Although ovulation was not looked for in the day 12 rats, it seems possible from the low LH concentrations in these rats that it had already occurred. We have in fact observed a tendency for ovulation to occur at the end of normal pseudopregnancy even before the vaginal smear pattern showed the characteristic sequence of changes seen in cyclic rats. Furthermore, the ovulation that occurs following cessation of progesterone treatment of cyclic rats is not necessarily preceded by a proestrous smear or a ballooned uterus (J. C. Hoffmann and N. B. Schwartz, unpublished observations). On the other hand, a drop in pituitary gonadotrophin concentration occurs on day 12 of pregnancy in rats(17) and on day 12 post partum (compared with day 6) in lactating mice(18), under circumstances in which ovulation probably does not take place. The point obviously needs to be settled in the case of pseudopregnancy by direct observation for ovulation.

Summary. The LH concentration (measured by the ovarian ascorbic acid depletion assay) in the pituitaries of pseudopregnant rats showed a fairly progressive rise from day 1 of the diestrus through day 11. On day 1, the level (2.0 μg/mg dry wt) was equivalent to that seen in cyclic rats in estrus or on day 1 of diestrus. The levels virtually equalled or slightly exceeded those characteristic of proestrus (4.2 μg/mg dry wt) from day 4 through 8. On days 9 through 11, the mean potency (5.4 μg/mg dry wt) somewhat exceeded proestrous levels. On day 12 the LH concentrations (1.5 to 2.0 μg/mg dry wt) were again typical of estrous levels in cyclic rats.

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1. Schwartz, N. B., Bartosik, D., *Endocrinology,* 1962, v71, 756.
2. Schwartz, N. B., *The Physiologist,* 1963, v6, 270.
3. Rothchild, I., *Endocrinology,* 1962, v70, 303.
4. Parlow, A. F., *Fed. Proc.,* 1958, v17, 402.
5. McCann, S. M., Taleisnik, S., *Am. J. Physiol.,* 1960, v199, 847.
6. Mindlin, R. I., Butler, A. M., *J. Biol. Chem.,* 1937, v122, 673.
7. Bliss, C. I., *Drug. Stand.,* 1956, v24, 33.
8. Gorski, R. A., Barraclough, C. A., *Acta Endocrinol.,* 1962, v39, 13.
9. Schmidt, I. G., *Endocrinology,* 1937, v21, 461.
10. Hill, R. T., *J. Physiol.,* 1935, v83, 129.
11. Makepeace, A. W., *Am. J. Obstet. and Gynec.,* 1937, v37, 457.
12. Kammlade, W. G., Welch, J. A., Nalbandov, A. V., Norton, H. W., *J. Animal Sci.,* 1952, v11, 645.
13. Santolucito, J. A., Clegg, M. T., Cole, H. H., *Endocrinology,* 1960, v66, 273.
14. Robertson, H. A., Hutchinson, J. S. M., *J. Endocrin.,* 1962, v24, 143.
15. Robinson, G. E., Nalbandov, A. V., *J. Animal Sci.,* 1951, v10, 469.
16. Hoffmann, J. C., Schwartz, N. B., *Fed. Proc.,* 1964, v23, in press.
17. Zeiner, F. M., *Anat. Rec.,* 1952, v113, 255.
18. Greenwald, G. S., *Gen. and Comp. Endocrinol.,* 1962, v2, 453.

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Exacerbation of Glycosuria by Partial Hepatectomy in the Partially Depancreatized Rat.* (29174)

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These experiments show that removal of 70% of the liver in mildly or moderately diabetic rats is followed by increased glycosuria for several days. The glycosuria of severely diabetic rats was not increased by partial hepatectomy.

Methods. 108 male rats of the Sprague-Dawley strain weighing approximately 300 g were partially depancreatized. Six to 8 weeks later each rat was placed in a metabolism

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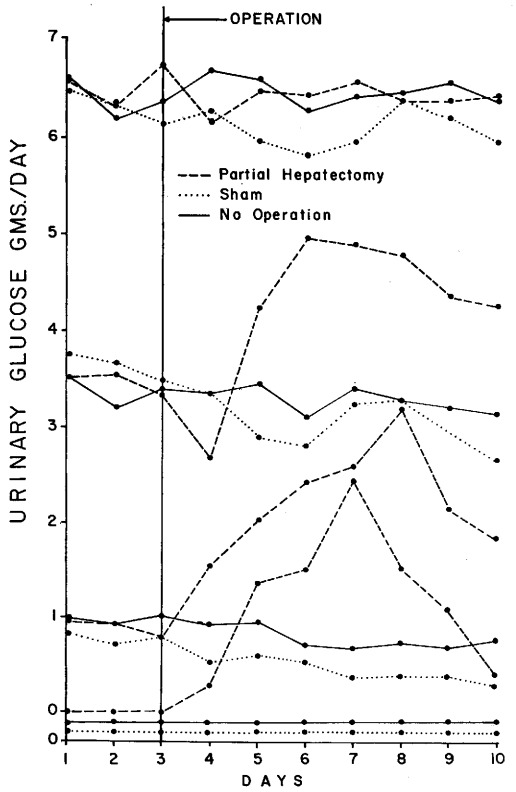

FIG. 1. Effect of removing 70% of liver on urinary glucose of partially depancreatized rats. Averages for 6 rats per group.

cage and tube-fed a medium carbohydrate diet(1), 13 cc each early morning and late afternoon. Urine was collected in a 250 cc volumetric flask containing a few crystals of thymol and 1 g of citric acid.

Urinary non-protein nitrogen (NPN) and glucose were determined for each 24 hours. Non-protein nitrogen was measured by the Kjeldahl procedure and glucose by the potassium ferricyanide-potassium ferrocyanide oxidation-reduction. Creatinine and uric acid, also reducing substances, were measured and the values subtracted from total reducing substances to give the approximate value for urinary glucose. All determinations were by the Technicon AutoAnalyzer. After the levels of urine glucose and NPN had been stable for at least 2 weeks, the rats were grouped according to the severity of the glycosuria and randomly selected for partial hepatectomy, sham operation, or non-operated controls. Approximately 70% of the liver (median and lateral lobes) was removed in the first group; the sham operation for the second group was an abdominal incision, and the third group were maintained as before. For the first 2 groups clean technique was used and all rats were treated with 5 mg of streptomycin and 5,000 units of penicillin daily for a week following surgery; infections did not occur.

Results. The data are in Fig. 1 and 2. Each curve represents an average of 6 rats. In Experiment 1 (Fig. 1) the rats are grouped according to the severity of the glycosuria prior to operation. Severely diabetic rats did not excrete increased amounts of glucose following partial hepatectomy, but moderately and mildly diabetic rats with no spontaneous glycosuria all showed a sharp increase in urinary glucose following removal of the liver. There was no increase in glycosuria in control or sham-operated rats. Twelve non-depancreatized rats were each subjected to removal of 70% of liver without causing glycosuria in any animal.

In Experiment 2 (Fig. 2) rats having either very mild glycosuria or none were treated as in Exp. 1 except that urinary NPN was measured. Sham operations and partial hepatectomy caused a rise in NPN, but only partial hepatectomy caused exacerbation of glycosuria.

Discussion. The liver is involved in the uptake of glucose from the blood (storage and conversion) as well as the genesis and release of glucose into the blood. If pancre-

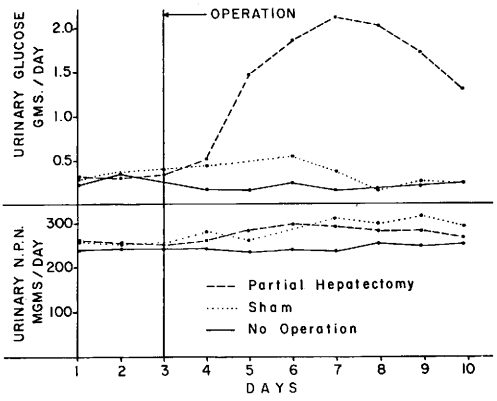

FIG. 2. Effect of removing 70% of liver on urinary glucose and NPN of partially depancreatized rats. Averages for 6 rats per group.

atic diabetes represented only overproduction of glucose, as was once claimed, removal of most of the liver should reduce hepatic gluconeogenesis and hence amount of urinary glucose. The exacerbation of glycosuria is consistent with the view that insulin deficiency causes failure of glucose utilization; the liver plays a primary role in uptake of dietary glucose, its storage as glycogen, and its conversion to fat. The fact that rats which were already wasting the equivalent of all the dietary carbohydrate into the urine did not develop more severe glycosuria following partial hepatectomy is also consistent with this hypothesis. Since partial hepatectomy was followed by an increase in NPN excretion which was roughly equivalent to that caused by a sham operation, it seems improbable that the glycosuria was caused by increased glyconeogenesis from protein. The explanation offered above, although plausible, is hypothetical. We have not studied the causes of the phenomenon and will not do so.

Summary. Partially depancreatized rats were tube-fed a medium carbohydrate diet. Removal of approximately 70% of the liver did not increase already severe glycosuria, but it did cause a marked exacerbation of glycosuria in moderately diabetic rats and in partially depancreatized rats without spontaneous glycosuria. A sham operation failed to cause excretion of more glucose although there was increased excretion of non-protein nitrogen.

1. Ingle, D. J., *Am. J. Physiol.*, 1953, v172, 115.

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Preparation of a Purified Vaccinia Virus Freed from Soluble Antigens. (29175)

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For the preparation of purified vaccinia virus material differential centrifugation, treatment with fluorocarbon and sedimentation in sucrose density gradients have been successfully employed(1,2,3). These preparatory methods were modified and used in the present study. For testing the degree of purity electron microscopy and the double diffusion-in-gel technique were employed.

Material and methods. Virus. The vaccinia virus used was a rabbit skin-adapted strain obtained from Dr. D. Peters, Inst. of Trop. Diseases, Dept. of Virology, Hamburg, Germany.

Production of crude virus material. The skin of rabbits weighing 3-4 kg was prepared and inoculated principally according to Hoagland *et al*(4). Each rabbit received 15 ml of a virus suspension containing approximately 10^8 plaque forming units (PFU) per ml. The skins were harvested 3 days after infection. The material obtained from each skin was suspended in 50 ml of 0.05 M Tris buffer pH 7.5 containing 200 IU of penicillin and 200 μg of streptomycin per ml.

Pooled suspensions from 6 rabbits were homogenized. They were treated 2 $\times$ 1 minute at maximum speed with an Ultra-Turrax homogenizer in an ice-bath. The homogenized material, the crude virus suspension, was designated preparation Cr.

Purification of virus. To 100 ml of the Cr preparation 5 ml of Trifluorotrichloroethane were added and the mixture was homogenized as mentioned above. The material was centrifuged at 1,500 *g* for 15 minutes; the sediment was homogenized with 4 volumes of buffer and then centrifuged. The supernatants obtained were pooled and designated preparation Tr.

The Tr preparation, 375 ml, was spun in the Spinco model L centrifuge, rotor 30, for