

*Summary and Conclusions.* From the data presented it is evident that there was considerable variation in the total amount of bile obtained in each animal. The mean flow of bile per hour per kilo of body weight was 3.4 cc in the first 2 hours in 47 rabbits, 2.64 cc in the second 2 hours in 33 of the 47 rabbits, and 2.33 cc during the third 2 hours of the experiments in 19 of the 47 rabbits. Thus there was a gradual decrease in the flow of bile as the experiments proceeded. This decrease was more marked in those experiments in which the bile was collected against a hydrostatic pressure greater than that of the atmosphere. During the entire period of each experiment the gallbladder was resorbing half the volume of its content per hour, *i. e.*, about 0.5 cc per hour per kilo of body weight,<sup>3, 4</sup> hence the amount of bile actually produced per hour by each rabbit was greater than that recorded.

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#### Reducing Substances of the Normal Urine. I. Effects of Insulin Injection.

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This paper embodies a study of the effect of insulin injection upon the excretion of the reducing substances of the normal urine, with special regard to the fermentable fraction. The observations were made on dogs.

There are two articles in the literature pertinent to our subject. Salmon<sup>1</sup> finds that, in man, there is a correlation between the blood sugar level and the normal excretion of reducing substances in the urine. Van Creveld and Van Dam<sup>2</sup> eviscerated frogs, except for kidneys and ureters, cannulated the latter, and perfused through the renal artery a fluid similar to Ringer's solution. To the perfusion were added various concentrations of glucose, and the glucose concentration of the recovered perfusion fluid and of the artificial urine was determined by the micrometric method of Bang. They noted that as the concentration of glucose in the perfusion fluid was

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<sup>1</sup> Salmon, U. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 823.

<sup>2</sup> Van Creveld, S., and Van Dam, E., *Nederland Tijdschr. Geneeskunde*, 1923, **66**, 11, 1498.

increased, there was a corresponding increase in the concentration of glucose in the artificial urine. Where insulin had been injected before the animal was eviscerated, or where insulin was added to the perfusion fluid, there was a profound fall in the concentration of glucose in the urine, and an increase in the concentration of glucose in the fluid recovered from the renal vein. These workers concluded that the presence of insulin in the kidney decreases the passage of glucose into the urine, and ascribed this action to the existence of a "renal attacking point" for insulin.

While the results of this experiment were dramatic and doubtless important, the mode of attack was such as to involve distinctly unphysiological conditions, notably in the use of an artificial renal environment. Further, the estimations involved the *concentration* of glucose in the urine and in the recovered fluid, rather than the *quantity* of glucose excreted per unit of time. We have found the latter method more suitable for the estimation of these urinary constituents.

We have used normal animals. Our method was first to obtain control figures for reducing substances in the blood and urine of the fasting animal. Having established the control levels, we administered glucose by stomach tube. Then one unit of insulin per kilo was injected. Further quantities of glucose were given at such intervals as to maintain the blood "sugar" above the control level. Urine samples were taken by catheter at hourly intervals and analyzed. Thus we were able to eliminate the hypoglycemic influence in our observations of the renal passage of reducing substances under the influence of insulin. Intravenous insulin injections gave the same results as those given subcutaneously.

Determinations of reducing substances were made by the Folin-Wu method.<sup>3</sup> Before analysis, the urine was treated with Lloyd's aluminum silicate, as recommended by Folin and Berglund.<sup>4</sup> In determining the fermentable fraction of the reducing substances in the urine, we employed the Folin-Berglund fermentation procedure.<sup>5</sup>

In 18 of 20 experiments the total reducing substances showed a sharp drop in the first hour. This effect persisted for 3 hours (Graph 1, Fig 1).

In each of 6 experiments in which we determined the fermentable fraction of the urinary reducing substances this fraction was practically abolished in the first 2 hours and was very much lower than the control level during the third hour (Graph 2, Fig. 1).

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<sup>3</sup> Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

<sup>4</sup> Folin, O., and Berglund, H., *J. Biol. Chem.*, 1922, **51**, 209.

<sup>5</sup> VanSlyke, D. D., and Hawkins, J. A., *J. Biol. Chem.*, 1929, **88**, 51.

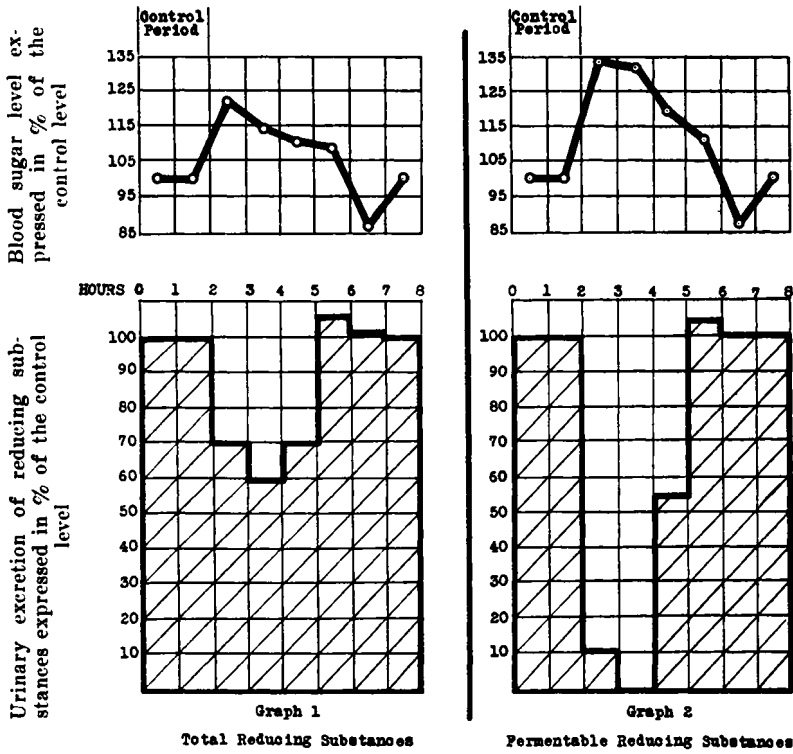


FIG. 1.

The explanatory graphs are arranged as composites of the values obtained from all experimental data, the values being expressed in percentages. The average initial blood sugar was 97 mg per 100 cc. The average initial total reducing substances was 14.4 mg per hour and the average initial fermentable reducing substances was 7.5 mg per hour, in the urine.

The data reported here seem to indicate that insulin acts to effect a diminution in the hourly excretion of fermentable reducing substances in the urine, by some mechanism other than its effect on the blood sugar.