LXX. POLARIMETRIC OBSERVATIONS ON SOLUTIONS OF GLUCOSE SUBJECTED TO CONTACT WITH INTESTINAL MUCOSA OF RABBIT.

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IN a paper by Hewitt and Pryde [1920] published in this Journal, three polarimetric experiments with previously equilibrated hypotonic solutions of glucose, which had been in contact with the mucosa of the small intestine of the rabbit for five minutes, are quoted with data which are claimed as indicative of a disturbance of the equilibrium of the solutions as a result of the intestinal contact.

An upward mutarotation of the solution in the polarimeter to the $\alpha - \beta$ equilibrium value is quoted for each of the three cases, the conclusion drawn being that a downward mutarotation had taken place earlier, *i.e.* during the period of contact.

The polarimetric observations were commenced from $3\frac{1}{2}$ to 10 minutes after removal of the solution from the gut, and the mutarotation effect had ceased to be evident from 15 to 25 minutes after removal.

The observers, however, state on pp. 399–400 of their paper that in some experiments these mutarotation effects were “ill-defined,” and in some “completely negative,” but the relative numbers of the experiments yielding the three types of results are not given.

As, so far as we are aware, the positive result of these observers has not been controlled, we have thought it advisable to repeat the experiment. One of us (D. S.) is responsible for the polarimetric and analytical work, and the other (E. W. R.) for the operative procedure.

The observer with the polarimeter took no part in the operation upon the animal, and entered a dark room ten minutes before taking an observation, for the double purpose of attaining a fair degree of dark adaptation of the eye for the earliest readings, and the necessary mental composure.

Since the question as to whether or no any mutarotation occurs in the glucose solution during the early period after its removal from the gut is, in this particular case, of more importance than quantitative values in relation to the concentrations of glucose given by analyses, no attempt at complete chemical removal of the small amounts of protein necessarily added to the solution from the intestinal glands during its sojourn in the gut, was attempted.

In cases such as that before us, we are, moreover, of opinion that chemical treatments may hold an element of danger.

Barring the necessary filtration, the only treatment to which we have ever subjected the solution has been to shake it, previous to filtration with a suspension of aluminium hydroxide. The p_H of the liquid of this suspension was 7.3 by the indicator method, and it was considerably less alkaline, by the same test, than the glucose solution as removed from the gut. The positively charged particles of this suspension are remarkably efficient in clarification, and the filtrate of gut contents so treated may give no "ring" with Tanret's reagent, and in all cases it is perceptible with difficulty.

In many experiments, as evident in the tables, we have purposely omitted the alumina treatment, but though such omission often entailed resort to a more powerful light source than that of sodium flame, it was found to be absolutely without effect on the order of the polarimetric readings, provided that the filtrate was clear enough for good readings.

Procedure. Rabbits, starved 24 hours, anaesthetised by ether, and tracheotomised were employed. About 30 inches (live length) of small intestine, which had been washed free of debris by Ringer-Tyrode solution at 38°, were used, and from 20 to 30 cc. of glucose solution at 38° were run in, distention of the gut being avoided.

No loss of epithelium ever occurred, and, during the contact period, cloths, wrung out in warm saline solution, protected any part of the gut which was not enclosed within the abdominal cavity.

Usually the solution was allowed to lie in contact with the mucosa for five minutes, and if peristalsis was not marked, an occasional gentle manipulation was employed to bring fresh parts of the contents into contact with the epithelium.

Occasionally the solution was kept in the gut for ten minutes, but no difference was noted, so far as mutarotation is concerned, in such instances. The time of removal of the solution from the gut was "clocked," and the clock handed in to the observer in the dark room, as soon as the filtration and filling of the polarimeter tube were completed.

Filtration. Since clarity is a *sine qua non* in a solution, the polarimetry of which is to be reliable, particular attention was devoted to this matter. Clarity of filtrate has, however, in this matter, to be combined with speed of filtration, and the latter condition rules out many otherwise excellent methods. The Balston "filter-hat" used by Hewitt and Pryde is quick, but we found it often at fault in the matter of clarity of filtrate.

We have used a pair of Gooch crucibles, with asbestos mats, built up freshly for each experiment on a disc of filter paper, the two crucibles being in parallel on the suction system, and used contemporaneously.

The number of crucibles in parallel can, of course, be increased, but we found two sufficient for the purpose of getting enough filtrate in a short time. The suction system stood ready with a partial vacuum of about 400 mm. of

mercury, the filter-flasks being clamped off. Once the crucibles were filled with solution from the gut, release of the clamps at once set going the filtration.

In the filter-flasks were set graduated tubes to receive the filtrate, and, by this device, the moment at which enough filtrate had collected to fill a polarimeter tube was known, and no time was lost in unnecessary prolongation of the filtering process.

The polarimeter tube, filled with filtrate, and the clock giving the time at which the original solution left the gut, were at once handed in to the observer in the dark room.

The filtrates, when alumina cream is used, are "glass clear," and the Tanret protein test is negative or only just perceptible. As the glucose analyses were made on the contents of the polarimeter tubes, the slight dilution due to the use of the alumina cream was of no moment in the matter of comparison of direct and calculated readings.

We do not, however, pretend that all laevo-rotatory substances of intestinal origin added to the original glucose solution are removed by this elementary procedure. With good working asbestos mats we often got a first reading on the polarimeter in two minutes after removal of the solution from the gut, but the time which must elapse to collect a sufficient filtrate is necessarily variable, by the "build" of the mat, and the amount of the secretion which has occurred in the gut affecting the viscosity of the solution submitted to filtration. The times will be found in the tables of experiments.

Polarimetry. The instrument was a Schmidt and Haensch three-field of the Lippich type, reading direct to 0.01° , and either one or two decimeter tubes were used. The zero value of the tube (water-filled) was independently taken for each experiment. To preserve, as far as possible, the dark adaptation of the observer's eye the light for scale reading was cut down to the minimum practicable.

Though sodium light served well for the filtrate which had undergone alumina cream clarification, difficulties in sharp reading were often met with when this treatment was omitted. As already noted, we have purposely omitted the alumina treatment in some cases, though it has not in any way affected the results. While a good sample of asbestos mat will give a clear enough filtrate, without any alumina clarification, others may give a filtrate too opalescent for proper observation by sodium light.

As, at the time, we had no arrangement for intense mercury-green light we had in those cases to resort to light-filters, and a 100 c.p. "Pointolite" lamp. The filters used for the light were Wratten's M. screens. $A + B + E$ in conjunction. The abandonment of monochromatic light in such cases, while affecting the comparison of calculated and observed rotations, does not, of course, affect the question under examination, viz. whether mutarotation is or is not to be observed in the solutions shortly after removal from the gut.

Glucose solutions and analytical method. Hewitt and Pryde do not state whether the solutions introduced into the gut were simple aqueous solutions

of glucose or solutions in some type of saline liquid. Most of our experiments were done with simple aqueous solutions; occasionally the glucose was dissolved in Ringer-Tyrode solution, and, in one instance, acid sodium phosphate was added to an equilibrated glucose solution before use, to try the effect of keeping the gut contents on the acid side throughout the experiment.

The concentration of the solution subjected to polarimetry was estimated by the micro-method of Shaffer and Hartmann [1921]. The limited volume of solution available for analysis necessitated the use of a reliable micro-method so that as many individual analyses as possible might be effected for a representative mean value.

As some laevo-rotatory material is, by the nature of the experiment, always present in the mixture of glucose and intestinal secretion subjected to polarimetry, the rotation calculated from the analysis is likely to be greater than that observed. In nine cases out of the thirteen, where observations were made by sodium light, this has been found to be the case. In the experiments where the protein tests were most distinct, this difference between calculated and observed rotations was greatest.

Furthermore, in such cases the opalescence of the solutions was sometimes sufficient to impair the reliability of the polarimetric readings.

An instance, not included in the tables, is given of a series of readings from 4 to 25 minutes after removal of the solution from the gut, in which the "switch-back" character of the readings, due to absence of clarity, makes the observation valueless.

Minutes since removal from gut	4	5	6	7	8	9	10	11	12	13	14
Observed dextro-rotation in degrees	.85	.82	.80	.80	.80	.79	.78	.79	.80	.79	.77
	15	16	17	18	19	20	21	22	23	24	25
	.78	.78	.76	.76	.75	.77	.77	.80	.80	.80	.77

The erratic up-and-down variation of the readings in this instance, beyond the limits of the error of reading on a clear tube, is obviously due to the absence of certainty in such a case in adjustment of the field of the instrument. Any results of this type have been rejected.

Effects of alkalisisation of contents of polarimeter tube. The addition of a drop of alkali to the contents of the polarimeter tubes, results, with one or two exceptions, in a distinct diminution of the dextro-rotation. We found, invariably, that the greater the amount of protein in the filtrate, the greater the diminution on addition of alkali, and, the clearer the filtrate, the smaller the effect of alkali.

We have also observed that the addition of weak alkali (2 % NaOH) results in no change or in a slight increase. On the other hand, the addition of a drop of strong alkali (40 % NaOH) results in a diminution sometimes of 0.1° or more in a 1-decimeter tube. These filtrates were alkaline enough, from added

Table II. Screened "Pointolite" Readings.

Dextrose concentration introduced into gut. Treatment of filtrate etc.	Mean dextro-rotations in degrees at minute intervals after removal from gut. Readings from 3—25 minutes																				Dextro-rotations in degrees							
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	(1) After standing one hour	(2) After adding alkali	(3) Calculated from analysis	% dextrose from analysis of tube	No. of tube
1 % dextrose in Ringer-Tyrode. No alumina. Filter hat. 1 d. tube	.24	.23	.23	.23	—	.24	.24	.24	.24	.24	.24	.24	.24	.24	.25	.25	.25	.24	.25	.24	.25	.25	.24	.24	.20	.21	.396	12
1.5 % dextrose in Ringer-Tyrode. Alumina. Asbestos filters. 2 d. tube	—	.53	.56	.56	—	—	.59	.58	.55	.55	.55	.55	.55	.55	.55	.55	.55	—	—	.55	.55	—	.55	.57	.56	.60	1.148	14
2 % dextrose in water. No alumina. Asbestos filters. 1 d. tube	—	—	—	.78	.79	.78	.78	.76	.76	.76	—	—	—	—	—	—	—	—	—	—	—	—	.76	.78	.67	.74	1.409	16 (a)
Ditto	—	—	—	.63	.62	—	.64	.64	.64	.64	.63	—	—	—	—	—	—	—	—	.64	—	.64	.63	.63	.65	.77	1.458	15 (a)
1 % dextrose in water. No alumina. Asbestos filters. 2 d. tube	.96	.97	.97	.97	.96	.96	.96	.96	.96	.97	.96	.97	.96	.97	.98	.97	.98	.98	.97	.97	.97	.98	.97	.96	.94	1.10	1.048	6
Ditto	1.08	1.07	1.08	1.08	1.09	1.09	1.08	1.08	1.08	1.08	1.09	1.09	1.08	1.08	1.10	1.08	1.09	1.08	1.08	1.08	1.08	1.08	1.08	1.08	.99	1.18	1.124	8
1 % dextrose in water. No alumina. Filter hat. 2 d. tube	.90	.90	.90	.89	.88	.90	.91	—	.88	.89	.87	.87	.87	.87	.89	.89	.91	.89	.88	.88	—	—	.88	.88	.80	.99	.942	9
1.8 % dextrose in water. No alumina. Asbestos filters. 1 d. tube	—	.69	.69	.70	.69	.68	.70	.70	.69	.69	.71	.69	.69	.68	.69	.69	.69	.69	.69	.69	—	.68	.67	.64	.58	.75	1.42	29
2 % dextrose in water. Alumina. Asbestos filters. 2 d. tube	—	—	—	—	—	—	*	1.32	1.36	1.34	1.34	1.35	1.33	1.36	1.34	1.34	1.34	1.35	1.35	1.35	1.35	1.35	1.34	1.36	1.22	1.44	1.368	3
1 % dextrose in water. Alumina. Asbestos filters. 2 d. tube	—	—	.91	.91	.91	.91	.91	.91	.92	.91	.91	.90	.91	.90	.90	.91	.91	.91	.89	—	.91	.91	.90	.91	.86	1.00	.952	4
Ditto	—	.84	.84	.83	.85	.84	—	—	.81	.81	.81	.81	.81	.81	.81	.80	.80	.81	.81	.82	.80	—	.80	.81	.74	.88	.84	5
2 % dextrose in water. No alumina. Gut washed with saline. Asbestos filters. 1 d. tube	—	—	—	.78	.78	.78	.77	.78	.78	.78	.79	.78	.78	.78	.79	.79	.79	—	—	—	.79	.79	.78	.79	.72	.87	1.65	22
2 % dextrose in water. No alumina. Asbestos filters. 1 d. tube	.82	.81	.80	.79	.79	.79	.80	.79	.80	.79	.80	.80	.79	.80	.79	.79	.79	.80	.80	.79	.79	.79	.80	.80	.69	.91	1.74	31

* Clogged filter.

intestinal secretion, to turn litmus paper blue before addition of the alkali. The same drop in rotation can be got by adding hydrochloric acid. An example of the effect of weak alkali can be seen in the Expts. 13, Table I, and 15 *a*, Table II, while the others all show the effect of a drop of the strong alkali.

These observations lead us to believe that this drop in rotation is due to the protein forming salts with alkali or acid, these salts being more laevo-rotatory than the natural protein—an observation made, among other workers, by Pauli [1914].

REMARKS.

It is evident that, so far as polarimetric evidence goes, our experiments give no support to the idea that a disturbance of equilibrium associated with the formation of a more reactive type of sugar occurs as a result of contact of a stabilised glucose solution with the mucosa of the small intestine of the rabbit.

It appears to us that, even if some reactive type were formed, it is very unlikely that evidence of its production would be obtained by the method adopted by Hewitt and Pryde.

Not only would one expect the special type of sugar formed to be at once taken up into the blood-stream, if the transformation was a necessary preliminary to absorption, and so to be removed from the solution used for experimental observation, but, even if traces of the special type still remained in the solution withdrawn from the gut, its existence in unstable form is likely to be momentary.

According to Irvine [1923] the gamma types of both glucose and fructose revert *instantaneously* to the stable form even in the presence of 0.1 % HCl.

Aqueous solutions of glucose which have been in contact with the gut wall for 5 minutes are always alkaline, so far as our experience has gone.

To prove the thesis that a reactive type is formed, some method of condensation to a stable derivative would appear to be necessary for, as Irvine remarks, "there is little prospect of detecting these fugitive isomerides by ordinary processes."

To effect such a "fixation" within the organism, preliminary to extraction and investigation, seems to be the problem before the searcher for evidence of the transitory presence of reactive types of sugar in metabolism.

CONCLUSION.

The experiment of Hewitt and Pryde has been repeated thirty-four times. In eight cases the solutions obtained from the gut had to be discarded as not clear enough for good polarimetry.

In the remaining twenty-six experiments in no single instance have we seen any sign of mutarotation in the earlier minutes after removal of the solution from the gut.

The rotation has remained steady within the observer's known error of adjustment of the field of the polarimeter on ordinary stable solutions of glucose in water.

REFERENCES.

- Hewitt and Pryde (1920). *Biochem. J.* **14**, 395.
Irvine (1923). *J. Chem. Soc.* **123**, 919.
Pauli (1914). *Biochem. Z.* **59**, 470.
Shaffer and Hartmann (1921). *J. Biol. Chem.* **45**, 377.