renal function.[3] The variations in the rate of excretion of leucocytes and epithelial cells (these are enumerated together because indistinguishable[1]) and to a lesser degree of casts (in both cases all the casts happened to be hyaline) were in general similar to those of the erythrocytes. The rate of excretion during the morning tended to be lower than at night followed by the mid-afternoon rise. During the late afternoon and evening as well as the night (with only an average observation between 10 P.M. and 6 A.M. we must guess at the latter) the rate of excretion of formed elements appears to be at a fairly steady level. As a practical point this would lend weight to the current practice of measuring the excretion rate of formed elements on the night urine whenever feasible quite apart from the fact that it is easier to obtain the desired urine concentration and acidity during the night with the least inconvenience to the patient. It should also be pointed out that from the experiments reported here it is obvious that the rate of excretion of the formed elements at any time of day if properly carried out would generally lead to the same conclusions about the patient for in spite of the considerable variations the number excreted tend to remain of the same order of magnitude.

8524 P

## Measurement of Insulin Action.*

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The insulin required to maintain a normal blood sugar level in depancreatized dogs has been studied by Holm[1] and in the laboratories of Houssay[2] and Soskin.[3, 4] Holm and Houssay found that 0.01 unit insulin per kilogram of body weight per hour was necessary. Soskin found values as low as 1-175th unit insulin per kg. per hour in dogs under pento-barbital sodium (Abbott).

[3] MacKay, E. M., *J. Clin. Invest.*, 1928, **6**, 505.

* We wish to express our sincere appreciation for the generous gifts of insulin and amytal from the Eli Lilly Co., and for nembutal from the Abbott Laboratories.

[1] Holm, *Arch. f. exp. Pathol. u. Pharmakol.*, 1927, **121**, 368.

[2] Houssay, B. A., Lewis, J. T., Foglia, V. G., *Compt. Rend. Soc. Biol.*, 1929, **101**, 241.

[3] Soskin, S., Allweiss, M. D., *Am. J. Physiol.*, 1934, **110**, 4.

[4] Soskin, S., Allweiss, M. D., Cohn, D. J., *Am. J. Physiol.*, 1934, **109**, 155.

We have determined the insulin requirement in dogs of different sizes which have completely recovered from the pancreatectomy. These dogs were maintained at a constant weight on a measured balanced diet and sufficient insulin to keep the urine nearly sugar-free. They received no food or insulin for 24 hours prior to a determination. No anesthetic was used. The initial high blood sugar was lowered to about 100 mg.% by an intravenous injection of 2-6 units of insulin, depending upon the size of the animal. A continuous or hourly intravenous injection of a weak solution of insulin in physiological saline was started about 4-6 hours following the initial injection. An amount is given which will keep the blood sugar at about 120 mg. %. Table I summarizes the results on 4 different animals.

TABLE I.

| Dog T. 9.5 kg. Depancreatized 3-7-35 | | Dog B. 9.5 kg. Depancreatized 12-5-35 | | Dog S. 15 kg. Depancreatized 9-27-35 | | Dog Bl. 15 kg. Depancreatized 10-24-35 | |
|---|---|---|---|---|---|---|---|
| Date | Units/-K.Hr. | Date | Units/-K.Hr. | Date | Units/-K.Hr. | Date | Units/-K.Hr. |
| 1935: | | 1936: | | 1935: | | 1935: | |
| 10-30 | .0095 | 1-16 | .0094 | 11-9 | .0126 | 11-15 | .014 |
| 11-5 | .0102 | | | 11-16 | .013 | | |
| 11-13 | .0105 | | | 11-23 | .014 | | |
| 12-4 | .0094 | | | 11-29 | .014 | | |
| 1936: | | | | | | | |
| 1-4 | .0094 | | | 12-6 | .014 | | |

Determinations of the insulin-glucose ratio were made. In one experiment the basal insulin requirement was increased from 0.21 unit insulin per hour to 0.41 unit and a continuous intravenous injection of 5% glucose was given. After 5 hours it was possible to inject 3.48 gm. of glucose per hour with the blood sugar constant at 125 mg. %. On the basis of the added insulin, the insulin-glucose ratio was therefore 1 unit to 17.4 gm. In another experiment 1 unit additional insulin per hour was added to the basal rate of 0.09 units insulin per hour, making a total of 1.09 units of insulin per hour. Eleven hours after starting the injection of glucose, 9.0 gm. per hour were being given with a blood sugar of 116 mg. %. More experiments of this nature are in progress with varying amounts of insulin.

Amytal and nembutal both apparently cause a marked lowering of blood sugar if given during the injection of the glucose and insulin. A 15 kg. dog received 4.0 gm. of glucose intravenously and 1.21 units of insulin per hour. Following the intravenous

injection of 400 mg. of sodium amytal, the blood sugar fell from 150 mg. % to 94 mg. % in 30 minutes. A rapid increase in the injection rate of glucose was possible, 12 gm. per hour being given 2 hours later with a blood sugar of 130 mg. %. In another experiment on the same dog insulin was given at the rate of 1.21 units, and 1.9 gm. of glucose per hour. In 20 minutes after the intravenous injection of 2 cc. of nembutal, the blood sugar had fallen from 140 mg. % to 110 and in 50 minutes to 88 mg. %. A fall of 40 mg. % in blood sugar in 30 minutes has also been demonstrated after nembutal in one of the 9.5 kg. dogs.

## 8525 C

## Urea as a Solvent in Preparation of Antigen Extracts.

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The development of the study of allergy in recent years, and particularly the search for the etiologic factors, has focused attention upon numerous protein containing substances. These include foods, pollens, molds, epidermal structures, orris root, house dust and such less common factors as the scales on sand flies or the stinging organ of bees, wasps, etc. The substance suspected may be tested on the patient in various ways: (a) by direct application of it to the skin, to the conjunctivae, or if a food, it may be ingested; (b) the juice may be expressed from certain of the substances and used by application to a scratch on the skin, or by intracutaneous injection. This method is naturally limited in its application. The ideal method is one which would concentrate the active substance, change its physical or chemical composition little or none, and one which could be used on all types of substances. By analogy with immunologic phenomena, and in view of the evidence which has accumulated in the field of allergy, one is led to believe that the active allergic agent is protein or closely attached thereto.

Proteins in general are rarely water soluble to any extent; this is especially true after they have undergone the procedures necessary to separate them from the various substances mentioned above. The simplest extraction methods employ a solution of NaCl in various concentrations. Alkalies, notably a bicarbonate or hydroxide, or a neutral phosphate buffer solution, find most general use. Recently a