Kodak) were made on Aug. 19th, 20th, 21st, 22nd, 23rd, and 24th, immediately following the vaginal smear. This experiment lasted until Sept. 4th, yet no effect on the estrous cycle was noted. The rats in Series A and B were offspring of our active breeding colony, and littermate controls were used throughout.

In Series C, 13 adult rats with perfectly regular estrous cycles were selected from stock rats by a process of elimination and, after a preliminary control smear period of 16 to 44 days' duration, were all injected subcutaneously for 4 successive days (Dec. 15th to 18th) with 60 mg. of choline chloride (Hoffmann-La Roche) per 100 gm. body weight. The rats were then smeared daily for 17 days following the final injection. Even at this high dose-level none of the rats showed inhibition of the estrous cycle.

On the basis of 3 series of experiments on a total of 98 rats it is concluded, contrary to the findings of Duncan, Gallagher and Koch,[3] that estrous cycles in the rat are not modified by: (a) 4 or 8 mg. of acetylcholine chloride injected intraperitoneally, (b) 40 or 80 mg. of choline chloride injected intraperitoneally, (c) 29 or 58 mg. of choline injected subcutaneously, or (d) 60 mg. of choline chloride per 100 gm. body weight injected subcutaneously.

9830 P

Effect of Cysteine on Action of Insulin.

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An earlier paper[1] showed that alloxan produces hypoglycemia in normal rabbits. Alloxan is an oxidizing agent credited with special affinity for sulphydryl groups. If alloxan and insulin induce hypoglycemia through the same mechanism, and if alloxan's effect is produced through interaction with sulphydryl groups of tissues, then one might surmise that the normal hypoglycemic action of insulin could be diminished by the administration of sulphydryl compounds. Specifically, a quantity of sulphydryl groups equal in amount, reactivity and availability to those already in the tissues should diminish the hypoglycemic effect of a standard dose of insulin to one-half.

This paper presents the results of experiments in which cysteine

[1] Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

was given with insulin. Normal young adult rabbits were fasted for 24 hours before each experiment. Blood sugar determinations were made according to Miller and van Slyke.[2] The individual reactions to insulin in a dosage of 3 units per kg. subcutaneously were first established. (Fig. 1, a, b, c, d, e, f.) About one week later the same animals were given the same dosages of insulin, but, in addition, were given cysteine hydrochloride in neutralized solution subcutaneously separately from the insulin. The cysteine hydrochloride was given in 3 doses: 0.5 gm. 5 minutes before, 0.5 gm. 5 minutes after, and the remainder of the dose about 20 minutes after the insulin injection. The corresponding blood sugar curves are also represented in Fig. 1 (A, B, C, D, E, F). The dosages of cysteine given in the figures are those of cysteine itself, not the hydrochloride. Care was taken to prepare the cysteine solutions just before the injections, to minimize oxidation in air, and to insure neutrality.

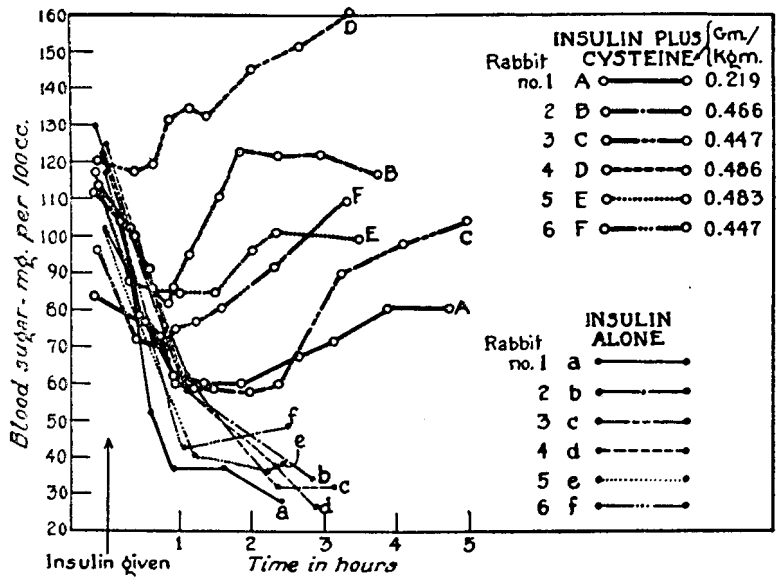

FIG. 1.
Blood sugar curves following insulin and insulin-plus-cysteine.

The effect of cysteine alone on the blood sugar level is shown in Fig. 2. The cysteine solutions were prepared and given in the same way as in the main experiment.

These experiments indicate that cysteine does actually diminish

[2] Miller, B. F., and van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

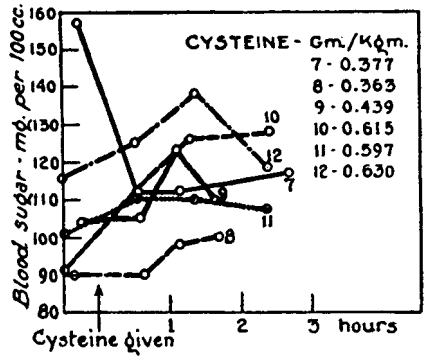

FIG. 2.
Blood sugar curves following cysteine alone.

the hypoglycemic effect of insulin. However, other amino-acids, such as glycine, alanine and glutamic acid[3, 4] are known to cause hyperglycemia and to diminish the effect of insulin. Furthermore, aliphatic compounds such as propionic acid are convertible into carbohydrate.[5] Before the specific influence of sulphydryl compounds on the hypoglycemic effect of insulin can be defined a number of non-aliphatic non-amino sulphydryl compounds must be studied as well.

In vitro sulphydryl compounds such as cysteine, glutathione, thiolactic acid and thioglycollic acid all readily inactivate insulin.[6-9] That the effect *in vivo* is related to this type of inactivation seems unlikely.

In vitro Experiments. When cysteine hydrochloride and insulin are mixed in a phosphate buffer (pH 7.3) in concentrations of 0.1-0.2 mg. per cc. and 0.1-0.3 units per cc. respectively (final strengths) the following observations can be made: (1) In the absence of oxygen (Thunberg tubes) at 37°C. methylene blue is decolorized up to 50% faster by cysteine-plus-insulin than by cysteine alone. The decoloration is further hastened by some alcohols (ethyl, n-butyl) but is markedly retarded by aldehyde. Insulin alone has a very weak bleaching action. (2) In the presence of oxygen, the nitroprusside reaction disappears faster from the cysteine-plus-insulin solution than from the cysteine solution alone. The nitroprusside reaction is preserved if oxygen is excluded. Both

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of these phenomena are explained by assigning the rôle of hydrogen transportation to the SH-insulin complex. These experiments, however, are inconclusive because insulin cannot be prepared chemically pure.

Conclusion. Cysteine diminishes the hypoglycemic action of insulin.

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Further Studies on Tongue Innervation.*

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This study constitutes an attempt to elicit proprioceptive impulses from the tongue musculature and to determine their pathway into the central nervous system. Since the cathode ray oscillograph has recently proven extremely valuable in the determination of the sensory innervation of the accessory musculature,[1] it was used in this study.

The observations of Langworthy[2, 3] (cat), Van der Sprenkel[4] (hedgehog), and Corbin, Lhamon and Petit[5] (monkey), suggest an afferent contribution to the hypoglossal nerve from the upper cervical dorsal root ganglia, proprioceptive in function. However, Hinsey and Corbin[6] obtained no evidence of myelinated fiber degeneration in the peripheral portion of the hypoglossal nerve of the cat after removing the upper 4 cervical dorsal root ganglia. Barron[7] obtained no evidence of proprioceptive activity in the hypoglossal nerve from traction on the tongue but did record action potentials, which he believed to be proprioceptive in origin, from the lingual nerve. Although Langworthy[1, 2] (cat) and Tarkhan[8] (rabbit) re-

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† National Research Council Fellow in the Medical Sciences.

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