ON THE FORMATION OF GLYCOGEN IN THE ARTIFICIALLY PERFUSED LIVER.

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LUCHSINGER was the first, and, as far as I can ascertain, until quite recently the only observer to study the question of glycogen formation in the liver by perfusion of this organ[1]. He found an increase of glycogen in the liver after perfusing blood which contained 2 % of added dextrose. Recently Kraus has used the perfusion method on the liver[2] in some experiments which he undertook with the object of studying the influence of peptone on sugar formation.

My first experiments, which were conducted upon cats, were all performed in the following way:—The animal was anæsthetised, the abdomen opened and threads passed under the portal vein in readiness for the introduction of the cannula. The animal was next bled from the carotid artery, its blood whipped and then mixed with fresh defibrinated sheep's blood and the whole filtered. No laking occurred, the corpuscles appearing quite normal microscopically. While the blood was being prepared by an assistant, a cannula was quickly tied in the portal vein, and the thorax having been opened a second cannula was tied in the inferior cava immediately above the diaphragm. The liver was then perfused with blood, using Brodie's[3] perfusion apparatus. The pressure employed varied from 20 to 40 mm. of mercury in the different experiments.

The first blood which issued from the inferior cava was collected separately, whipped, filtered, and then returned to the main receiver. As soon as the artificial circulation was established, 30 grms. of blood were drawn off, and after weighing, at once thrown into alcohol. This

[1] *Physiologie und Pathologie des Glykogens.* Inaug. Diss. Zurich, 1875.

[2] *Pflüger's Archiv*, xc. p. 630. 1902.

[3] Brodie. This number of the *Journal*, p. 266.

was used for the determination of the sugar, which was effected by Pavy's[1] method.

In the early experiments nothing was added to the blood, and the sugar was determined before and after the perfusion with the object of ascertaining whether the liver cells remained alive and in a normal state with respect to their glycogen metabolism. If much sugar were found in the blood after perfusion, this would indicate that the cells were no longer normal, or even that some or all of them were dead.

Although the various stages of the operation were performed as quickly as possible, some minutes necessarily elapsed between the bleeding of the animal and the commencement of the perfusion, and it was doubtful whether the cells would be found in a completely normal state after this break in their blood-supply.

Asp ascertained that the liver was still able to secrete bile after it had been cut off from its blood-supply for ten minutes[2], but, as will be seen presently, the liver seems to retain its secreting power for a longer time than that of forming glycogen. In fact, post-mortem sugar formation in the liver apparently begins very soon after it has been cut off from its blood supply. This is clearly evidenced by the results given in the following table, in which the figures for the dextrose are in all cases given in parts per thousand:—

		1	2	3	4	5
		Amt. of sugar in blood before perfusion	Amt. after perfusion	Amt. in blood kept at same temp. during perfusion time	Amt. of added sugar	Duration of perfusion
Exp. 1.	15. xi. 00	1·67	5·85	—	—	2 hrs.
,, 2.	16. xi. 00	1·60	3·86	—	—	2 hrs.
,, 3.	21. xi. 00	1·22	2·05	0·90	—	2 hrs. 15 mins.
,, 4.	22. xi. 00	1·00	3·50	0·86	—	2 hrs. 50 mins.
,, 5.	6. xii. 00	4·50	6·30	4·10	5·00	2 hrs.
,, 6.	7. xii. 00	5·30	4·50	5·00	5·00	2 hrs.

From this it is seen that in all the experiments, with the exception of the last, the amount of sugar was found higher after perfusion. The condition of the liver in these early experiments was approximately the same in all: it kept its normal appearance for a short time, about twenty minutes, except that it always looked rather darker than normal. After some time it gradually became harder, darker and œdematous.

[1] Pavy. This *Journal*, xxiv. p. 479.

[2] *Arbeiten aus dem physiolog. Institut zu Leipzig*, 1873, p. 470.

In one experiment, special attention was paid to the gall-bladder, which had been entirely emptied before perfusion was started. After the perfusion it was tightly distended with bile which was not mixed with blood, as was ascertained by microscopical examination.

The general results not proving satisfactory the experiments were modified. Two of the most likely sources of error being the anæsthetic and the delay in establishing the artificial circulation, the experiment was performed in the following way:—a large supply of blood having been obtained and placed in the receiver, the animal was killed by pithing, the abdomen opened and a cannula quickly tied in the portal vein and the circulation at once started. The inferior vena cava was then cannularised and the heart and lungs removed. Before the perfusion was started a small lobe of the liver was ligatured and cut away for the determination of the glycogen, which was effected in the following manner, and is a slight modification of the method described by Pavy[1].

The weighed portion of liver was broken down in a mortar with a little alcohol and then brought into about 300 c.c. of methylated spirit, stirred and allowed to stand over night. The beaker was then heated in a water-bath and thoroughly boiled, the alcohol decanted off through linen and the residue squeezed and rubbed down in a mortar to a fine powder. Extraction with alcohol was then twice repeated. The mixed alcoholic extracts were distilled under diminished pressure and treated in the same way as a blood extract. The residue was boiled for half-an-hour under a reflux condenser with 50 c.c. of a 10 % solution of potash and then poured into three to four times its volume of alcohol, the flask being well washed with alcohol. The precipitated glycogen was then collected on an asbestos filter, and washed into a boiling flask with hot 2 % hydrochloric acid, using about 50 to 60 c.c. The glycogen was hydrolysed by boiling for an hour and a-half in a reflux condenser. The liquid was then cooled, neutralised and made up to 100 c.c., filtered and titrated.

The results are given in the following experiments:—

Exp. 7. 9. i. 01. Cat. Sheep's blood to which dextrose (approximately 1 %) had been added was used.

Amount of blood-sugar.	Before perfusion		0·48 %
,, ,,	After ,,		0·69
,, glycogen in the liver expressed as glucose.		Before perfusion	3·6
,, ,, ,, ,, ,,		After ,,	2·04

[1] Pavy. *Physiol. of Carbohydrates*, p. 63.

Exp. 8. 10. i. 01. Cat. Sheep's blood to which dextrose was added (approximately 1 %). Perfusion at a pressure of 25 mm. Hg. Small lobe of liver tied and cut off before perfusion.

Amount of glycogen before perfusion	2·9 %
,, ,, after ,,	1·4

Exp. 9. 11. i. 01. Cat. To the blood dextrose was added = 1 %.

Amount of glycogen before perfusion	2·31 %
,, ,, after ,,	0·98

Exp. 10. 17. 1. 01. Cat, anæsthetised with A.C.E. Abdomen opened and cannula tied in portal vein. Thorax opened and cannula tied into inferior cava. The artificial circulation started. There elapsed only a very short interval between the stoppage of the normal blood-flow and the beginning of the perfusion. To the blood used, dextrose was added (approximately to the amount of 1 %). As soon as the artificial circulation was started a small lobe of the liver was tied and cut off. The result of the experiment was as follows:

Amount of sugar found in liver. Before perfusion	1·64 %
,, glycogen ,, ,, (determined as glucose). Before perfusion	1·50
Total amount of carbohydrate before perfusion	3·14
Amount of sugar found in liver after perfusion	1·42 %
,, glycogen ,, ,,	2·27
Total amount of carbohydrate after perfusion	3·69

This result distinctly showed the possibility of conducting an experiment by the perfusion method in which the liver would retain its normal function so far as glycogen formation was concerned. The reason why a better result was obtained in this last experiment was, apparently, because there was much less delay in starting the artificial perfusion than in any of the preceding, consequently the following modification in the method was made and ultimately adhered to. A cat was anæsthetised with A.C.E., the abdomen opened in the middle line and a cannula tied in the splenic vein and connected to the perfusion apparatus, while a ligature was passed under the portal vein below the entrance of the splenic vein, but left untied. A small lobe of the liver was then ligatured and cut off, weighed and put into alcohol for analysis. Next the thorax was opened and a cannula quickly tied into the inferior vena cava; artificial circulation was started at once and at the same time the ligature round the portal vein was tied. The heart and lungs were then removed and a cannula tied into the aorta to collect any blood that escaped through it. In this way scarcely any time elapsed between the moment the normal blood-supply to the liver was stopped, and that at which the perfusion began. The artificial

circulation was maintained at a pressure of 20 to 30 mm. of mercury. The results obtained in a perfusion lasting 2 hours were as follows:—

Amount of sugar found in the liver before perfusion	0·89 %
,, glycogen ,, ,,	1·58
Total amount of carbohydrate	2·47
Amount of sugar found in the liver after perfusion	0·80 %
,, glycogen ,, ,,	2·38
Total amount of carbohydrate	3·18

The results of similar experiments are given in the following protocols:

23. i. 01. Cat. Duration of perfusion 2 hours.

Amount of sugar found in liver before perfusion	1·04 %
,, glycogen ,, ,,	2·07
Total amount before	3·11
Amount of sugar found in liver after perfusion	1·19 %
,, glycogen ,, ,,	2·78
Total amount after	3·97

24. i. 01. Cat. Duration of perfusion 2½ hours.

Amount of sugar found in liver before perfusion	1·23 %
,, glycogen ,, ,,	0·46
Total amount before	1·69
Amount of sugar found in liver after perfusion	1·44 %
,, glycogen ,, ,,	1·73
Total amount after	3·17

31. i. 02. Cat. Duration of perfusion 2 hours.

Amount of sugar found in liver before perfusion	1·89 %
,, glycogen ,, ,,	0·74
Total amount before	2·63
Amount of sugar found in liver after perfusion	1·40 %
,, glycogen ,, ,,	2·04
Total amount after	3·44

Conclusion.

The results, thus far, show that in a properly conducted perfusion the liver retains the power, which it is well known it possesses in life, of forming glycogen from dextrose. The method can therefore be

adopted for studying many important points in glycogen formation, and I hope to publish further results shortly. I must point out that the experiments thus far described suffer from one great disadvantage, viz.,— that a foreign animal's blood was in all instances used, a condition, which, as pointed out by Brodie from other perfusion experiments, possesses great disadvantages. In some future experiments I hope to avoid this source of error.

I am much indebted to Dr Brodie, at whose suggestion the work was undertaken, for much help given and suggestions made while carrying out these experiments.