The Distribution of Glycogen in the Liver of Rabbits

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Attention has recently been called by Gomori & Goldner (1947) to a strikingly uneven distribution of glycogen in the liver of rabbits. According to these authors, variations exceeding several 100 % may be observed in simultaneous biopsies taken from the same or from different liver lobes.

In the present investigation, a survey of the distribution of liver glycogen has been carried out on rabbits submitted to a variety of experimental conditions. It has not been possible to confirm Gomori & Goldner's (1947) observations. Only relatively small intralobar and interlobar differences were observed, not exceeding those described in other animal species.

Only the distribution of glycogen will be considered here. The effects of the various experimental conditions on the glycogen content will be described elsewhere.

METHODS

Ten rabbits were chosen for the survey by sampling a very heterogeneous population, including fed and fasting (24 hr.) animals, and fasted animals injected with anterior pituitary extract, or with glucose + insulin or with both (two animals in each group). Their liver glycogen content varied between 0.32 and 8.55 %.

The animals were killed by intravenous injection of 500 mg. sodium-Evipan. Samples weighing approximately 100 mg. were taken immediately after death and immersed in tared centrifuge tubes containing 2 ml. of 30 % (w/v) KOH. They were analysed according to Good, Kramer & Somogyi (1933), the final glucose determination being made by the method of Nelson (1944).

The sampling was made on the three main lobes of the liver: 1=right lobe (+gall-bladder), 2=middle lobe, 3=left lobe. Three samples were taken from distant sites in each lobe. Three contiguous samples were further taken from one of the lobes for estimation of the error of determination.

Interlobar and intralobar variations were determined statistically by making a variance analysis of the results (Mather, 1946). In order to homogenize the variances from animals which were not immediately comparable, the analysis was made on the logarithms of the results, using natural logarithms. An estimation of the relative variations of the glycogen content throughout the liver was thereby obtained. It may be pointed out that the absolute differences between comparable results were found to increase with increasing glycogen contents, whereas the relative differences remained approximately constant.

RESULTS

The results of the variance analysis are summarized in Table 1.

Table 1. Variance analysis of distribution of liver glycogen

Origin of variance	Degrees of freedom	Sum of squares	Mean square	P	Relative S.D.* (%)
Rabbits + treatment	9	36.9722	—	—	—
Lobes	20	0.2558	0.0128	<0.001	8.6
Position in lobe	60	0.2332	0.0039	<0.001	6.4
Analytical procedure†	60	0.0692	0.00115	—	3.45

* Gives the equivalent of the logarithmic standard deviation from the mean, in percentage of the mean.

† Since only one of the nine determinations made on each animal was done in triplicate, the estimation of this factor could not be included in the general variance analysis, but was effected separately.

For the purpose of illustration, the results of the two experiments in which the largest discrepancies were observed have been recorded fully in Table 2.

Table 2. Distribution of liver glycogen

Rabbit no.	Treat-ment	Lobe	Glycogen content (g./100 g. wet wt.)				Mean of lobe	Mean of liver
			Site A	Site B	Site C			
8	Fasting (24 hr.)	1	1.34	1.00	1.21	1.18	1.24	
		2	1.40 1.35 1.20	1.32	1.29	1.22		
		3	1.24	1.13	1.39	1.25		
9	Fed	1	5.70	5.10	4.76	5.19	5.24	
		2	5.10 5.51 5.60	5.40	6.35	5.71		
		3	4.71	4.52	4.93	4.72		

(a) Error of determination. This includes possible local differences between contiguous pieces of liver tissue. As is shown in Table 1, the standard deviation

from the mean of three results obtained on contiguous samples amounts to 3.45 % of the mean value.

(b) *Intralobar distribution.* When three pieces of liver are taken from distant sites in the same lobe, the variations are significantly larger than those arising only from determination errors (relative s.d. = 6.4 %, $P < 0.001$). These variations are, however, much smaller than those described by Gomori & Goldner (1947). It may be calculated that the probability of finding a local glycogen content more than 16 % higher or lower than the average content of the lobe is smaller than 0.01. Out of the sixty determinations of this kind which have been made, none shows a deviation of that magnitude and only three deviate more than 10 % from the mean lobar value, 10, 14.4 and 15.5 % respectively.

(c) *Interlobar distribution.* Interlobar variations are significantly higher than the intralobar ones (relative s.d. = 8.6 %, $P < 0.001$), but here again the differences are not very important quantitatively. It may be computed from the data presented that a single glycogen determination, performed on 100 mg. of hepatic tissue, would, under the conditions of our experiments, be representative of the average glycogen content of the whole liver with a standard error of 8.6 %. The probability that the result obtained would exceed the average content by more than 25 % is smaller than 0.01.

Closer analysis of the individual results shows that the heterogeneity of the lobar distribution is almost entirely due to the middle lobe (lobe 2), whose glycogen content is systematically higher than those of the two others, which are usually in close agreement (average increase for lobe 2: 5.9 %; limits for $P = 0.01: 2.4$ and 9.5 %).

(d) *Individual differences and differences due to treatment.* It should be mentioned here that the results were first analysed separately for each individual rabbit and then by groups of two similarly treated animals. Comparison of the results of the

individual and group analyses showed that the relative distribution of glycogen in the liver was not significantly affected either by individual differences nor by differences due to the treatment. A bulk analysis of the homogenized variances of all the animals was thereby justified. Only the results of this analysis have been given in Table 1.

DISCUSSION

In view of the data presented here, there seems to be no reason to exclude the rabbit from studies involving determinations of liver glycogen. An analysis performed on three pieces taken at random in the three lobes of the liver should furnish a very satisfactory estimation of the average glycogen content of that organ.

It is difficult to reconcile these results with those of Gomori & Goldner (1947). However, the conclusions of these authors are partly based on histological evidence, and one may wonder to what extent microscopic heterogeneity is liable to affect the average glycogen content of a macroscopic sample of tissue.

SUMMARY

1. The distribution of glycogen in the liver of rabbits subjected to a variety of experimental conditions has been studied chemically and the results analysed statistically.

2. The distribution in the same lobe and in different lobes has been found to be significantly, though moderately, heterogeneous.

3. The relative s.d. of a single determination with respect to the average glycogen content of the whole liver was found to be 8.6 % under the conditions of our experiments.

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