. There is no increase in the amount of ferrocyanide formed if the reaction is carried out for 100 minutes instead of for 10 minutes or if the rate of reaction is increased by increasing the amount of ferricyanide 25 times and the amount of Duponol 10 times or by raising the temperature from 37° to 100° C.

Urea[7] and guanidine,[8] like Duponol, promote the

1 Magoun, Ranson and Hetherington, *Amer. Jour. Physiol.*, 119: 615, 1937.

2 Harrison, Wang and Berry, *Amer. Jour. Physiol.*, 125: 449, 1939.

1 M. L. Anson, "The Chemistry of Amino Acids and Proteins," Chapter IX. Edited by C. L. A. Schmidt, Springfield, 1938.

2 A. E. Mirsky and M. L. Anson, *Jour. Gen. Physiol.*, 18: 307, 1935.

3 R. Kuhn and P. Desnuelle, *Zeits. Physiol. Chem.*, 251: 14, 1938.

4 J. P. Greenstein, *Jour. Biol. Chem.*, 125: 501, 1938.

5 A. E. Mirsky and M. L. Anson, *Jour. Gen. Physiol.*, 19: 451, 1936.

6 M. L. Anson and A. E. Mirsky, *Jour. Gen. Physiol.*, 15: 341, 1932.

7 I have used urea for many years to promote the reaction between protein-reducing groups and the uric acid reagent.

8 Greenstein (see note 4) has studied the effect of guanidine on the oxidation of denatured egg albumin by porphyrindin. I shall discuss his results elsewhere.