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.

THEODORE KOPPANYI
CHARLES R. LINEGAR

GEORGETOWN UNIVERSITY

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.

ferricyanide reaction. They are much less effective reagents than Duponol.

Ferricyanide, iodine and iodoaceticacidamide all react with the SH groups of simple SH compounds like cysteine, which is a constituent of egg albumin. Ferricyanide, although it reacts with denatured egg albumin, does not react with native egg albumin.[5] Iodine and iodoaceticacidamide, nevertheless, do react with native egg albumin. 10 mg of native egg albumin treated with 1 cc of 0.0015 N iodine at pH 3.2 and then neutralized and denatured by Duponol, no longer reduces dilute ferricyanide. 10 mg of native albumin treated with 0.01 mM of iodoaceticacidamide at pH 9 and then denatured reduces only half as much ferricyanide as untreated albumin.

In connection with the observation that native egg albumin reacts with iodoaceticacidamide but not with ferricyanide, it is interesting to note that urease is inactivated by iodoaceticacidamide[9] but not by ferricyanide.[10] The effect of iodoaceticacidamide on viruses is now being tested.

The fact that ferricyanide does not react with native egg albumin does not prove that the SH groups of native egg albumin are linked or inaccessible. On the other hand, it can not be assumed, without independent evidence, that in the present experiments ferricyanide, iodine and iodoaceticacidamide are reacting only with SH groups. All three of these reagents can, under suitable conditions, react with protein groups other than SH groups. It is possible, furthermore, that there are reducing groups in denatured egg albumin which are not SH groups but which are oxidized by dilute ferricyanide only in the presence of SH groups.

M. L. ANSON

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN IMPROVED TYPE OF KETENE GENERATOR

RECENTLY Herriott[1] described a ketene generator which was improvised from that previously suggested by Ott, Schroter and Packendorf.[2] In our studies of the acetylation of pituitary hormones we have developed a new design of generator, giving a more efficient and workable apparatus. A diagram is given in Fig. 1.

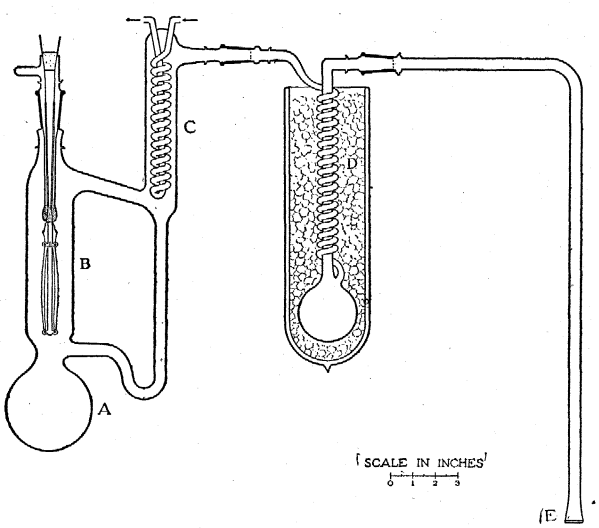

FIG. 1

Vaporized acetone from flask A is decomposed by the heating filament B, which is kept at a bright-red heat. The mixed vapors pass through the condenser C in order to remove the unchanged acetone vapor and most of the ketene polymers. More complete removal of these two substances is then effected by the trap D, which is immersed in a freezing mixture of salt and ice. Ketene then passes into the solution through a sintered glass plate E. The sintered glass plate is particularly useful for bubbling gas; it gives more effective contact between the solution and the gas, and it stirs the solution sufficiently to make unnecessary a mechanical stirrer as generally suggested.

The removable filament-support is the same as described by Herriott. The 40 mill tungsten leads are sealed permanently into the glass and the replaceable tungsten filament (15 mill) may be attached to them in the manner illustrated in the figure. The rate at which ketene is generated can be controlled by varying the current which passes through the heating filament.

CHOH HAO LI

INSTITUTE OF EXPERIMENTAL BIOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY

A SIMPLE AND EFFICIENT PRECIPITATE DRYER

IN the course of preparing enzymes, protein was precipitated with ammonium sulfate at 70 per cent. saturation. The filtered, wet precipitate contained a large amount of mother liquor. If the precipitate was allowed to dry on the filter paper more than half its dry weight would be salt and not the active material desired. Drying first on blotting paper and then on a porous plate was tried. By this method all the water was absorbed; but as it began to evaporate,

[1] R. M. Herriott, *Jour. Gen. Physiol.*, 18: 69, 1934.
[2] E. Ott, R. Schroter and K. Packendorf, *Jour. Prakt. Chem.*, 130, N.S.: 177, 1931.
[9] C. V. Smythe, *Jour. Biol. Chem.*, 114: 601, 1936.
[10] L. Hellerman and M. E. Perkins, *Jour. Biol. Chem.*, 107: 241, 1934.