

is no difference between glucose and levulose in producing aketosis.

Thus the "fire of carbohydrates" is not always necessary to oxidize completely the end products of fat metabolism in the phlorhizinized dog, but a mere increase in the quantity of glucose is sufficient to do so, possibly, by contact action between it and the ketone bodies. From a general standpoint it is interesting to note that a substance, such as sugar, which is not retained nor oxidized but is slowly and completely eliminated from the body, nevertheless, by its mere presence in even small amounts (as little as 20 grams) is able to produce a complete oxidation of fat to its normal end products, associated with a sparing action upon protein, and at the same time to improve, sometimes to a high degree, the clinical state of the dogs in diabetic coma.

### 203 (2726)

#### Ramon flocculation test for determining potency of antiscarlatinal serum.

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The Ramon flocculation test has proven quite successful for determining the potency of antitoxic diphtheria serums. It is used now at the Research Laboratory of the City Health Department for the routine study of the progress of diphtheria horses; these tests need to be verified only here and there by guinea pig inoculations.

The above results with anti-diphtheritic serums led to attempts to apply the flocculation test, if possible, for determining the potency of antiscarlatinal serums, as compared with clinical neutralization tests.

For titration of the anti-scarlatinal serum, or of toxin, we rely on skin reactions. The strength of the toxin is expressed by the number of skin doses per cc. required for a reaction; and the potency of the serum is estimated by the amount of serum required to neutralize the number of skin doses per cc. Thus if

the titrated toxin contains 40,000 skin doses per cc. and flocculation occurs with 2 cc. of the tested serum, we would estimate that the serum contains 20,000 neutralizing skin doses per cc. A table can be then constructed, with a titrated toxin, in the same manner as for the diphtheria Ramon tests, on the basis of units. Thus in the above cited case the serum contains  $\frac{1}{2}$  unit per cc.; if one cc. of serum were required for neutralization, the serum would contain 1 unit; if  $\frac{1}{2}$  cc. is used, 2 units, etc.

Though the flocculation tests for scarlet fever serum were undertaken many months ago, no visible results were obtained until lately. The explanation is possible, that though we had good toxins, we did not have strong enough serums to make the test workable in the test tube. At present our own horses as well as the horses from different laboratories have furnished us with stronger serums, and the potency of some of them can be demonstrated by flocculation test, and compared with the skin neutralization tests in humans.

The favorable results thus far obtained are: Our own scarlatinal serum horse No. 50, whose serum continued negative for a long time by the flocculation test, shows in the last successive bleedings, flocculation with amounts from 4 cc. to 2 cc. with one cc. of toxin containing 40,000 skin doses per cc. This closely corresponds to the skin reactions. Corresponding results were obtained with two other weaker toxins. Four sera from outside laboratories which were labelled "15,000 neutralizing doses per cc." gave flocculations with the above 1 cc. of toxin in amounts from 3 cc. to 2 cc. of serum. One serum also from an outside laboratory gave an instant flocculation with anti-scarlatinal, with diphtheria toxin, as well as with plain broth, though the control of the serum itself remained clear. It is possible the preservative used in the last serum gave the non-specific reactions. Controls were also made with anti-streptococcus serum and a few normal serums, with negative results. On the other hand almost every anti-scarlatinal horse serum showed flocculation with diphtheria toxin, when the serums used were in large amounts (from 5 to 3 cc.) These amounts of serum would detect from  $\frac{1}{4}$  to  $\frac{1}{3}$  unit of diphtheria antitoxin per cc. Such small amounts of diphtheria antitoxin is normal for horses, especially as some might have been old diphtheria horses, and the specificity for scarlet fever may not necessarily be questioned.

The above cited tests though encouraging are by no means conclusive or definite as yet. Much more work must be done to be sure of the specificity of the flocculation test for scarlet fever serums and toxins, as in the case of diphtheria.

## 204 (2727)

### Note on the relationship between insulin and trypsin.

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Epstein<sup>1</sup> and his co-workers have recently claimed that insulin is not digested by trypsin and pepsin. It is supposed to form an inactive addition product with trypsin, which is stable at an alkaline pH, and from which the insulin can be reactivated by acidifying. A similar reaction occurs with pepsin, but under opposite conditions as far as the pH is concerned. The physiological and clinical aspects of these *in vitro* experiments were followed up by Epstein<sup>2</sup> and his co-workers by *in vivo* experiments and perfusion studies of the pancreas.

Epstein's results, however, as far as the perfusion experiments go, were contradicted by W. S. Collens,<sup>3</sup> and his *in vivo* experiments could not be supported by D. A. Scott.<sup>4</sup> As to Epstein's *in vitro* experiments, Scott was able to confirm that insulin forms an inactive addition compound with trypsin, from which it can be reactivated. But he found, in accord with previous investigators, that trypsin digests insulin. The fact that acid reagents reactivate insulin from its inactive addition compound with trypsin, led Scott to point out other results which indicate that insulin may exist in the pancreas and in the body in an inactive form.

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<sup>1</sup> Epstein, A. A., and Rosenthal, N., *J. Am. Med. Assn.*, 1924, lxxxii, 1990; Epstein, A. A., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 9; Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1924, lxx, 225.

<sup>2</sup> Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1925, lxxi, 316.

<sup>3</sup> Collens, W. S., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 367.

<sup>4</sup> Scott, D. A., *J. Biol. Chem.*, 1925, lxxiii, 641.