

In another investigation silver oxide was isolated in the decomposition products of silver nitrite, although it had not been found by E. Divers<sup>2</sup>. The probable formation was indicated in a thesis mentioned in *British Chemical Abstracts* (1, 245; 1942); the details were not published and reference to the thesis shows that the oxide was isolated when silver nitrite was heated in a current of dry air at 550°, when the decomposition is extremely rapid. His silver oxide may have been a secondary product; silver, in a finely divided state, can react with the oxygen of the air<sup>3</sup>. Oza, Oza and Thaker have isolated silver oxide in the decomposition of silver nitrite at 130° in an atmosphere of oxygen at 11 cm. mercury pressure. Addition of finely divided silver to the decomposing nitrite did not alter the results. It is obvious that the atmosphere immediately in contact with the decomposing nitrite is a mixture of nitrogen dioxide and oxygen, which can produce only nitrate but not oxide. The presence of oxygen in the atmosphere surrounding the decomposing nitrite has the effect of removing nitric oxide, a product of the decomposition, which, if left as such, destroys the silver oxide.

The action of nitric oxide on oxides of silver, mercurous and mercuric mercury, and calcium has been investigated by Oza and Thaker and Oza and Oza. The oxides were heated, at suitable temperatures (< 300°), for varying periods of time, in an atmosphere of nitric oxide at different pressures. The experiments demonstrated the formation of nitrite as the primary product of the reaction. Nitrogen and nitrogen dioxide are produced in the gas and their formation is greatest when nitric oxide is at a pressure tending to zero. There seems little doubt, therefore, that the molecule of nitric oxide decomposes and the nitrogen dioxide found in the gas arises in this way. The formation of nitrite may also involve a prior orientation of the nitric oxide molecule on the surface of the metal oxide to bring about what is essentially a peroxide structure<sup>4</sup>. Nitrogen is also formed from nitric oxide in contact with nitrite<sup>1</sup>. In the case of silver oxide, which has, among the oxides now studied, the most readily detachable oxygen, this provides the most noteworthy reactions.

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<sup>1</sup> Oza, T. M., and Oza, V. T., *J. Chem. Soc.*, 909 (1953).

<sup>2</sup> Divers, E., *J. Chem. Soc.*, 24, 85 (1871).

<sup>3</sup> Benton, A. E., and Drake, L. C., *J. Amer. Chem. Soc.*, 54, 2186 (1932).

<sup>4</sup> Addison, C. C., and Lewis, J., *J. Chem. Soc.*, 1874 (1953).

### A Simple Method for the Determination of Glycogen in Liver

THE methods used for the determination of liver glycogen can be divided into two general groups: the glycogen can be determined in its original state<sup>1,2</sup>, or after its hydrolysis to form simple sugars<sup>3,4</sup>.

Recent investigations indicate that liver glycogen is built up of two physiologically different components. One of these is protein-bound, constant and can be extracted by boiling with alkali. The other is 'free', and physiological alterations are mainly associated with this fraction, which can be extracted with trichloroacetic acid<sup>5,6</sup>. The latter method has been recently regarded by several authors as an

accurate method for the determination of quantitative changes in the liver glycogen<sup>2,6,7</sup>.

On the basis of our experiments carried out during the past year, we recommend the following method for the determination of glycogen. We use 10 per cent (v/w) trichloroacetic acid, physiological saline and absolute alcohol. 2-3 gm. aliquots of the livers of rats killed by decapitation are quickly homogenized in a mortar previously weighed and containing 2 ml. of trichloroacetic acid. The weight of the liver sample is established by reweighing the mortar, and the quantity of trichloroacetic acid made up to 1 ml./gm. liver. To this 1 ml./gm. liver mixture physiological saline is added and the homogenization completed. After filtration through filter paper, 1.5 ml. of alcohol is added to 1.2 ml. of the filtrate. (In the case of high liver-glycogen concentrations, appropriate dilutions of the filtrate may be used). The turbidity arising on shaking and after standing for three hours is measured nephelometrically with a yellow filter in a suitable colorimeter. The original (or the diluted) filtrate in a 1.2-1.5 dilution with water serves as blank control.

Calibration curves were established with dilutions of a commercial sample of glycogen and with three samples originating from different groups of rat livers. The regressions of all the four samples proved to be identical. This shows a great advantage above the van der Vies iodine method<sup>2</sup>, as with the latter even livers of single animals of the same species gave different colour reactions. We have ourselves investigated the use of this method<sup>8</sup>.

In recovery experiments, 103.6 ± 3.85 per cent of added glycogen was regained (average of nine determinations). The values obtained by our method were compared with the values for the same livers as determined by the hydrolytic method<sup>3,9</sup>. According to our experiments, in the case of low liver glycogen, the relative proportion of trichloroacetic acid glycogen proved to be low, whereas in the case of high liver glycogen the two methods gave nearly the same results. This observation is in accordance with the results of other authors<sup>3,10</sup>.

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### In vitro Synthesis of Ribonucleic Acid in Reticulocytes

WE have been studying the concurrent synthesis *in vitro* in rabbit reticulocytes of haem, globin and the non-haemoglobin proteins. Using methylene-labelled (<sup>14</sup>C) glycine the degree of ribonucleic acid synthesis also could be measured. The technique devised may be useful to others faced with the