to 120 mm. Hg. diastolic). With this episode the blood sugars rose from 25 mg. % to 45 mg. % with a subsequent slow rise to normal. There were no changes in blood pressures in the adrenalectomized dogs. From these investigations we concluded that insulin hypoglycemia is an adequate method for studying changes in blood pressure, and that this hypoglycemic condition calls forth a secretion of adrenalin which is responsible for the sudden elevation in blood pressure and the initial rise in blood sugar. As far as adrenalin is concerned, the response of the hypertensive person is excessive, whereas the response in the patients with Addison's disease is negative.

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Diazotization of Proteins.

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Proteins treated with nitrous acid are said to be "diazotized" insofar as, like true diazonium compounds, they couple in alkaline reaction with substances containing an aromatic OH or NH_2 group, often forming a colored compound resembling the azo dyes. This reactivity of proteins is puzzling, since there is no known primary aromatic amine in protein which would react with HNO_2 to form a diazonium compound. Flick[1] contended that the reactivity of wool treated with HNO_2 was due to the formation of a nitroso rather than a diazonium compound. More recently, Morel and Sisley,[2] confirming previous work by Landsteiner[3] with salicylic acid, have reported that tyrosine treated with nitrous acid forms a diazonium compound which couples with naphthol to form azo dyes. They therefore ascribed the reactivity of diazotized protein to its tyrosine content. Their contention remains plausible even if tyrosine forms a nitrosophenol instead of a diazonium compound on treatment with HNO_2, as found in the case of phenol by Baeyer and

[1] Flick, *Bull. Soc. Chem.*, 1899, **69**, 221. Quoted from Morel and Sisley.

[2] Morel, A., and Sisley, P., *Bull. Soc. Chem. de France*, 1927, **41**, 1217; 1928, **43**, 881.

[3] Landsteiner, K., *Centralbl. Physiol.*, 1895, **14**, Oct. 5.

Caro[4]; for nitroso compounds might also couple with phenols and aromatic amines.

The following data, however, indicate that tyrosine is not the only constituent of protein responsible for its diazotization. Instead, there is reason to believe that tryptophane is also responsible for the fact that protein treated with HNO_2 couples with aromatic amines and hydroxyls.

When tryptophane (or indole) was treated with nitrous acid in the cold, under the same conditions which lead to the "diazotization" of protein, a highly reactive compound was formed which gave a brilliant red color when added to α-naphthol in alkaline reaction (Table I). The HNO_2 probably reacted with the indole NH to form a nitrosamine.

TABLE I.
"Diazotization" of Tryptophane and Proteins, and Failure of Either Tyrosine or Protein Devoid of Tryptophane to Diazotize when Similarly Treated.

Substance tested	4N $NaNO_2$, cc.	N/1 HCl, cc.	Time of incubation	Max. dilution of neutralized mixture giving definite red coloration with α-naphthol
Tryptophane: 5 cc. neutral N/7 solution	.2	0.8	1 hr. room temp.	1:100
Tyrosine as above	.2	0.8	a. ½ " " " b. 1 " " " c. 12 " 2-5°C.	No color
Horse serum, 5 cc. 1:2 dilution (4% protein)	.8	4	12 " 2-5°C.	1:16
Casein: 5 cc. 4% solution	.8	4	12 " 2-5°C.	1:24
Egg albumin: 5 cc. 4% sol.	.8	4	12 " 2-5°C.	1:8
Gelatin: 5 cc. 4% sol.	.8	4	12 " 2-5°C.	Trace of color
Zein: 5 cc. 4% sol. in alcohol	.8	4	12 " 2-5°C.	No color

Moreover, tyrosine failed to develop any diazo-like reactivity on treatment with nitrous acid under the conditions here used; zein, which contains tyrosine but no tryptophane, also failed to form a diazo compound; and gelatin, deficient in both tryptophane and tyrosine, developed only a trace of diazo-like reactivity as compared with serum protein, egg albumin or casein similarly treated. The slight reactivity of "diazotized" gelatin may have been due to impurities in the gelatin.

It is therefore suggested that the reactivity of "diazotized" protein is in part, and perhaps largely, due to its tryptophane content.

[4] Baeyer, A., and Caro, H., *Ber. d. deutsch. chem. Gesellschaft.*, 1874, **7**, 967.