THE PRODUCTION OF PARTIAL LIVER INSUFFICIENCY IN RABBITS.

By PHILIP D. McMASTER, M.D., AND D. R. DRURY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Reasons enough for the existence of the liver are known. A host of functions have been ascribed to the organ already. Nevertheless the clinical condition of liver insufficiency is still a riddle. For the better understanding of it one must distinguish between functions which are essentially hepatic and those which can be carried out somewhere else in the body when the liver fails.

The recent work on dogs by Mann (1–9) and his colleagues has greatly increased our knowledge of liver functions. But much remains to be learned, and the method of these authors is laborious. For this reason we venture to submit a simple procedure for inducing extreme liver insufficiency in rabbits.

To approach the problem of liver insufficiency in animals three general types of procedure or their combination are available; total extirpation, partial ablation, and derangement of the organ by tying the bile duct or by chemical injury. The method of total extirpation, though it has served most excellently (1–9), still leaves much to be desired. Death follows the operation so rapidly that scant time remains for study of its onset. The use of drugs to bring about liver derangement is questionable for one must assume that the substance employed acts only on the liver parenchyma and not elsewhere as well. The evidence for and against these methods has been reviewed by Marshall and Rowntree (10) and by Mann (9).

The employment of partial ablation has also been open to objection. Investigators are agreed (9, 11, 12) that the liver possesses a "factor of safety" so great that it is well-nigh impossible to remove enough parenchyma to produce insufficiency without fatally obstructing the portal flow. Workers partially ablating the liver in dogs and rats

have found this difficulty insurmountable (9, 11, 13). In the rabbit our efforts have led to success. One can remove slightly more than 90 per cent of the animal's liver without untoward obstruction to the portal blood flow, with result in an extreme degree of hepatic insufficiency. The animals unless treated succumb in 12 to 18 hours, with convulsions and low blood sugar. If given glucose they live for varying periods up to 5 days, eventually dying with symptoms like those exhibited by dogs deprived of the entire liver (2, 9). For a study of the problems of liver insufficiency the method presents many advantages. Not the least of these is the ease of the ablation. It can be accomplished in less than 10 minutes, by a single operator, unassisted.

Anatomical Peculiarities of the Rabbit's Liver.

The arrangement of the rabbit liver in well-nigh separate lobes permits the removal of much of the hepatic substance by simple ligations and excision. Three large cephalad lobes, comprising what has been termed the "main liver" (14–16), constitute about 70 per cent of the organ. The remainder, almost separate from these, the "posterior lobe mass" (14, 15), is situated further from the head and to the right of the portal vein. Unlike the lobes of the "main liver" the "lobe mass" is intimately attached to the vena cava and cannot be wholly ablated without injury to this vessel. But a deep cleft exists on the cephalad surface of the mass and here a ligature may be thrown about the lobe and tied in such a way that it lies close to the vena cava but yet not close enough to impede the flow of blood. The half of the lobe beyond this ligature can now be removed, leaving a neat small stump. The small caudate lobe on the other side of the cava possesses a flattened, tail-like portion springing from a narrow base which can be readily severed after ligation. There remains about half the substance of the lobe, that portion which extends along the sheath of the portal vein between it and the spinal column.

In our attempts to induce liver insufficiency in the rabbit we employed three types of sub-total hepatectomy. Ablation of the "main liver" was the first. To this operation there was next added excision of the removable part of the caudate lobe, leaving the stump and the whole "posterior lobe mass" intact. Finally removal of the "main

liver" and half of the "posterior lobe mass" was practiced, leaving only the stump of this latter and the small caudate lobe intact. This last operation alone resulted in true liver insufficiency.

Estimation of the Relative Amounts of Liver Tissue Removed by the Three Types of Sub-Total Hepatectomy in the Rabbit.

In twenty-five normal rabbits we have separated the portions of the liver, discussed above, weighing the "main liver," the excisable portion of the "posterior lobe mass," its remaining stump, the removable bit of the caudate lobe, and its stump. Table I shows, in terms of percentage of total liver, the relative amounts of the tissue remaining and removed by each of the three procedures outlined above. Further, the table gives some idea of the relative variability in individual instances. Simple ablation of the "main liver" (see Column I of the table) withdraws on the average about 73.6 per cent of the liver tissue, a figure well in accord with the findings previously published by Rous and Larimore, 72.3 per cent (15), and by Ponfick, 74.7 per cent (16). As will be seen below, this procedure does not induce apparent liver insufficiency. "Main liver" removal plus partial ablation of the caudate lobe offers no great additional advantage over the former method, for the caudate lobe adds but little to the percentage of tissue removed (Column II). Ablation of the "main liver" and half the "posterior lobe mass" is the method of election (see Column III of the table). By this procedure an average of slightly over 90 per cent of the liver tissue is removed, the remaining stump of the "posterior lobe mass" and the intact caudate lobe constituting but 9.6 per cent of the original total.

Method.

Rabbits weighing 1500 to 4500 gm., previously kept on a mixed diet, were fasted but allowed water for periods of 24 to 48 hours. They were then operated upon under ether anesthesia. The three large cephalad lobes of the liver, the "main liver," were removed together with that portion of the "posterior lobe mass" which lies to the right of the vena cava.

To accomplish this, the "posterior lobe mass" of the liver was exposed and the ligament attaching its lower medial pole to the sheath of the vena cava severed. A heavy silk ligature was thrown around the lobe, placed in the deep incisura on its cephalad surface, and tied, care being taken to avoid all puckering of the vena cava or inclusion of its sheath in the knot. The liver portion to the right of the

TABLE I.

The Relative Amounts of Liver Removed by the Three Methods of Sub-Total Hepatectomy Described in the Text.

	No.	Body wt.	Liver percent-age of body wt.	I "Main liver" out — Per cent of total liver Out	In	II Posterior lobe in — Per cent of total liver Out	In	III Caudate lobe in — Per cent of total liver Out	In
		gm.							
	1	2570	2.32	76.8	23.2	78.6	21.4	93.4	6.6
	2	1960	2.70	72.7	27.3	76.1	23.9	90.8	9.2
	3	2150	2.21	68.1	31.9	72.2	27.8	90.7	9.3
	4	2957	2.41	76.5	23.5	81.2	18.8	90.2	9.8
Freshly fed	5	2250	2.88	81.2	18.8	83.9	16.1	89.4	10.6
	6	2010	2.22	76.2	23.8	81.0	19.0	89.1	10.9
	1a	4275	2.48	73.7	26.3	77.2	22.8	91.9	8.1
	1b	2375	2.36	80.1	19.9	84.2	15.8	91.2	8.8
Average.........		2568	2.45	75.7	24.3	79.3	20.7	90.8	9.2
	1	1980	4.38	73.1	26.9	76.8	23.2	91.4	8.6
	2	2310	4.61	75.3	24.7	80.2	19.8	90.6	9.4
Fasted 24	3	2621	4.74	69.8	30.2	74.9	25.1	90.6	9.4
hrs.	4	2210	3.67	72.0	28.0	77.2	22.8	90.3	9.7
	x	1800	3.99	70.4	29.6	75.2	24.8	88.7	11.3
	y	2100	3.80					90.6	9.4
	z	2050	3.76					90.2	9.8
Average.........		2153	4.14	72.1	27.9	76.9	23.1	90.3	9.7
	1	2250	3.17	75.2	24.8	78.4	21.6	92.4	7.6
	2	1770	2.53	69.8	30.2	73.6	26.4	90.1	9.9
Fasted 48	3	1450	3.12	66.8	33.2	71.8	28.2	89.5	10.5
hrs.	4	1960	3.20	68.1	31.9	73.7	26.3	88.1	11.9
	*	2150	2.25	76.8	23.2	82.2	17.8	88.3	11.7
Average.........		1916	2.85	71.3	28.7	75.9	24.1	89.7	10.3
	1	1509	2.83	75.9	24.1	78.1	21.9	92.1	7.9
	2	1500	2.87	74.2	25.8	77.9	22.1	91.3	8.7
Fasted 5	3	1550	3.02	74.3	25.7	80.3	19.7	90.3	9.7
days	4	1600	2.63	72.6	27.4	75.5	24.5	89.5	10.5
	5	1750	2.75	72.8	27.2	76.6	23.4	89.1	10.9
Average.........		1582	2.82	74.0	26.0	77.7	22.3	90.5	9.5
Average of all...................				73.6	26.4	77.7	22.3	90.4	9.6

* Fasted 3 days.

ligature was then cut away. After severing the ligaments attaching the "main liver" to the diaphragm, a ligature was placed about it. This ligature, tied just ventral to the diaphragm without inclusion of the vena cava, permitted excision of the "main liver."

In some instances interference with the circulation of the remaining liver tissue resulted in necrosis of these parts. Rarely the vena cava was obstructed by the ligatures about the "posterior lobe mass" and very rarely a bile leak or hemorrhage occurred. Occasionally, too, animals developed a postoperative pneumonitis. Such instances have been ruled out in considering the evidences of liver insufficiency induced by this method. They have amounted in all to about 15 per cent.

After excision of nine-tenths of the liver tissue, in the rabbit the remaining portions appeared slightly congested but never turgid and tense as they do after the removal of but three-fourths of the organ in dogs and rats (11, 13). The venous channels became moderately dilated and a fatty infiltration of the parenchyma developed. Furthermore changes indicative of a fatty degeneration were found histologically, this serving to increase further the degree of liver insufficiency obtained by the operative procedure. Autopsy yielded no evidences of severe portal obstruction. There was no pronounced dilatation of the portal tributaries, and no marked congestion of the gastric or intestinal mucous membranes or engorgement of the spleen. Even the remaining portions of liver were not greatly distended with blood. Nevertheless we have asked ourselves, Can death have been due to obstruction to the flow of portal blood occasioned by the operation? To answer this question a series of control experiments were carried out in which an equivalent or greater obstruction of the portal blood flow was induced with but a negligible removal of liver parenchyma.

Control Experiments.

In seven rabbits, the portion of the "posterior lobe mass" of the liver lying to the right of the vena cava was removed under ether anesthesia as in the operation for nine-tenths liver ablation. The portal vessels to the "main liver" were then ligated, leaving intact all branches of the bile ducts and the hepatic artery. In this way the portal blood to 90 per cent of the liver was shunted through the caudate lobe and the remaining stump of the "posterior lobe mass." 4 to 8 days later, India ink was injected into the portal vein of the surviving rabbits to determine how great an amount of portal obstruction had actually been obtained. In

five instances the ink flowed only to the 10 per cent of liver which, according to the conditions of the experiment, should have received portal blood. In the two remaining rabbits some ink reached the main liver, showing that the portal blood had some access to it.

These animals in which a diversion of 90 per cent of the portal blood to the liver had been successfully effected had all survived in good condition.

In another group of four rabbits the branch of the portal vein which leads to the "posterior lobe mass" was ligated following ablation of the "main liver." This procedure caused an even greater blocking of the portal blood flow than that above described; yet the animals did not die until about 5 days after the operation. The sole road for the portal blood had been through the small caudate lobe. Marked hypertrophy of this lobe was found with dilatation of its channels and one may infer that obstruction to the portal flow had progressively diminished from the time of the operation. Death was preceded by the typical signs of liver insufficiency, to be outlined below.

It is evident that death of rabbits when 90 per cent of the liver has been removed by our method cannot be ascribed to the portal obstruction incident to the operation. As will be reported in a succeeding paper, we have found it possible to occlude in the rabbit from 9/10 to 19/20 of the lumen of the portal vein together with 4/5 to 9/10 of the lumen of the vena cava without jeopardizing the animal.

The Evidences of Liver Insufficiency after Ablation of 90 Per Cent of the Rabbit Liver.

The Fall in Blood Sugar.—Following removal of nine-tenths of the liver the animals made a rapid recovery from the anesthetic, drank water, and appeared to be in excellent condition. In a few hours, however, they were to be found with drooping head, sprawling legs, and obvious muscular weakness. The heart rate was rapid, rising to 285 beats per minute from a preoperative average of about 140. In 6 to 12 hours the animals became prostrate and convulsions supervened, in one of which death occurred about 8 to 18 hours after operation. Mann and Magath have described (2, 3) a rapid fall in the blood sugar concentration of dogs deprived of the entire liver, with result in convulsions and death a few hours after the operation. So too in our rabbits deprived of 90 per cent of the organ the blood sugar fell rapidly to a level below the minimum compatible with life.

In numerous instances we have followed the blood sugar concentration in rabbits before and after removal of nine-tenths of the liver,

employing for the purpose the Hagedorn-Jensen method (18, 19).
Text-fig. 1 shows the rapid decrease of blood sugar resulting from the
liver loss, in fourteen of these animals.   In the instances depicted by
the solid lines, the blood sugar concentration fell to a level incompat-
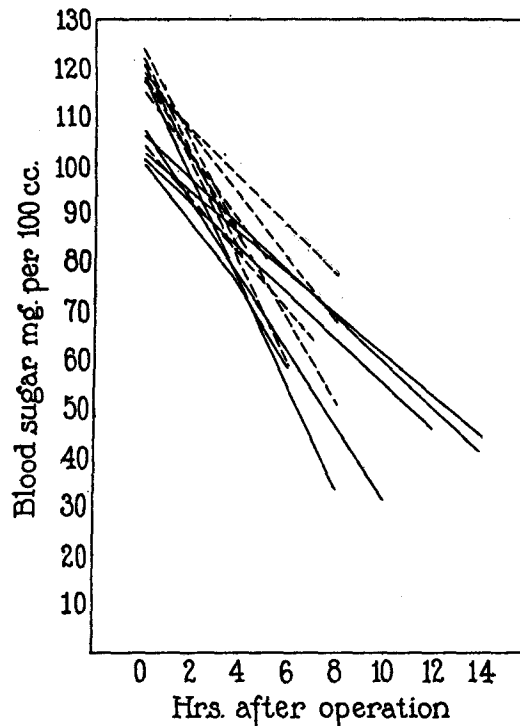
TEXT-FIG. 1. *Fall in the Blood Sugar Concentration in Rabbits Following
Ablation of 90 Per Cent of the Liver.*

The blood sugar findings in fourteen rabbits deprived of 90 per cent of the liver
are plotted in terms of milligrams of glucose per 100 cc. of blood. In all the
animals a rapid fall in the amount of blood sugar occurred. In the instances
depicted by continuous lines, typical hypoglycemic symptoms developed and the
animals died. The dotted lines show that a similar fall in blood sugar concen-
tration took place in eight animals, until they were given glucose at the points
where the lines are discontinued.

ible with life; convulsions and death followed.   The dotted lines show
an equally rapid initial fall in the blood sugar of other rabbits, which
was checked by injections of glucose given at the points indicated by
the discontinuance of the lines.

*The Insufficiency in Rabbits Given Glucose after Ablation of 90 Per Cent of the Liver.*

Mann and Magath showed (3) that administrations of glucose to dogs deprived of the total liver preserved life for many hours. Eventually the animals died, in spite of the maintenance of a high blood sugar level, in a condition characterized by weakness and flaccid paralysis. The same phenomenon more prolonged was found in our rabbits given glucose after ablations of about 90 per cent of the liver. The substance was usually administered by stomach tube, but often by intravenous injection, and at times subcutaneously, in varying doses and at various intervals of time.

The effect of glucose, especially by intravenous injection, in rabbits deprived of 90 per cent of the liver is as startling as in completely hepatectomized dogs (3, 9). Prostrated, moribund animals will be found sitting up or moving about freely a few minutes after a single injection of 10 to 20 cc. of 5 or 10 per cent dextrose solution. The pulse and respiratory rates are slowed and the animal appears normal again. The effect, however, is not long lived. 30 minutes to several hours later the rabbit again becomes weak, the pulse and the respiration rate are increased, the blood sugar is low, reflexes are exaggerated, and convulsions may occur. Again and again the animal may be restored by administrations of glucose but finally, in spite of these, death comes on, heralded by extreme asthenia, coma, and respiratory failure.

Derangements in Urea and Uric Acid Metabolism.—It is now well known that urea formation ceases in the liverless dog (20). This phenomenon is accompanied by an accumulation of uric acid in the blood, with a consequent increased excretion of the substance if kidney function is maintained. The change from a normal urea metabolism does not appear in dogs deprived of as much as 70 per cent of the liver after an Eck fistula operation (9, 21), although uric acid destruction may possibly fail to occur. Indications of a lack of urea formation or of cessation of uric acid destruction in dogs may be taken as definite evidence of liver insufficiency (9). Such evidence has been obtained in our partially hepatectomized rabbits.

Procedure.—Freshly fed rabbits were fasted for 24 hours, given 50 to 75 cc. of 5 per cent glucose solution by gavage, and later allowed to drink the same solution

at will. A copious secretion of urine resulted. 12 to 20 hours later the animals were catheterized, the bladder washed thoroughly with water, and a blood sample taken from an ear vein. They were then placed in clean metabolism cages for 17 to 24 hours. At the end of this period another blooa specimen was taken, and the catheterization and bladder washing repeated. The urine thus obtained and that collected during the previous 17 to 24 hour period was analyzed for urea and uric acid. At once after taking the second catheter specimen 90 per cent of the liver was removed under ether anesthesia, and for varying periods thereafter
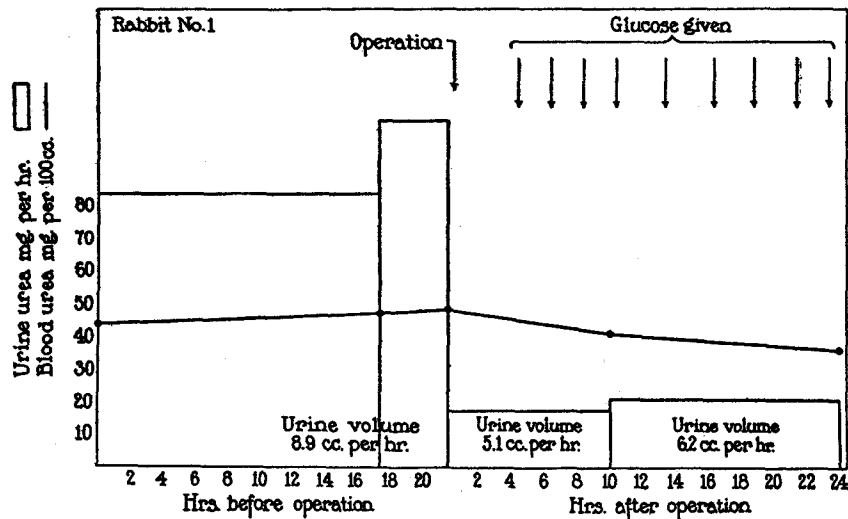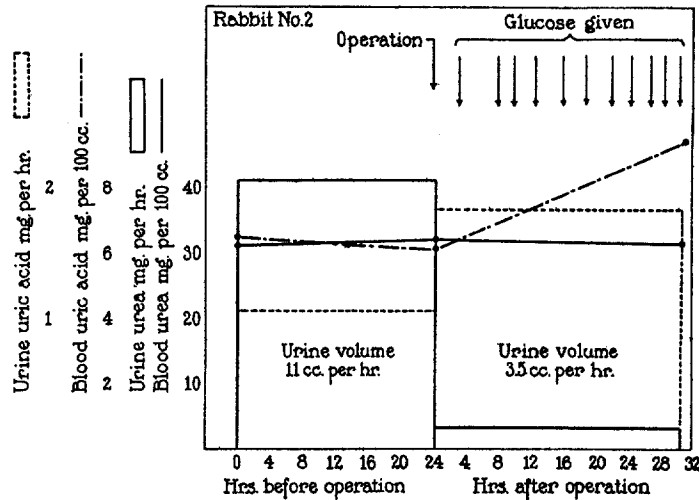

Text-Fig. 2. *Changes in Blood Urea Concentration and Urea Output in the Urine after Removal of 90 Per Cent of the Liver.*

Uric acid studies were not made. The changes shown are similar to those described in Text-figs. 3 to 6.

the animals were kept in metabolism cages. From time to time they were given water or 5 per cent glucose solution to maintain diuresis. At intervals they were catheterized and the urine and bladder washings added to the cage urine specimens to be analyzed for uric acid and urea. Blood specimens taken during or immediately before the catheterizations were used for blood urea and blood uric acid analyses. For the determinations of urea concentration in blood and urine, the method described by Addis (22) was employed. In some instances (Text-figs. 2, 4, and 5) the technique of Van Slyke (23) was used as well. Folin's method (24) served for the estimation of uric acid in the blood, and the procedure of Folin and Wu (25) for its determination in urine.

In all five instances depicted in Text-figs. 2 to 6 adequate diuresis was maintained. In all a striking decrease of urea output in the urine
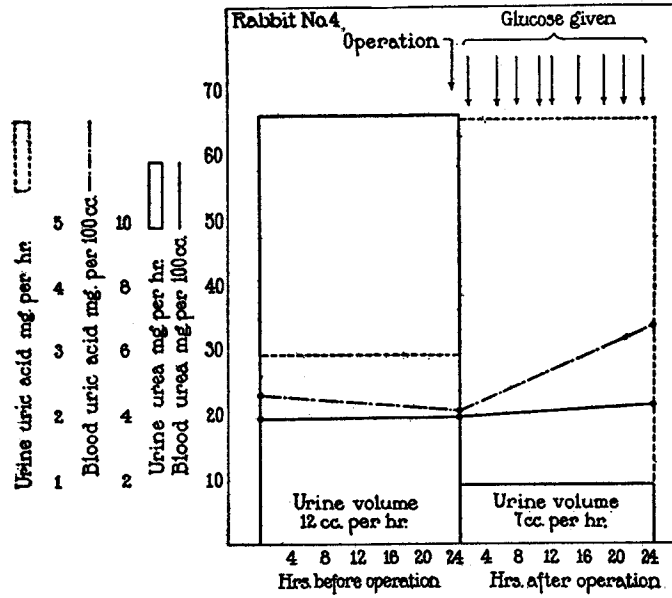
TEXT-FIG. 3.

TEXT-FIGS 3 to 6.   *Changes in the Urinary Output and Blood Concentration of Urea and Uric Acid in Rabbits Deprived of 90 Per Cent of the Liver.*

Text-figs 3 to 6 depict the uric acid and urea findings in the blood and urine of rabbits before and after ablation of 90 per cent of the liver.   In all, the urea output of the urine decreased profoundly after the ablation while the blood urea concentration remained almost unchanged.   In all, an increased urinary output of uric acid appeared, together with a greater concentration of the substance in the blood.
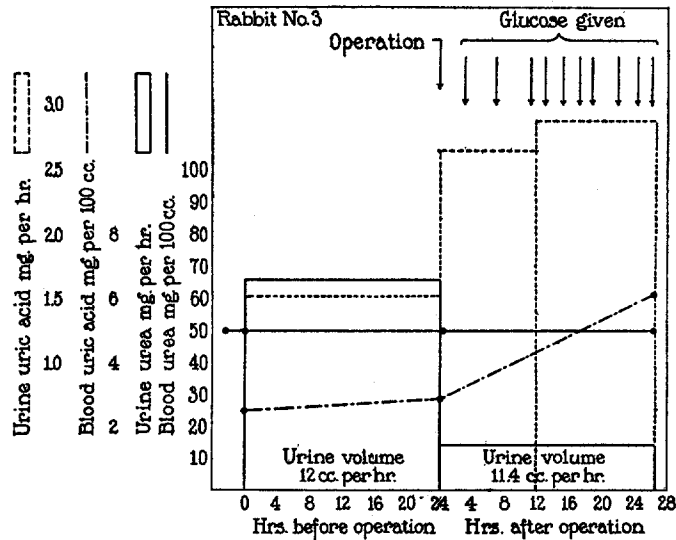
TEXT-FIG. 4.

TEXT-FIG. 5.
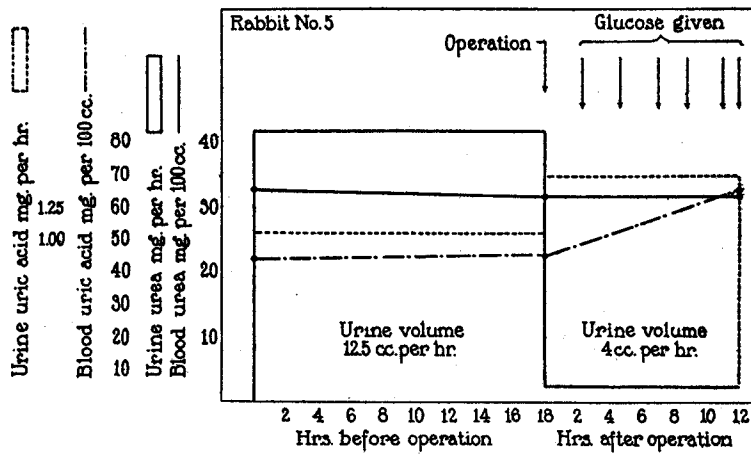
TEXT-FIG. 6.

occurred. Despite this lack of urea output the blood urea concentration remained unchanged in three of the animals, fell in one (Text-fig. 2), and rose but slightly in two, affording thereby evidence of greatly decreased urea formation. The uric acid studies made on four of these animals, Text-figs. 3 to 6, showed an increase of this substance in both blood and urine after ablation of 90 per cent of the liver.

In considering the evidences of liver insufficiency in these animals it is to be noted too that a mild tissue icterus appeared, accompanied by an output of bile pigment in the urine, as shown by the Van den Bergh test.

Liver Insufficiency in Rabbits Deprived of 80 Per Cent of the Liver.

Similar studies were made on three animals deprived of but 80 per cent of the liver. The findings were not clear-cut. The almost complete absence of urea excretion observed after ablation of 90 per cent of the liver was not present in these instances. In two of the animals the urinary output of urea per hour amounted to about half that of the preoperative period, while in the third animal no decrease was observed. Despite this the blood urea concentration of all rose notably, yielding evidence of a normal formation of urea in the case of the animal last mentioned, and of only a slight decrease in the others. In but one of the three experiments was an increase in urinary uric acid noted and this appeared in the instance showing normal urea formation. From these findings one might suppose that by removal of 80 per cent of the organ a borderline condition had been obtained in which there might or might not be liver insufficiency, in respect to these functions.

The Degree of Liver Insufficiency in Rabbits Following Ablation of 70 Per Cent of the Organ.

Prior to the adoption of the method described above, the "main livers" of several rabbits were ablated in the hope that the animals would develop a true liver insufficiency, for the operation entails a loss of 70 per cent of the organ. In 1889 Ponfick (16) reported his results with this procedure, stating that practically all the animals died. Of twenty-one rabbits operated upon in our series, seven survived in

good health for several weeks, when they were autopsied. Two others died of postoperative pneumonia and five others lived about 6 days, succumbing from undetermined causes. In the remaining seven instances, death occurred in about 20 hours accompanied by very low blood sugar concentration and convulsions. Ordinarily one finds after removal of the rabbit's "main liver" a transient fall in the blood sugar which may be sufficient to bring about death. The other evidences of liver insufficiency, the clinical picture of asthenia and prostration with faulty urea and uric acid metabolism, are in our experience lacking.

DISCUSSION.

The evidence presented herein clearly shows that a fatal liver insufficiency develops in rabbits deprived of 90 per cent of the organ. Following this great reduction in parenchyma the blood sugar falls to the lethal point. If this be avoided by the administration of dextrose the insufficiency manifests itself in a partial failure of the organ to form urea and to transform uric acid. Eventually the animal dies, of causes unknown.

After ablation of 90 per cent of the liver a mild jaundice appears, showing that there is a deficiency in the function of bile elimination. Earlier work from this laboratory, on the biliary obstruction required to produce jaundice in dogs, is in agreement with this finding. McMaster and Rous showed that jaundice of biliary obstruction does not appear after ligating several of the branches of the hepatic duct unless the drainage from 95 per cent or more of the liver is obstructed (12).

The fact is important that the rabbit deprived of 90 per cent of its liver behaves like the liverless dog in all ways that have thus far been tested. The blood sugar concentration falls, convulsions occur, urea formation ceases, uric acid metabolism is disturbed, and even when the blood sugar is artificially maintained at a high level death occurs, with symptoms similar to those arising in the liverless dog (9).

SUMMARY.

A rapid and simple method for the production of marked liver insufficiency in rabbits has been described. The necessary operation can be carried out by an unassisted operator in a few minutes. The method should further the study of liver physiology.

The changes as concerns blood sugar, urea formation, and uric acid metabolism would appear to be the same in the rabbit suffering from hepatic insufficiency as in the dog.

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