

jelly was collected at the end of 1, 2 or 3 days and the cells immediately re-grafted and returned to the bees. Each group of bees was used for 6-7 days after which time the confined bees became distressed to the point that no further queen rearing was possible. Two collections of jelly at 3-day intervals were found to give an optimum yield. Radioactivity in the jelly did not decrease up to 7 days after feeding. A total of 8.8 gm. (fresh weight) of labelled royal jelly was produced.

A sample of each collection of royal jelly was dried and checked for radioactivity on a Nuclear-Chicago model D47 gas-flow detector. The acids from the royal jelly were isolated and separated chromatographically by the method of Brown and Freure<sup>2</sup>. The chromatograms were checked for radioactivity. Only the spot corresponding to 10-hydroxydec-2-onoic acid ( $R_F$  0.70) was found to be active. The spot was eluted and a total of 80 mgm. of radioactive royal jelly acid was recovered from 6 gm. of whole royal jelly. The recovered acid was shown to be identical with an authentic sample of inactive royal jelly acid by comparison of infra-red absorption spectra.

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<sup>1</sup> Law, J. H., and Weaver, N., *Nature*, **188**, 938 (1960).

<sup>2</sup> Brown, W. H., and Freure, R. J., *Canad. J. Chem.*, **37**, 2042 (1959)

### Variations in the Blood Saccharoid Fraction in Diabetes

RECENT reports have stressed the advantages of using highly specific 'glucose oxidase' methods for the clinical determinations of blood sugars<sup>1-3</sup>. Traditional methods, such as those of Folin and Wu, measure the total reducing capacity of the blood, and include the so-called saccharoid fraction as well as the true glucose-level. The exact nature of this fraction, and its significance in pathological conditions, is not yet fully understood.

Clinical investigations have suggested that in a variety of conditions affecting blood sugar there is little correlation between blood glucose and the saccharoid fraction, and that this latter fraction is erratic and unpredictable during glucose tolerance tests. Saifer and Gerstenfeld<sup>1</sup> believe that this fraction is significantly increased in diabetic patients, and Marks<sup>2</sup> has shown a reduction in the saccharoid fraction following insulin therapy, although the fall is delayed and erratic compared with that of the true glucose fraction. Similar clinical studies are being carried out in this department, together with corresponding determinations on normal, alloxan-diabetic and insulin-treated alloxan-diabetic rats. Preliminary results of this latter investigation are shown in Table 1 and Fig. 1. Diabetic rats had been treated 3-4 months previously with 180 mgm. alloxan per

Table 1. BLOOD GLUCOSE-LEVELS OF NORMAL AND DIABETIC RATS MEASURED BY FOLIN-WU AND GLUCOSE OXIDASE METHODS. The values are the mean of 9 normal and 6 diabetic animals

	Folin-Wu (mgm. per cent)	Glucose oxidase (mgm. per cent)	Saccharoid fraction (by difference)
Normal rat	136.9 ± 4.7	79.4 ± 2.6	57.4 ± 3.8
Diabetic rat	484.0 ± 48	253.8 ± 17	230.0 ± 30

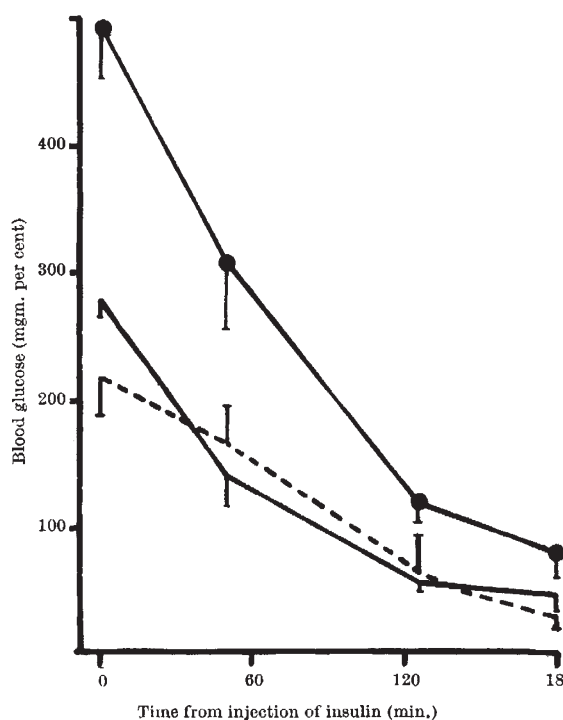


Fig. 1. Glucose-levels in blood of alloxan-diabetic rats following the injection of insulin. Each point represents the mean of three animals. The vertical lines indicate the S.E. ●—●, Folin-Wu; —○—, glucose oxidase; ---, 'saccharoid fraction' (difference)

kgm. body-weight (2 per cent solution, citrate-phosphate buffer, pH 4) and had blood sugars greater than 200 mgm. per cent (glucose oxidase). Table 1 shows that the true glucose-level increases from a mean of  $79 \pm 2.61$  to a mean of  $253 \pm 17.8$ . This represents an increase of 3.2 times. The saccharoid fraction (obtained by subtraction) shows a parallel increase, although the factor is somewhat larger (4.0 times). When diabetic rats are treated with insulin (100 units/kgm.) this trend is reversed as shown in Fig. 1. It would appear that in the case of the alloxan-diabetic and insulin-treated rat, corresponding changes occur in both the true glucose fraction and the saccharoid fraction. It is of interest that in the rat approximately half the value obtained by the Folin-Wu method of estimation is due to substances other than glucose. This is higher than the corresponding fraction in the human, where they represent approximately 25 per cent of the Folin-Wu value. It appears that there may be a marked and characteristic species difference and it is interesting that Hernandez and Coulson found no evidence of a saccharoid fraction in the alligator<sup>4</sup>.

These results emphasize the importance of standardizing methods of glucose estimation, not only clinically but also in experimental animal investigations.

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<sup>1</sup> Saifer, A., and Gerstenfeld, S. J., *J. Lab. Clin. Med.*, **51**, 448 (1958).

<sup>2</sup> Marks, V., *Clin. Chim. Acta*, **4**, 395 (1959).

<sup>3</sup> Middleton, J. E., *Brit. Med. J.*, **1**, 824 (1959).

<sup>4</sup> Hernandez, T., and Coulson, R. A., *Biochem. J.*, **79**, 596 (1961).