XCV. STUDIES IN THE METABOLISM OF TISSUES GROWING *IN VITRO*.

IV. EFFECT OF FRUCTOSE, GALACTOSE AND XYLOSE UPON THE AMMONIA AND UREA PRODUCTION OF EMBRYO KIDNEY TISSUE.

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PREVIOUS work has shown that by using the tissue culture technique the production of ammonia and urea by growing and non-growing cultures can be estimated. By comparison with controls it has been found that the growing cultures produce considerable amounts of these substances whereas the non-growing ones do not [Holmes and Watchorn, 1927], and that the presence of glucose, while favouring the growth of the tissue, inhibits the production of ammonia and urea during this growth [Watchorn and Holmes, 1927]. It seemed that it might be of interest to compare the effect of other sugars with that of glucose, in order to determine whether the utilisation of a carbohydrate necessarily has this "protein sparing" effect, or whether it depends upon the type of carbohydrate utilised and thus upon the method of breakdown.

The use of the tissue culture method has much to recommend it for work of this type. In the first place, it is valuable to know that one is dealing with the metabolism of growth and activity, and not with autolytic processes, or with the metabolism of cells surviving under conditions which are likely to upset its normal balance. Even in tissue culture the conditions are unnatural, but they are not sufficiently so to prevent the continuation of growth in the embryonic tissue taken. By noting the effect upon this growth of various substances added to the medium and utilised by the cells, it is possible to judge whether the breakdown of these substances is favourable or unfavourable to the activity of the tissue. Thus, in the earlier work with glucose, we found that the presence of the sugar prevented the ammonia and urea formation which is otherwise characteristic of growth, and also that this did not inhibit the growth, but encouraged it.

EXPERIMENTAL.

As in previous work embryonic rat kidney tissue has been used, and the details of technique have been the same as those previously described [Holmes

and Watchorn, 1927, 1929; Watchorn and Holmes, 1927]. The cultures were made in pyrex flasks, and the conditions of culture of the growing and non-growing tissues were identical, except that the growing fragments were allowed to rest upon very fine wisps of cotton-wool, while in the non-growing preparations the fragments were floated in a shallow layer of the medium. It is known that fragments floating freely in a medium do not grow to any appreciable extent. When necessary the system of controls described in our first paper was used, but in the experiments dealing with ammonia and urea production it was usually sufficient to compare the growing tissues with the non-growing ones, as these latter never formed any ammonia or urea and therefore gave exactly the same result as the controls.

At least two kidneys were used in each flask, and in order to obtain large enough differences, it was necessary to use four when the utilisation of carbohydrate was estimated. As it was obviously important to use only kidneys taken from the same litter throughout each experiment, the number of preparations that could be set up each time was limited by the size of the litter. When the nitrogen metabolism of cultures with and without sugar was being compared, the media were both made from the same embryo extract and were equally diluted, in the one case with Ringer's solution containing the carbohydrate and in the other with plain Ringer's solution. 2 cc. of the medium were used in each flask, and the cultures were incubated for 2 days.

The solutions of carbohydrate were sterilised by passing through a filter candle. We are inclined to believe that the method of sterilisation of the carbohydrate is important, and that after sterilisation by steaming there is likely to be a bigger disappearance of a sugar in culture than when the unheated sugar is used. The large breakdown of glucose observed in our earlier work may have been due to the fact that the medium was sterilised by heat. This idea is born out to some extent by an experiment with adult rat kidney tissue, in which the disappearance of steamed and unsteamed glucose in the presence of the tissue was estimated. It was found that there was a larger disappearance of the sugar which had been steamed. The sugar may or may not have been broken down by the tissue. Two or three years ago, one of us (B.E.H.) attempted in collaboration with A. Pirie to repeat the work of Borsook and Wasteneys [1925] in which they showed that glucose in the presence of an amino-acid and phosphate could reduce methylene blue under anaerobic conditions. We found that we could not repeat this unless we used, as did Borsook and Wasteneys themselves, glucose which had been sterilised by heating. With fresh glucose no reduction of the methylene blue was obtained. In these tissue culture experiments, therefore, it seemed safer to avoid complications by using filtered instead of steamed solutions.

Other details of technique will be found in earlier papers.

Growth¹ of tissue.

In most series one or more small control dishes were included, containing small amounts of tissue growing in the same medium as that used in the experimental flasks. This tissue could be stained and used for microscopical purposes. We have not adopted any accurate method for estimating growth, but the presence of fructose, galactose, and even xylose seemed to have an unmistakably favourable effect upon the growth of the tissues. This was particularly noticeable when the growth without added sugar was poor. From this it seemed almost certain that each of these sugars was utilised by the tissue, and we found later that definite amounts of the carbohydrate disappeared during the course of the experiment.

Effect on production of ammonia and urea.

Fructose behaved like glucose in that it inhibited either partially or completely the production of ammonia and urea by growing tissue in spite of the increased growth promoted by the sugar. In the presence either of glucose [Watchorn and Holmes, 1927] or of fructose (Table I) there was sometimes an actual disappearance of the urea already present, and as this did not appear as extra ammonia it seems possible that it was used by the growing tissue.

Table I. *Effect of fructose upon ammonia and urea production.*

Exp. no.	Non-growing tissue		Growing tissue		Non-growing tissue + fructose		Growing tissue + fructose		Increase in $\text{NH}_3\text{-N} + \text{urea-N}$		Effect of fructose
	$\text{NH}_3\text{-N}$ (mg.)	$\text{NH}_3\text{-N} + \text{urea-N}$ (mg.)	$\text{NH}_3\text{-N}$ (mg.)	$\text{NH}_3\text{-N} + \text{urea-N}$ (mg.)	$\text{NH}_3\text{-N}$ (mg.)	$\text{NH}_3\text{-N} + \text{urea-N}$ (mg.)	$\text{NH}_3\text{-N}$ (mg.)	$\text{NH}_3\text{-N} + \text{urea-N}$ (mg.)	Without fructose	With fructose	
									(mg.)	(mg.)	
80	0.034	0.051	*0.040 0.044	0.070 0.065	0.030	0.058	0.030 0.033	0.066 0.061	0.019 0.014	0.008 Nil	Inhibition
81	—	0.056	—	0.118 0.092	—	0.055	0.036	0.081	0.062 0.036	0.026	Partial inhibition
82	0.031	0.060	0.038	0.071	0.030	0.062	0.030	0.055	0.011	Nil	Inhibition
84	0.040	0.097	0.059 0.050	0.163 0.117	0.040	0.081	0.057 0.040	0.077 0.088	0.066 0.020	0.016 0.007	Partial inhibition
86	0.043	0.066	0.060	0.105 0.072	0.039	0.067	0.043	0.054 0.056	0.039 Nil	Nil Nil	Inhibition (possible urea utilisation)

* Where two sets of figures are given, these refer to two separate preparations in the same experimental series, the control therefore being the same for both.

Quite unlike the results obtained with fructose and xylose those with galactose were markedly irregular. This irregularity persisted, although the experiments were repeated a great many times. Sometimes the production of ammonia and urea was inhibited, sometimes it was unaffected or even slightly increased (the increase probably being due to the relatively greater growth). Perhaps there is more than one path of galactose breakdown possible in the cultures, and the effect upon the nitrogen metabolism depends upon the path taken. It is certain, however, that the presence of galactose could

¹ By "growth" we mean increase of area, which is due to wandering of cells as well as to actual cell division.

at times have an inhibitory effect upon the formation of ammonia and urea, and when concentrations of sugar as high as 0.2 % or more were used, this inhibition was likely to be found in the majority of cases. Some results are given in Table II.

Table II. *Effect of galactose upon ammonia and urea production.*

Exp. no.	Non-growing tissue		Growing tissue		Non-growing tissue + galactose		Growing tissue + galactose		Increase in NH ₃ -N + urea-N			Effect of galactose
	NH ₃ -N + urea-N		NH ₃ -N + urea-N		NH ₃ -N + urea-N		NH ₃ -N + urea-N		Without galactose (mg.)	With galactose (mg.)		
	NH ₃ -N (mg.)	urea-N (mg.)	NH ₃ -N (mg.)	urea-N (mg.)	NH ₃ -N (mg.)	urea-N (mg.)	NH ₃ -N (mg.)	urea-N (mg.)				
76	0.030	0.045	0.024	0.039	0.029	0.043	0.037	0.072	Nil	0.029	Increase	
79	0.030	0.045			0.026	0.045	0.025	0.036		Nil	No effect	
	0.032	0.063	0.055	0.073	0.032	0.063	0.039	0.073	0.010	0.010	No inhibition	
			0.055	0.088			0.025	0.056	0.025	Nil	Inhibition	
			0.045	0.074			0.037	0.056	0.011	Nil	Inhibition	
90	0.057	0.090	0.089	0.136	0.062	0.116	0.062	0.118	0.046	Nil	Inhibition	
91	—	0.083	—	0.084	—	0.085	—	0.112	Nil	0.027	Increase	
93	0.074	0.135	0.098	0.172	0.055	0.103	0.095	0.115	0.037	0.052	Increase	
98	0.029	0.083	0.041	0.099	0.029	0.083	0.036	0.090	0.016	0.007	Partial inhibition	
Higher concentrations of galactose (approx. 0.2 %).												
118	—	0.108	—	0.123	—	0.108	—	0.103	0.015	Nil	Inhibition	
								0.033		0.025	Small increase	
119	—	0.088	—	0.136	—	0.097	—	0.090	0.048	Nil	Inhibition	

In no case have we found any inhibition of ammonia or urea formation by xylose, although there seems to be very little doubt that the tissue can cause this sugar to disappear from the medium. Xylose therefore is an interesting case of a carbohydrate which is in some way utilised without affecting the nitrogen metabolism of the tissue as measured by the production of these substances (Table III).

Table III. *Effect of xylose (0.2 %) upon ammonia and urea production.*

Exp. no.	Non-growing tissue		Growing tissue		Non-growing tissue + xylose		Growing tissue + xylose		Increase in NH ₃ -N + urea-N		Effect of xylose
	NH ₃ -N (mg.)	NH ₃ -N + urea-N (mg.)	NH ₃ -N (mg.)	NH ₃ -N + urea-N (mg.)	NH ₃ -N (mg.)	NH ₃ -N + urea-N (mg.)	NH ₃ -N (mg.)	NH ₃ -N + urea-N (mg.)	Without xylose (mg.)	With xylose (mg.)	
99	0.032	0.091	0.049	0.119	0.040	0.097	0.052	0.121	0.028	0.024	No inhibition
							0.055	0.121		0.024	
101	0.026	0.056	0.030	0.078	0.036	0.066	0.040	0.100	0.022	0.034	Slight increase
			0.035	0.082					0.026		
102	0.024	0.048	0.031	0.067	0.030	0.063	0.045	0.079	0.019	0.016	No inhibition
120	—	0.070	—	0.113	—	0.080	—	0.129	0.043	0.049	No inhibition
121	—	0.102	—	0.135	—	0.083	—	0.114	0.033	0.041	No inhibition

Disappearance of the sugars from the medium.

Apart from the added sugar the medium contained very little reducing substance. This is to be expected, since it was made from chopped embryo tissue which had already been once washed with Ringer's solution, and was finally diluted with twice its volume of Ringer's solution. The amount of reducing substance was about 0.1 mg. to 0.15 mg. in each culture. This is

unimportant when compared with the amount of sugar added (2.0 mg. approximately in each culture). We did not find that it changed appreciably during the experiment, and in any case it was not sufficient to account for the changes observed. We therefore ignored the reducing substances already present, and estimated the added sugar as reducing substance. The small amount of protein present was removed with colloidal ferric hydroxide, and the sugar estimated by the method of Hagedorn and Jensen. In order to obtain larger percentage differences we have made the final concentration 0.1 % instead of the 0.2 % used in most of the other experiments.

There seemed to be very little doubt that all the sugars added, fructose, galactose and xylose, could be utilised by the tissue, and it will be seen that the non-growing generally used less than the growing tissue. The control was a culture which had been kept in the ice-chest throughout the experimental period (Tables IV, V, VI).

Table IV. *Utilisation of fructose (estimated as glucose).*

Exp. no.	Control at 0° (mg.)	Non-growing tissue (mg.)	Growing tissue (mg.)	Control - growing tissue (mg.)
114	1.61	(1) 1.25 (2) 1.23	(1) 1.23 (2) 1.20	(1) 0.38 (2) 0.41
110	1.38	1.22	1.10	0.28
109	3.52*	(1) 3.20	(1) 3.23	(1) 0.29

* Very little tissue used.

It is interesting to find a small but definite disappearance of fructose, as it has been suggested [Campbell and Markowitz, 1927] that fructose is not utilised except by the liver.

Table V. *Utilisation of galactose (estimated as glucose).*

Exp. no.	Control at 0° (mg.)	Non-growing tissue (mg.)	Growing tissue (mg.)	Control - growing tissue (mg.)
115	1.39	1.02	(1) 1.06 (2) 0.93	(1) 0.33 (2) 0.46
116	1.35	0.99	0.67	0.68*

* Grew for 3 days.

It has been shown that galactose can give rise to lactic acid [Wierzechowski and Laniewski, 1931] and can raise the respiratory quotient and the heat production of dogs [Wierzechowski, 1931], while Sherif and E. G. Holmes [1930] have observed an increased oxygen uptake with mammalian nerve in the presence of galactose. It is perhaps worth noting that on one occasion the rise in blood- and urine-lactic acid found by Wierzechowski and Laniewski was much greater than in the other two experiments; some such irregularity may explain the lack of consistency in the effect of galactose on nitrogen metabolism in our experiments.

Table VI. *Utilisation of xylose (estimated as glucose).*

Exp. no.	Control at 0° (mg.)	Non-growing tissue (mg.)	Growing tissue (mg.)	Control - growing tissue (mg.)
113	2.03	—	1.70	0.33
106	1.81*	1.77	1.73	0.08
122	2.00†	1.60	1.46	0.54
108	1.99	1.77	1.64	0.35

* Very little tissue used.

† In this experiment the sugar was estimated by McCance's [1926] method for pentoses, and we are glad to have this opportunity of thanking him for help.

McCance and Madders [1930] showed that xylose was retained in the tissues after injection or absorption from the intestine, and Russell [1923] showed that pentoses were oxidised by tumour tissues, but that normal nerve tissues, including kidney, cannot oxidise them. The only exception was liver, which oxidised arabinose. He did not however use any embryonic tissue, and we think that the fact that we used embryonic tissue in an active condition influenced the results with xylose and with the other sugars.

SUMMARY.

1. The tissue culture method has been used to test on embryo kidney tissue the "protein sparing" action of fructose, galactose and xylose.
2. Each of these sugars tends to increase the growth of the tissue and each of them is utilised by the tissue, but while fructose constantly inhibits the production of ammonia and urea by the growing cells, galactose does so only irregularly and xylose not at all.

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