Table 1.

Extract	Volume ml	AU/ml	Total AU	mg N/ml	AU/mg N
I	2800	90	252000	4.0	22
II	650	380	251000	2.0	190
III	650	350	228000	0.6	580
IV	650	250	162000	0.4	600
IV+Mn	650	450	292000	0.4	1100
V	50	2100	110000	3.0	700
V+Mn	50	5000	250000	3.0	1650
VI	180	900	160000	1.2	750
VII	70	2000	140000	2.6	780

AU signifies arginase units, a term adopted by Safwat Mohamed and Greenberg [1].

By AU/ml is meant arginase units per ml of extract. AU/mg N = arginase units per mg N in 1 ml of extract.

f) Supernatent (IV) is mixed at 3° C with 0.7 volumes cold acetone. Let stand for 8 h and centrifuge in cold room.

g) Supernatent + 0.5 volumes cold acetone and left at 3° C for 8 h Centrifuge and the greenish blue precipitate is taken up in distilled water (V).

h) The precipitate formed in (e) contains considerable arginase. Take up in phosphate buffer, pH 7.5. Let stand with shaking overnight and centrifuge. Extract (VI) is mixed at 3° C with 1.2 volumes cold acetone, centrifuge after 8 h. Precipitate was taken up in phosphate buffer, pH 7.0 (VII).

Extract (V) contains very active arginase which is strongly activated by Mn^{++}. Electrophoresis at pH 7 and pH 6.0 showed the presence of a single homogeneous protein. The protein was precipitated several times with acetone in the cold (1.2 volumes). A greenish blue protein was always recovered. It dries into a white powder with bluish tinge.

Electrophoresis of (VII) showed the presence of three peaks with arginase forming 85—90 % of the total protein present.

From Table 1 it is evident that part of the arginase is split up, under the effect of heat and Pb^{++} ions, into smaller fragments still very active and can be much further activated by Mn^{++} ions. This fraction (V) was separated in a pure, yet not crystalline, form. In fraction (VI) the remainder of the enzyme, probably intact, could be separated along with two other proteins; arginase forming 85—90 %.

In a later publication a more detailed report will be presented.

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1. Safwat Mohamed, M., and Greenberg, D. M. *Arch. Biochem.* **8** (1945) 349.
2. Thompson, C. B. *Science* **104** (1946) 576.
3. Thompson, C. B. *Experientia* **4** (1948) 432.

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On the Reducing Sugars in Sera from Pregnant and Lactating Women

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The statement by Lövgren [1] that sera from pregnant and lactating women contain lactose only and no glucose as determined by his osazone formation method seemed to the present authors so surprising that it was desirable to check its validity.

It is well known that the microscopical identification of osazone crystals can be very difficult. This is especially the case if more than one osazone-forming sugar are simultaneously present or if the mother liquor is contaminated with other crystal-

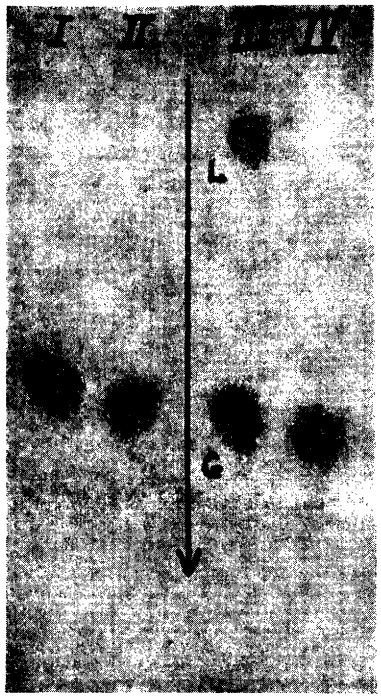

Fig. 1. Chromatogram showing reducing sugar in serum from woman in pregnancy (I), puerperium (II) and lactation (IV). Reference sugars (III): lactose (L) and glucose (G). Solvent: butanol-acetic acid. Developer: $AgNO_3$-ammonia.

lizing or noncrystallizing material (inorganic salts, peptides, lipids *etc.*). Thus instead of using the method of Lövgren we have carried out the analyses for reducing sugar by means of paper partition chromatography.

Method: 1 ml serum was precipitated with 9 ml 95 per cent ethanol and centrifuged. (This concentration of ethanol was shown not to precipitate a 200 mg per cent lactose solution). The supernatant liquid was decanted and evaporated to dryness *in vacuo*. Water was added and the solution desalted in the apparatus used by de Verdier and Ågren [2], and finally brought to a small volume *in vacuo*. A few drops of this solution were applied to the paper strip. For the details of the chromatographic technique see Partridge [3] and Werner and Odin [4].

In tests with lactose added to serum we found that by this method a lactose content in serum of about 5—10 mg per cent could be detected.

30 sera from women in late pregnancy and puerperium and 3 from women who had been lactating for a few months were investigated. *Glucose was found to be the dominating sugar in all these cases.* Only in one of the sera from women in puerperium and one from those in lactation did the chromatograms show traces of lactose. (A typical chromatogram is shown in Fig. 1.) Thus it seems probable that lactose, if at all present, generally does not reach a concentration exceeding 5—10 mg per cent.

It is well known that the urine in cases of pregnancy and lactation may contain lactose in an amount sufficient to give a positive Almén's test. Even that, however, can be explained by a lactose content in serum of less than 5—10 mg per cent.

The presence of lactose in serum in this low concentration can easily be explained by a slight resorption to the blood from the mammary glands. It certainly does not imply any profound alteration in the carbohydrate metabolism as suggested by Lövgren.

1. Lövgren, T. *Svensk Kem. Tid.* **59** (1947) 67.
2. de Verdier, C. H., and Ågren, G. *Acta Chem. Scand.* **2** (1948) 783.
3. Partridge, S. M. *Biochem. J.* **42** (1948) 238.
4. Werner, I., and Odin, L. *Upsala Läkareförens. Förh.* **54** (1949) 69.

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