epinephrine no significant change in plasma potassium can be observed.

*Conclusions.* The concentration of potassium in the plasma increases as the circulatory volume decreases. This is a reversible process since the potassium concentration returns to normal if the circulatory volume is increased subsequently by reinjection. Saline is as effective as whole blood in causing the decrease of potassium concentration in this way. The tissue cells are reversibly permeable to potassium ions. The injection of epinephrine has only a transitory effect on the plasma potassium but histamine causes a more lasting increase.

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## On the Absence of Thiolhistidine in Insulin.

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Considerable evidence has pointed to the possibility of the presence in insulin of sulfur other than that attributable to cystine, in which form the major portion of the sulfur is present. That thiolhistidine might account for part of this sulfur was a possibility to be considered. We therefore undertook studies to ascertain whether or not this amino acid is a constituent of the insulin molecule. On the basis of reports in the literature that the sulfur of thiolimidazoles could be split off as sulfate by boiling with ferric chloride and that the sulfur of cysteine was stable, we attempted to test for the presence of thiolhistidine. However, in testing this reaction with cystine, we found that ferric chloride will oxidize the sulfur of cystine to sulfate.[1] This reaction could not, therefore, be utilized to detect thiolhistidine in the presence of cystine in an insulin hydrolysate and some other method of approach was necessary.

In Barger and Ewin's early paper on the betaine of thiolhistidine, ergothioneine it was shown that the sulfur of this compound was

[1] Sifferd, R. H., and du Vigneaud, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 332.

oxidized by bromine to inorganic sulfate.[2] In addition, it is a well recognized fact that cystine upon treatment with bromine is converted to cysteic acid and that no inorganic sulfate is formed.[3] It occurred to us that these 2 observations might form the basis of the desired test but before applying this reaction to insulin for the detection of thiolhistidine we felt that it was necessary to test the reaction on the amino acid itself rather than depend on the known behavior of its betaine. This amino acid was, therefore, synthesized by Harington's method,[4] using aspartic acid as starting material. We were able to check Harington's procedure in all essential details and obtained approximately the same yields reported in the various steps recorded by him. Upon treatment of the synthetic thiolhistidine with bromine the sulfur was converted into sulfate. As was to be expected, cystine yielded no sulfate under the same conditions. The same was true of glutathione, methionine, and homocystine. Furthermore we found that zein, in which the presence of thiolhistidine had been suspected,[5] did yield sulfate after similar treatment with bromine. Recently the bromine oxidation of thiolimidazole as a test for this type of sulfur in protein has been independently developed by Blumenthal and Clarke[6] and applied with excellent results to a series of proteins. In addition to zein they found that other proteins, particularly keratin, yielded sulfate by this method of oxidation.

After the feasibility of this method of approach was established, the reaction was applied to crystalline insulin. Five hundred mg. of the insulin was hydrolyzed by refluxing it with 50 cc. of 20% HCl for 8 hours. The sample was evaporated to dryness, the residue was taken up in 10 cc. of water and bromine water was added until a slight excess of bromine was present. The test for sulfate ion with barium chloride was negative. Knowing that the sulfur in hydrolyzed insulin was mainly if not all in the disulfide state and thinking that this might make some difference in the behavior of a thiolimidazole which would conceivably be present in a disulfide form, we reduced a sample of hydrolyzed insulin and then treated it with bromine. Likewise no sulfate was obtained. Finally the reaction was applied to the unhydrolyzed insulin and again as in the previous tests no sulfate could be detected.

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[2] Barger, G., and Ewins, A. J., *J. Chem. Soc.*, 1911, **99**, 2336.

[3] Andrews, J. C., *J. Biol. Chem.*, 1933, **102**, 263.

[4] Harington, C. R., and Overhof, J., *Biochem. J.*, 1933, **27**, 338.

[5] Eagles, B. A., and Vars, H. M., *J. Biol. Chem.*, 1928, **80**, 615.

[6] Blumenthal, D., and Clarke, H. T., *J. Biol. Chem.*, 1935, **110**, 343.

On the basis of these results we feel that the conclusion is justified that thiolhistidine is not present in the insulin molecule.

## 8377 C

## Resistance of the Spider Monkey (Ateles ater) to Infection with the Virus of Acute Anterior Poliomyelitis.

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Monkeys are the only animals definitely known to be susceptible to poliomyelitis and generally speaking, varieties of old world monkeys seem more susceptible[1, 2] than those of the western hemisphere. Of the new world monkeys members of the genus *Cebus* (ringtail) have been thoroughly investigated. Although transmission of the experimental disease to a *Cebus* monkey has been reported in one instance[1] this South American species can now be considered to be completely refractory.[2, 3, 4] Although not an inexpensive species, the spider monkey like the ringtail can be obtained with relative ease on the Pacific Coast and the shortage of *Macacus rhesus* specimens for titering serum in the 1934 poliomyelitis epidemic in Los Angeles made it desirable to reexamine the susceptibility of the spider.

We are indebted to Doctor Simon Flexner for *Macacus rhesus* cord infected with his well-known mixed (M. V.) virus. This was used as a 10% suspension in buffered saline. Inoculations were made intracerebrally in the usual manner. Typical symptoms and paralyses occurred in all of the *Macacus rhesus* specimens which were inoculated. When no symptoms had developed in the spider monkeys after 10 days a second or accelerating dose of 1 cc. of the suspension was injected.[5, 6] One spider monkey was observed for 30 days after the first injection and the other 2 for 60 days without showing symptoms. At the end of 60 days one was killed and a

[1] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, **12**, 227.

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[4] Jungeblut, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

[5] Flexner, S., *Science*, 1931, **74**, 520.

[6] Flexner, S., *Science*, 1933, **77**, 413.