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Metabolic Consequences of Portacaval Shunting in the Rat*

Effects on Weight, Food Intake, Intestinal Absorption and Hepatic Morphology

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Summary. In order to study systematically the physiologic significance of blood from the gastrointestinal tract reaching most tissues *indirectly*, after prior exposure to the liver, end-to-side portacaval shunting was carried out in rats. A technique derived from that described by Lee and Fisher was evaluated in more than 140 animals, first as to general applicability and its effects on overall health, body weight, food intake, intestinal absorption and hepatic morphology. In the hands of the authors, and provided that all animals where any doubt as to the patency of the shunt arises during operation are immediately discarded, the procedure used proved reproducible and satisfactory. Specifically, there was good evidence for continued shunt patency for many months postoperatively, while there was no evidence for portal hypertension, for significant collateral circulatory bypass of the shunt, or for hepatic pathology, although liver weight was diminished by about one third, as expected. After an initial loss of 5 to 10% in body weight during the first 2 weeks postoperatively, weight gain resumed and paralleled that in the sham-operated and control animals. The initial weight loss resulted from decreased food intake, not from decreased absorption of one or several components of the diet. The importance of precisely controlled environmental conditions for achieving such limited initial weight loss with prompt resumption of weight gain and good general health has been stressed. The operative procedure used and the conditions selected for the subsequent maintenance of the portacaval shunted animals would thus appear well-suited to the systematic and more detailed analysis of the metabolic and endocrine consequences of portacaval shunting in the rat.

Key-Words: Portacaval shunting — Liver and glucose tolerance — Liver and insulin — Intestinal hormones and insulin release.

Zusammenfassung. Die vorliegende Arbeit schafft die Grundlagen für eine systematische Untersuchung der physiologischen Bedeutung der Gegebenheit, daß aus dem gastrointestinalen Trakt stammendes Blut die meisten Gewebe *indirekt*,

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erst nach Filtrierung in der Leber erreicht. Hierzu wurden Ratten mit Porta cava-Anastomosen benutzt, wobei vorerst über 140 Tiere dazu dienten, die technische Durchführbarkeit der Operation sowie den allgemeinen Gesundheitszustand der über viele Monate beobachteten operierten Tiere zu beurteilen. Vorausgesetzt, daß wirklich alle Tiere, bei denen während der Operation Zweifel an der Funktionstüchtigkeit der Anastomose entstehen, sofort verworfen werden, erwies sich das Vorgehen als befriedigend. Insbesondere konnte die gute Funktion der Anastomose über viele Monate dargestellt werden, während keine Hinweise auf portale Hypertonie, auf das Entstehen einer collateralen Zirkulation oder irgendwelche morphologisch erfaßbare Leberpathologie erkannt werden konnten. Das Lebergewicht war, wie zu erwarten, um etwa ein Drittel vermindert. Während der ersten 2 Wochen nach der Operation nahm das Körpergewicht um 5—10% ab, Gewichtszunahme stellte sich hiernach wieder ein, und zwar etwa parallel der Gewichtszunahme von leer-operierten oder Kontrolltieren. Die anfängliche Gewichtsabnahme ließ sich auf verminderte Nahrungszufuhr, ohne verminderte Resorption zurückführen. Genau kontrollierte Bedingungen der Haltung der operierten Tiere sind notwendig, um eine so bescheidene und nur vorübergehende Gewichtsabnahme sowie einen allgemein guten Gesundheitszustand zu erreichen. Die gewählte Operationstechnik und die für die Haltung der operierten Tiere gewählten Bedingungen scheinen sich somit für systematische Untersuchung der Stoffwechselfolgen von Porta cava-Anastomosen gut zu eignen.

Schlüsselwörter: Porta cava-Anastomose — Leber- und Glucose-Toleranz — Leber und Insulin — Intestinale Hormone und Insulinsekretion.

There has been increasing interest over the last decade for the endocrine role of the gastrointestinal tract. More specifically, it has become evident that among gastrointestinal factors released into the blood stream, one must consider not only the accepted functions of such well-known intestinal messengers as secretin and pancreozymin, but also the participation of these and other substances in more general endocrine-metabolic regulation, such as the control of the secretion of insulin and glucagon from the endocrine pancreas [5, 9, 17, 20]. Furthermore, recent morphological evidence suggests the presence in gastrointestinal mucosa of a number of distinct types of cells, all endowed with the characteristics usually associated with endocrine activity [8].

In analyzing the endocrine-metabolic function of the gastrointestinal tract, particularly as related to the endocrine pancreas and energy metabolism, an interesting consideration is that of appraising the importance of such secretions reaching the liver directly through the portal system, as would normally be the case. Accordingly, we have engaged in a long-term programme of evaluation of the differences existing between normal animals and animals with complete portacaval shunting, all secretions into the portal vein from the gastrointestinal tract of the latter being bound to reach all tissues only through the general circulation.

We have decided to use the rat as experimental animal because this species is devoid of pre-existing shunts that might bypass the operative

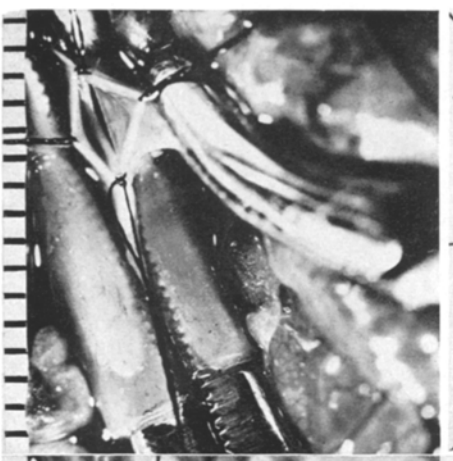
interruption of portal venous flow to the liver. Also, a convenient feature of rats is their inability to tolerate rapid constriction of the portal vein to a diameter below 50% of normal. If the decrease in portal vein diameter exceeds 50%, animals usually die within 2 hrs, while a lesser decrease results in evident portal hypertension with clearly demonstrable collateral circulation [13, 14]. Rats are thus well-suited to detect inadequacy in the functioning of portacaval shunts.

Materials and Methods

All animals used were male Wistar-derived rats bred in our laboratory. Two series of approximately 70 rats each were operated successfully, the animals in the first series weighing between 325 and 360 g, those in the second series between 290 and 325 g. At that point, our rats had generally reached the end of their most rapid period of weight gain and their age varied between 13 and 15 weeks.

Surgical Technique for Termino-lateral Portacaval Shunting. The rats were not fasted overnight and operated on between 8 a.m. and 1 p.m. The technique used was adapted from that described by Lee and Fisher [10, 11]. Ether was used for narcosis. After opening the abdominal wall, the intestines were displaced to the left in order to expose the portal vein and the vena cava. The portal vein was dissected free, from as close as possible to the hepatic hilus, up-stream to as close as possible to its first branching point. The vena cava was carefully dissected free in the area of its confluence with the right renal vein. It was then clamped parallel to its flow, using a curved hemostat with both branches covered with teflon catheter tubing. A longitudinal 3 mm slit was then made in the isolated part of the vena cava and traction threads were placed at its extremities, using 7—0 double armed silk (Ethicon K-800), these threads to be used later for the portacaval suture. The portal vein was then ligated at the liver hilus using a 4—0 thread and clamped with a protected hemostat as far from the hilus as possible. The portal vein could now be sectioned between the clamp and the ligature and its mobile part, approximately 2 mm long when empty, was attached to the 7—0 traction threads on the vena cava. This stage is shown in Fig. 1. The two traction threads were now used for uninterrupted sutures of the inferior, then superior edge of the anastomosis (Figs. 2 and 3). The clamps were then removed and repeated application of wet cotton wool was used for hemostatis. After a few minutes, the extent to which the anastomosis had become functional could be estimated and its diameter measured (Fig. 4). The time during which the clamps occluded the abdominal circulation did not exceed 15 min. The abdominal cavity was then closed with a Fig. 8 suture. The technique used was clean but not sterile, all material used being sterile but no attempt being made to preserve continued sterility of the operative field. At the end of surgery 100,000 units of penicillin G were administered intraperitoneally.

Whenever there was doubt as to the full patency of the anastomosis, as measured visually, the animal was discarded. After the first 30 procedures, this occurred only exceptionally. Death from hemorrhage during the operation occurred in about 15% of cases. After successful completion of the operative procedure, the overall mortality rate within 24 hrs was approximately 8% for a total of over 150 procedures. The criteria used for full patency of the shunt were visual inspection of the shunt, anatomical control for collateral circulation and splenoportograms. Sham operations were carried out as follows: the abdominal cavity was opened to the same extent, the intestines displaced to the left in the same manner, and the area which would



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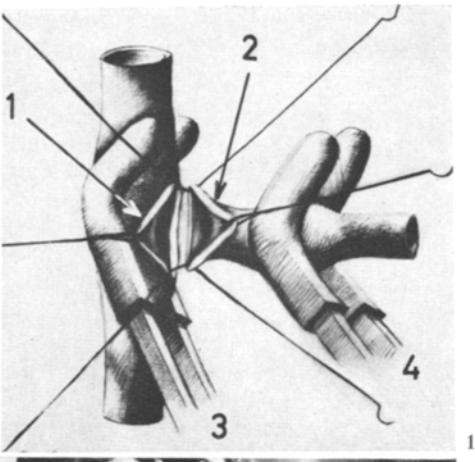

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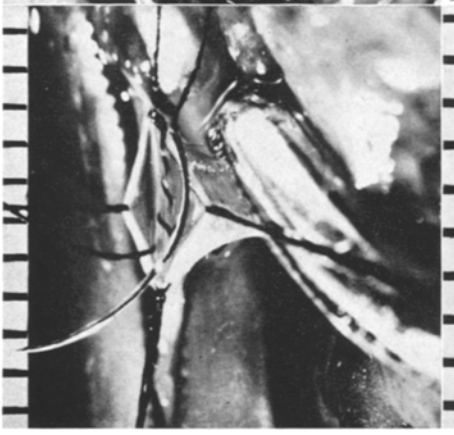

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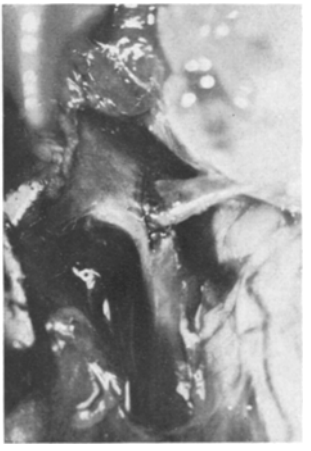

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Figs. 1—4. Portacaval shunting operation:

Fig. 1 a and b. Suturing of the portal vein to the vena cava. *1* Lumen of the incision in the vena cava. *2* Lumen of the portal vein. *3* Clamp on the vena cava. *4* Clamp on the portal vein. Scale at left is in millimetre

Fig. 2. Uninterrupted suture of the posterior edge of the anastomosis

Fig. 3. Suture completed, but anastomosis still clamped

Fig. 4. Functional anastomosis

have been used for the shunt was intermittently handled for the same duration as that needed for establishing the shunt in the successfully operated animals. In both instances, the abdominal cavity was open for approximately 50 min.

Splenoportograms. These were performed on 15 animals at different time intervals after portacaval shunting or sham operations. They were carried out after 5 days, and after 3, 6, or 8 months. 1 ml urografin "U 60" was injected into the spleen and radiographs were taken at intervals of 1 sec for 6 sec after the beginning of the injection. 12 sec after the last of these exposures, another 3 radiographs were taken at intervals of 2 sec.

Maintainance of the Portacaval Shunted (PCS) Animals. As will be discussed further on, we consider the environmental control of the operated animals an essential condition for obtaining generally healthy animals with portacaval shunts. Only in such healthy animals can we be certain that subsequent alterations truly relate to the new circulatory state and its endocrine-metabolic consequences, rather than to inadequate diet or intercurrent health problems. The number of animals per cage should be sufficient for body contact and elective warmth, but avoid crowding: we settled on 6 rats per cage, the dimensions of the cages being: 40 cm long, 25 cm wide, and 17 cm deep. The animal room was fully air conditioned at a temperature of 25 $\pm$ 0.5°C, with humidity maintained and controlled at 60 $\pm$ 5%. There was no source of outside light and the abundant fluorescent tube lighting was kept constant at 14 hrs per day. Whether necessary or not, each animal was handled daily.

The importance of environmental conditions for the PCS animals was well illustrated by their reaction to transfer from their usual cages to metabolism cages. Although we used the rather large, round (21 cm diameter) plastic metabolic cages developed by Grass and Bunter and available through Maryland Plastics Inc., Federalsburg, Maryland, with only one rat per cage, a training period of 10 days proved necessary to achieve weight stabilization. This training period began 5 days after shunting, on the one hand, and after 3 months, on the other. Accordingly, the periods under scrutiny were that from 15 to 25 days postoperatively, and that from 3½ to 4 months postoperatively. Comparison was between PCS and sham operated animals for the first of these two periods, and between PCS and non-operated animals of the same weight for the second period, since it was felt that the 15% weight differential between PCS and sham operated animals after 3½ months was an undesirable feature.

Composition of Food and Feces. PCS and control animals were maintained on normal laboratory chow on which we have maintained many generations of our Wistar-derived strain. The pelleted food termed "Altromin R" was purchased from Kunath Co., Aarau, Switzerland; it is a standard rat chow of good quality with composition, including that of vitamins and minerals available from the manufacturer and guaranteed. Prior to analysis, food samples, as well as feces samples were dried for 2 hrs at 120°C. The measurements made were total combustion heat, in Cal. per gram, according to the technique of Dewar; total lipid content, using chloroform-methanol extraction; and total nitrogen content according to Kjeldahl. The nitrogen values were transformed into combustion heat (Cal. per gram) due to nitrogen-containing substances by multiplying the values expressed in grams by 27.4 which is the coefficient intermediate between that for a gram of vegetal nitrogen and that for a gram of animal nitrogen. For lipids, the combustion heat, in Cal. per gram was obtained by multiplying the total lipid value by 9.1. The combustion heat due to carbohydrate (mostly cellulose) was then obtained by subtracting from the total combustion heat that due to protein and that due to lipids.

Digestive Utilization Coefficient. This coefficient [3, 4] expresses the percentage of ingested food which is absorbed, and is obtained by subtracting the dried weight of feces from the dried weight of ingested food. It can also be calculated for each of the three major food components, carbohydrates, proteins and lipids.

Urine measurements included daily volume and semiquantitative estimations of glucose (Tes-Tape, Lilly) and ketone bodies (Acetest, Ames).

Histology. For histological examination, samples of liver were fixed in formaldehyde, embedded in paraffin and stained with hematoxylin-eosin, or according to van Gieson for the detection of collagen. Intravenous injection of carbon (India ink) was used to demonstrate reticulo-endothelial cells, with subsequent staining with hematoxylin-eosin.

Results

Patency of the Shunt. Of the some 140 shunt operations included in the two series 40 were killed at intervals from 5 days to 1 year postoperatively in order to test shunt patency. The width of each shunt was measured and evidence of collateral circulation was looked for, but not found. In 15 animals spleno-portography was carried out at intervals of 3 months postoperatively. This procedure is well suited to detect even minimal collateral circulation in the rat. An example is shown in Fig. 5. In no instance was there any evidence for the development of collateral circulation.

Body Weight. As shown in Fig. 6 for 15 PCS animals, there regularly was a 5 to 10% weight loss during the first 2 weeks following shunting,

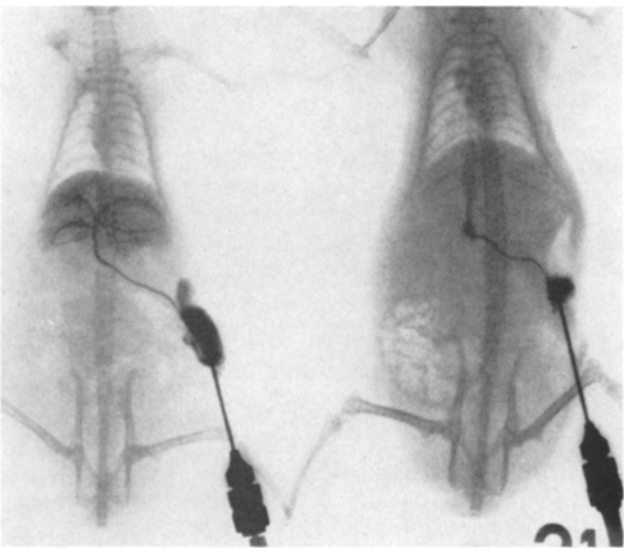


Fig. 5. Splenoportograms. Right, 2 months after P.C. shunt. Left, control rat. Both animals were injected and X-rayed (2 sec after injection) simultaneously

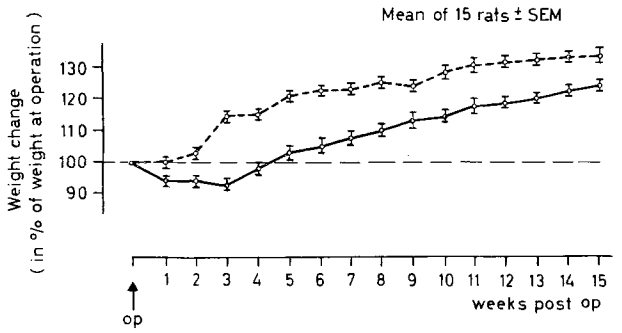


Fig. 6. Comparative post-operative weight gain in PCS rats (full line) and sham operated controls (dotted line)

Table 1. *Liver weight of PCS and control rats*

Time post-op.	Relative liver weight (% of body weight)		
	Controls	PCS	Difference
5 days	2.90	2.25	— 22%
11 weeks	2.65	1.84	— 31%

with return to pre-operative weight at between 4 and 5 weeks. Thereafter, there was no difference in the weight curve of PCS and sham operated animals. Note also that there was little difference in the individual variability between the two groups, again suggesting homogeneous group behaviour among the shunted rats.

Liver Weight and Histology. As shown in Table 1, the relative weight of the liver decreased 22% within 5 days postoperatively, with a much slower subsequent weight loss, reaching 31% at 11 weeks postoperatively. Despite this, however, there was little evidence to support the presence of hepatic pathology other than this moderate overall atrophy. Routine histological controls have been carried out between 5 days and 1 year after shunting, using both hematoxylin-eosin and van Gieson stains. As illustrated in Fig. 7, evidence for hepatic pathology was not found. There were no nuclear alterations, no abnormal cytoplasmic appearance, no proliferation of connective tissue or increased deposition of collagen. Furthermore, as shown in Fig. 7c, India ink injection did not reveal abnormal activity or distribution of reticulo-endothelial cells. There was no evidence of ischemia and the portal vessels were filled with

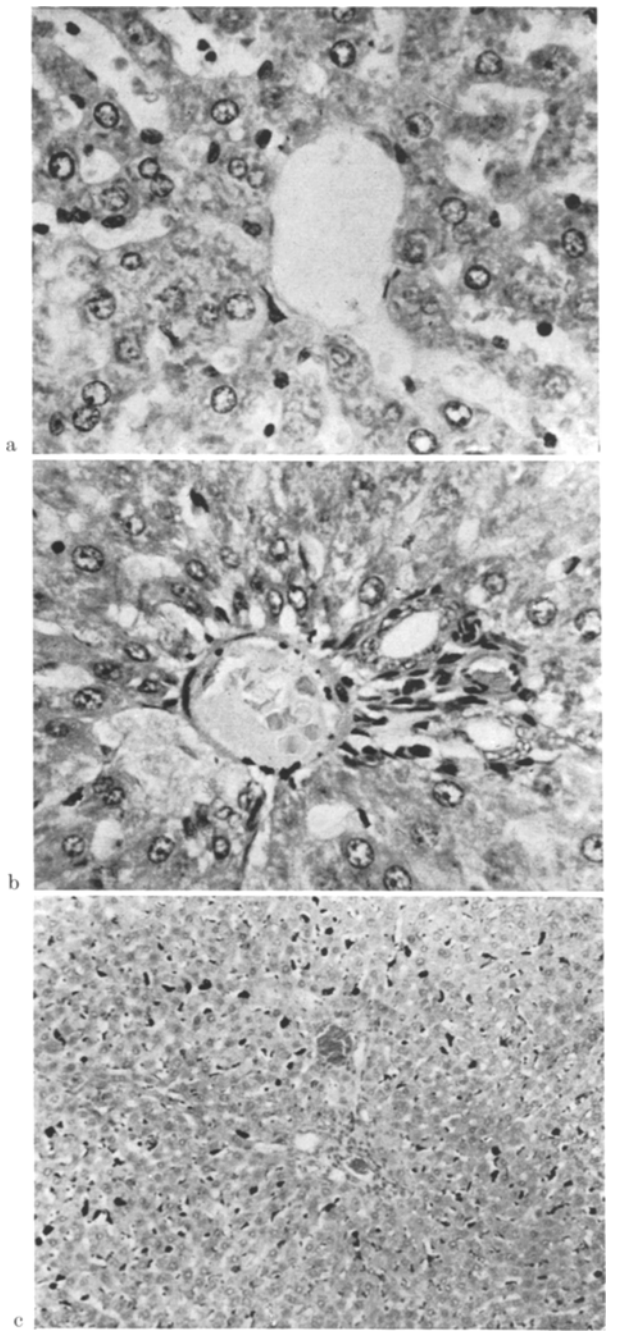
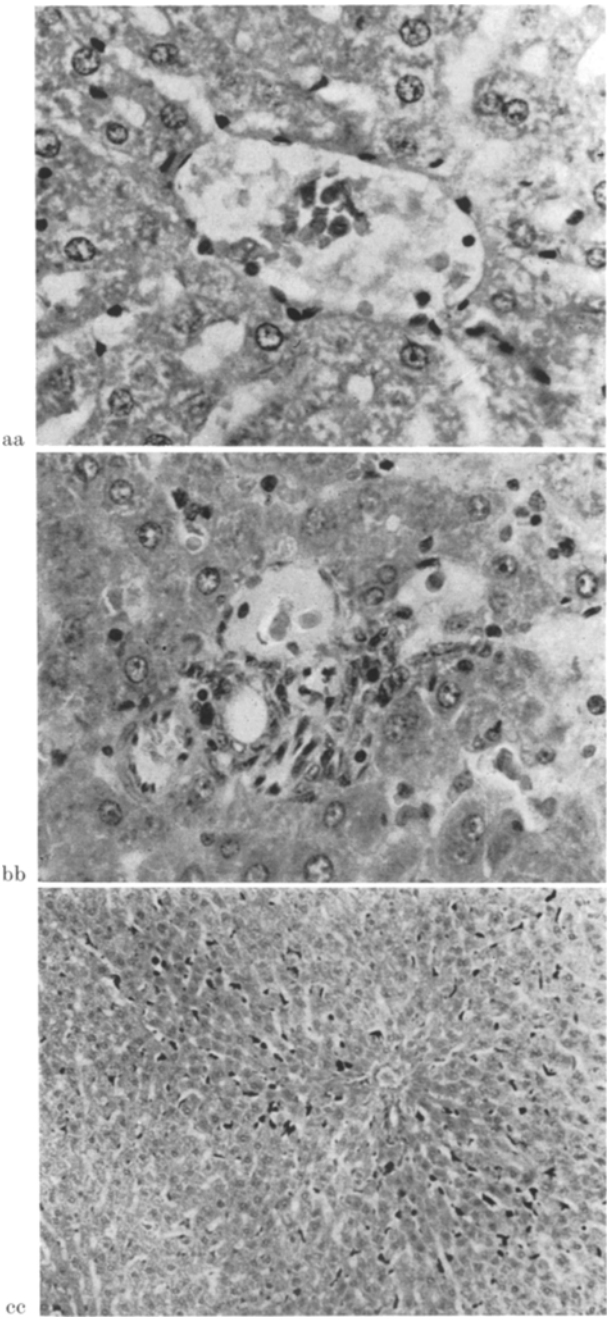


Fig. 7a—c. Histology of livers from portacaval shunted (right, aa, bb, cc) and control (left, a, b, c) rats, 14 months after shunting or after sham operation.



a, aa Hematoxylin-eosin, 160×; b, bb van Gieson, 160×; c, cc Hematoxylin-eosin after i.v. carbon injection, 50×

Table 2. *Nutritional balance of PCS and control rats*

	Time after operation			
	15 days		3 months	
	Controls	PCS	Controls	PCS
Days in metabolic cages	14	14	10	12
No. of animals	6	12	6	12
Ingested food (g/day/100 g rat)	5.3	*3,4*[a]	5.3	5.3
Resorbed food (g/day/100 g rat)	3.7	2.4	3.7	4.0
Digestive utilization coefficient (% ingested food)	70	69	71	*76*[a]

[a] Significance of difference from controls < 0.005.

Table 3. *Composition of food and feces 3 months postoperatively in 12 PCS and 6 control rats*

Component	Food Composition (mg/g dry weight)	Feces	
		Controls (mg/g dry weight)	PCS
Carbohydrate	797	721	740
Protein	160	189	*160*[a]
Lipid	62	61	68
Combustion heat (Cal/g)	4,644	4,448	4,453

[a] Significance of difference from controls < 0.005.

Table 4. *Digestive utilization coefficients 3 months postoperatively for 12 PCS and 6 control rats*

Food component	Digestive utilization coefficients (% ingested food)		
	Controls	PCS	% of controls
Carbohydrate	74	78	+ 4
Lipid	71	74	+ 4
Protein	66	*76*[a]	+ *15*[a]

[a] Significance of difference from controls < 0.005.

Table 5. *Caloric value of resorbed food components 3 months postoperatively in 12 PCS and 6 control rats*

Food component	Caloric content (Kcal/100 g rat/day)	
	Controls	PCS
Carbohydrate	13.10	13.65
Lipid	2.01	2.16
Protein	2.35	2.68
Total	17.46	18.49

blood, presumably through intrahepatic connections with the hepatic arterial circulation. When slides from PCS and sham operated animals were submitted without identification to neutral observers, these were unable to recognize which came from PCS rats. Note that the examples shown in Fig. 7 are from an animal autopsied 14 months postoperatively.

Nutritional Balance Studies. As shown in Table 2, both the amount of food ingested, and the amount of food resorbed (food weight minus feces weight) was significantly decreased 15 days after portacaval shunting when compared with sham operated animals. 3 months postoperatively, however, food intake had returned to normal while the food resorbed was slightly increased, resulting in a significantly greater digestive utilization coefficient. 15 days postoperatively it is noteworthy that weight loss was exclusively the result of decreased food intake since the digestive utilization coefficient remained normal. Table 3 further shows the distribution between carbohydrate, protein and lipid in food and feces of PCS and control rats. The data indicate that there was no major difference in the effectiveness of absorption of the three food classes from the intestinal tract of shunted or control animals, although we again find, as shown in Table 4, a slight overall increase in digestive utilization coefficients, particularly for protein. Since protein accounted for only approximatively 15% of food calories in these animals, however, as shown in Table 5, the overall increase in digestive utilization was only a minor feature. Urine glucose and ketone bodies were routinely tested throughout, but never found to be measurably increased.

Discussion

Since the present study provides the baseline information for subsequent endocrine and metabolic comparisons between PCS and suitable control animals, it was essential to establish that the procedure used

here did not result in portal hypertension and the development of collaterals, alterations which are known to be associated with disturbed intestinal absorption [6, 18] and abnormalities in the circulating level of some nutritional [15, 16] or hormonal components such as adrenalin and noradrenalin [19]. Clearly, when discarding all animals where some doubt as to the patency of the shunt arose during the operation, the procedure used here led to highly reproducible and adequate portacaval shunts. Our findings confirm that the rat is a particularly suitable animal because of its incapacity to rapidly develop collaterals and because the demonstration of portal hypertension is easily made. In all of the animals studied, there was no evidence for portal hypertension, for developed significant collateral circulation, or for hepatic damage which might be expected to result from either portal hypertension or inadequate blood supply. There was no proliferation of connective tissue and both function and distribution of Kupfer cells were normal.

Of particular interest and particularly gratifying to us was the remarkable good general state of health of all PCS animals which were kept beyond the first few postoperative weeks. We apparently did not encounter the difficulties described by several groups, with persistent weight loss throughout the postoperative period, even the preoperative weight being reattained only in a few instances [1, 2, 12]. One of us (R. L.) has observed rats of identical weight and apparent state of health at operation, identically operated, but maintained in his laboratory in Lyon [12]. 10 weeks after operation, their average weight loss was 50% of preoperative weight while at that time the weight of our animals had increased by 14%. The rats operated either in Geneva or in Lyon were of the same strain and it would seem that the major differences concerned the environmental conditions under which the animals were kept. While there was no difference in food or caging, air conditioning with constant temperature and humidity, as well as constant duration of daily artificial light were available only in Geneva. We believe that environmental postoperative care is the main element conditioning the favorable behaviour of the PCS animals reported on here, as also suggested by similar findings reported by others [7].

Our PCS rats nevertheless did loose up to 10% of their preoperative body weight during the first 2 weeks following operation. It would appear that this weight loss resulted from decreased food intake and not from any anomaly of gastrointestinal absorption of one or several food classes. The small increase in the efficiency of absorption of food observed 3 months after shunting is unlikely to be sufficient to significantly alter the endocrine-metabolic response to nutritional stimuli.

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