A SIMPLE COLORIMETRIC METHOD FOR ESTIMATING SUGAR IN URINE OR MILK

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The method described below is a modification of Fine's (1934) method by which the latter is adapted to the colorimeter so as to give the amount of sugar in urine or milk within 2 g. per litre (0.2 g.%) of that determined by Folin's method. Several hundred samples of urine sugar were simultaneously determined by this method and by that of Folin. The results of the new method were found just as satisfactory as those of the latter. Fewer samples of milk were tried, though, the results were fairly good. As a quantitative test for sugar in urine the method is most suitable as a clinical test where minor differences of 1 to 2 g. per litre are of no importance either to the physician or to the patient. It is also most suitable and most convenient for routine work, because qualitative tests can rapidly be made quantitative (Harrison, 1947).

Urine or milk is heated with Benedict's qualitative reagent, and the precipitate is removed by centrifuging. The blue colour of the supernatant solution is read colorimetrically.

Procedure for Urine Sugar

Mix 5 ml. of Benedict's qualitative reagent and 0.25 ml. of urine, heat in a boiling-water bath for five minutes, and then centrifuge. If the supernatant fluid is not blue, repeat with 0.1 ml. of urine. If the supernatant fluid is still not blue, dilute the urine 1 in 2 or more and repeat (alternatively, use 10 ml. of Benedict's qualitative reagent mixed with 0.1 ml. of urine).

Reading with a Duboscq Type of Colorimeter

Take about 2 ml. of the supernatant fluid without disturbing the precipitate of cuprous oxide. Set the unknown in the colorimeter at 10 mm. and read the standard. The standard is prepared from the same Benedict qualitative reagent used for the unknown by taking 5 ml. of Benedict + 15.6 water—i.e., Benedict solution 97% diluted 1 in 4 in water (Fine, 1935).

From the Table or Fig. 1 the amount of sugar in urine (grammes per litre) can be directly read for the colorimetric reading of the standard. The table and chart were made from several accurate readings of the standard against various sugar concentrations treated as above.

FIG. 1

If it is desired not to use a table or graph the following formula for calculating the amount of sugar in the urine is applied.

Setting the unknown at 10, suppose that the reading of the standard is R, then with 0.25 ml. of urine $40 - R =$ urine sugar in grammes per litre. With 0.1 ml. of urine the formula $(40 - R) \times 2.5$ gives an approximate figure, within 1 or 2 grammes of that in the table.

It must be admitted that with the upper part of the table—i.e., when R = 1 or 2 or even 5—it is very difficult and some-

TABLE

Colorimetric Reading of the Standard	With 0.25 ml. of Urine	With 0.1 ml. of Urine
1	39	99
2	38	96
3	37	92
4	36	90
5	35	86
6	34	85
7	33	83
8	32	81
9	31	78
10	30	76
11	29	73
12	28	71
13	27	70
14	26	68
15	25	64
16	24	61
17	23	59
18	22	56
19	21	54
20	20	51
21	19	49
22	18	46
23	17	44
24	16	41
25	15	39
26	14	36
27	13	33
28	12	31
29	11	28
30	10	26
31	9	23
32	8	21
33	7	18
34	6	16
35	5	13
36	4	11
37	3	8
38	2	5
39	1	
40	0	0

times impossible to make good colorimetric readings: On the lower part of the table, however, where R equals 38 or 39, the calculation of the amount of sugar in urine may be fallacious unless there is a definite precipitate of cuprous oxide; again, the reduction of copper may not be due to sugar at all.

Reading with a Photo-electric Colorimeter (Lumetron)

Mix 2 ml. of supernatant fluid with 8 ml. of water and put in the colorimeter tube (1 in. (2.5 cm.) calibre). Transmission is read through the orange filter (850 Lumetron), setting water at 100% transmission.

Calibration.—Glucose concentrations of 100, 80, 60, 40, 30, 20, 10, and 0 g. per litre are prepared. Mix 0.25 ml. of each of the 40, 30, 20, 10, and 0 g. per litre concentrations with 5 ml. of Benedict's qualitative reagent and treat as before. Mix 0.1 ml. of 100, 80, 60, 40, 20, and 0 g. per litre concentrations with 5 ml. of Benedict's reagent and treat as before. Transmission of the above concentrations is plotted on semilog

FIG. 2

paper as in Fig. 2 in two separate lines. The reading with 0.25 ml. of 40 g. per litre and with 0.1 ml. of 100 g. per litre should be somewhere about 100% transmission. 0 concentration with either 0.1 or 0.25 ml. should read somewhere between 51 and 52% transmission. From the lines drawn on the semi-log paper a table can be prepared similar to the table above.

Determination of Milk Sugar

The proteins of milk are precipitated by adding 1 ml. of sodium tungstate 10%, 1 ml. of $\frac{1}{2}$ N sulphuric acid, and 2 ml. of water to 1 ml. of milk, thus diluting milk to 1 in 5. Then

filter through good-quality filter paper. The filtrate should be clear. Add 0.5 ml. of the filtrate to 5 ml. of Benedict's qualitative reagent and treat as before.

With a Duboscq colorimeter set the unknown at 10 and read the standard. Let the reading be R.

$$\therefore \frac{(40 - R) \times 5 \text{ (dilution)}}{2 \text{ (for using 0.5 instead of 0.25)}} = \frac{(40 - R) \times 5}{2} \text{ grammes per litre}$$

That is, we can read the amount direct as if we are using 0.1 ml. of urine (from the table and Fig. 1).

With the Lumetron 2 ml. of the supernatant fluid is mixed with 8 ml. of water (as usual). The transmission is read on the line of 0.1 ml. of urine. For the amount of sugar in milk divide by 10 to get grammes %. The result is multiplied finally by 67/50, the reduction factor for lactose.

REFERENCES

- Fine, J. (1934). *British Medical Journal*, 2, 167.
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Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed., p. 128. Churchill, London.

Medical Memoranda

Surgical Treatment of Rabies

Cauterization of bites by rabid animals, as recommended by the Pasteur Institutes, might advantageously be combined with the modern surgical technique of excision of contaminated wounds. Complete destruction of the virus by cauterization alone is at best fortuitous, and unlikely in extensive, deep, and punctured wounds.

I have recently seen three fatal cases of rabies following dog-bites. One patient received no prophylactic treatment, but the other two (Anglo-Indian children aged 2 years 3 months and 2 years 5 months) had their wounds thoroughly cauterized shortly after infliction. They were also given a full course of anti-rabic vaccine (5 ml. subcutaneously every day for 14 days), although there was an unavoidable delay of two days in instituting vaccine treatment in one case, and of nine days in the other. The risk of rabies in these two patients was admittedly serious, as the bites were facial and of considerable severity.

I suggest the following procedure in the treatment of wounds inflicted by rabid or presumably rabid animals:

(1) Thorough cauterization of the wound with concentrated nitric or pure carbolic acid. This is done immediately, and on no account delayed until preparations for operation have been completed.

(2) The skin is carefully sterilized over a wide area with pure rectified spirit, and then with tincture of iodine.

(3) The aim of surgical treatment is complete excision *en bloc* of all lacerated and potentially infected tissue. All dissection takes place in uncontaminated tissue-planes, with an ample margin of clean tissue to act as a barrier, a fresh knife being used for each wound. Scrupulous care is required to keep contaminated instruments separate (e.g., those used to put tension on the edges of the original wound), and at the earliest moment all contaminated instruments are treated with pure lysol, washed, and re-sterilized.

(4) The surgical wound resulting from excision is then cauterized with the same agent that was used originally. After haemostasis has been secured the wound is left unsutured and dressed.

This technique is open to certain objections. Dr. N. Veeraraghavan, director of the Pasteur Institute, Coonoor (personal communication), observes in experimental animals that rabies is produced as quickly by inoculation of the sciatic nerve as of the subdural space. He raises the question of similar inoculation of nerve trunks during surgical excision of the wound, although it seems likely that this danger could be avoided by immediate cauterization of the surgical wound and careful technique to avoid contamination in the operative field by the surgeon and his instruments.

There seems ground for experimental work to establish the value or otherwise of the above procedure. If proved to be effective, the method would be indicated in cases with strong

presumptive evidence of infection with rabies; in cases where the wounds were amenable to radical excision; and in centres possessing adequate surgical facilities. In more remote areas the present method of treating wounds by cauterization alone would continue to be employed. In either case, a full course of anti-rabic vaccine would be necessary.

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Hypnosis and Suggestion in Obstetrics

Below are given the histories of three cases in which use was made of the power of suggestion to the subconscious mind so that the activity of the normal conscious levels and the concomitant highly developed critical faculties were temporarily suspended.

CASE REPORTS

Case 1.—The patient, a primigravida aged 21, was admitted to hospital as an emergency with a diagnosis of obstructed labour, having been in the second stage of labour for six hours after a first stage of about twenty-four hours. On examination rather painful uterine contractions were still present, although they were much less severe than they had been, the vertex was engaged but a large caput had formed, and there was excessive moulding of the foetal head. The cause of the delay appeared to be a relative disproportion between the presenting part and the outlet, together with incomplete anterior rotation of the occiput. The head was fitting extremely tightly in the birth canal and seemed completely "stuck." A condition of light hypnosis was induced and the patient was instructed to relax, which she did sufficiently well to enable the occiput to be rotated more to the front and the forceps to be applied correctly. During delivery of the baby the mother was able to co-operate and use her own expulsive muscles while traction was being made. She later stated that she had had no actual pain but that she felt "like another person." Although the tissues of the perineum were very thick, only a very small episiotomy was required. The placenta was expelled naturally after 20 minutes, and the baby, which cried lustily, weighed just over 10 lb. (4.5 kg.).

Case 2.—A primigravida aged 32 with a twin pregnancy was admitted to hospital as a booked case, having regularly attended the antenatal clinic. Her membranes ruptured two hours after labour pains had started, and progress was very slow, as is often the case with multiple pregnancies, so that towards the end of the third day of labour she was much distressed and exhausted in spite of the administration at intervals of pethidine hydrochloride in doses of 100 mg. each and other sedatives such as chloral hydrate and potassium bromide. The cervix was then a little more than two-thirds dilated but was very oedematous, and the patient constantly reiterated her strong desire to bear down with each contraction. Doubtless this inability to relax properly during the first stage was partly responsible for the oedema and slow dilatation of the cervix. In spite of the presence of painful uterine contractions therapeutic suggestion was attempted, and after about fifteen minutes the patient began to respond and became much quieter and more relaxed. A few minutes later she was sleeping, although strong contractions were still occurring. The suggestion of "No pain" was continued at intervals for another ten minutes or so, and she remained asleep for some hours. When re-examined later, on awakening, her condition was much improved, the cervix being nearly fully dilated and the oedema having disappeared. She then stated that she felt much refreshed and that all she could remember was a feeling of drowsiness associated with a sensation of being "far away—as if I were floating in space." The rest of the labour progressed normally and satisfactorily and both babies were born in good condition.

Case 3.—A primigravida aged 24 was admitted to hospital owing to a prolonged first stage and an impending uterine inertia. She had been in labour for between two and three days and was becoming exhausted and depressed. On examination the cervix was nearly three-quarters dilated and the membranes were still intact. A light hypnotic sleep was then induced and the suggestion made that she would sleep until it was time to "push the baby out." She slept, although slightly restless now and then, for some hours, and soon after waking second-stage contractions began and labour then continued normally and uneventfully.

These three cases illustrate to some extent what can be achieved if the so-called "subconscious mind" can be utilized in aiding the removal of fear and tension and the promotion of relaxation both of mind and of body.

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