the past to reconstitute the *M. phlei* particles with *Azotobacter* supernatant were unsuccessful by our methods of preparation. This may also reflect on the differences in DNP sensitivity of these various supernatants.

We should like to express our appreciation to Dr. ONCLEY of the Physical Chemistry Laboratories for the use of the Spinco preparative centrifuge, and to Mr. ROBERT THOMAS and Miss SARAH BOND for their technical assistance.

ARNOLD F. BRODIE
CLARKE T. GRAY

Leonard Wood Memorial Laboratory,
Department of Bacteriology and Immunology, Harvard Medical School,
Boston, Mass. (U.S.A.)

1 A. F. BRODIE AND C. T. GRAY, *Biochim. Biophys. Acta*, 17 (1955) 146.
2 A. F. BRODIE AND C. T. GRAY, *Bacteriol. Proc.* (May 1955).
3 A. F. BRODIE AND C. T. GRAY, *J. Biol. Chem.* (in press).
4 Manuscript to be published.
5 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
6 W. D. WOSILAIT, A. NASON AND A. J. TERRELL, *J. Biol. Chem.*, 206 (1954) 271.
7 C. MARTIUS, *Angew. Chemie*, 67 (1955) 161.
8 W. D. WOSILAIT AND A. NASON, *J. Biol. Chem.*, 208 (1954) 785.
9 A. TISSIERES AND E. C. SLATER, *Nature*, 176 (1955) 736.

Received November 18th, 1955

Demonstration of direct effects of insulin on the isolated perfused diabetic rat liver*

Although administration of insulin to a diabetic animal is followed by correction of the characteristic defects in hepatic lipogenesis and glycogenesis, liver slices from diabetic rats are not influenced by insulin which is added *in vitro*[1,2]. Furthermore, no direct effect of insulin on net glucose uptake by isolated normal or diabetic liver tissue has yet been reported. LUNDSGAARD, in whose laboratory liver perfusions of short duration (three hours or less) were performed[3,4], states in a recent symposium, "I am not convinced that it has ever been proved that insulin has a direct effect on liver tissue"[5].

Development of a method in this laboratory for maintaining the isolated perfused rat liver for at least 6 hours[6] has permitted us to reinvestigate hepatic metabolism in diabetes with special reference to the action of insulin.

Rats of the Wistar strain, fasted 48 hours and weighing between 175 and 225 grams, were made diabetic by a single subcutaneous injection of 5% alloxan, 140 mg per kg, one hour after an injection of 1 unit/kg of insulin[7]. They were then maintained on daily injections of protamine zinc insulin for at least three weeks before use as liver donors, with no insulin being given them for 40 hours preceding an experiment. Normal and diabetic rats were allowed to eat a stock Purina pellet ration until sacrifice.

Liver perfusion apparatus and operative technique were essentially as described in previous reports from this laboratory[6,8]. Prior to incision of the abdomen, a specimen of tail blood was removed for determination of blood sugar content. Ketonuria was estimated semi-quantitatively through the use of Acetest tablets (Ames Co., Elkhart, Indiana). The initial liver glycogen was determined from the right lateral lobe which was ligated and removed during the operation. At the end of the perfusion two pieces of tissue weighing about 300 mg each were removed from different lobes of the liver for estimation of the final glycogen content[9]. The NELSON method[10] was used for all glucose determinations.

Total liver fatty acids were isolated by the usual methods and their radioactivity measured with a dynamic vane electrometer after oxidation to carbon dioxide[11].

In all experiments cited in the accompanying table, livers from rats weighing between 200 and 330 grams were perfused with approximately 140 ml of rat blood diluted one-third with Ringer's solution and containing a total of 60 mg of heparin. To the perfusing blood were added 500 mg of glucose and 35 mg of sodium acetate-1-^{14}C which had a total radioactivity of 2200 arbitrary units**. In experiments No. 173, 197 and 274 the entire labelled substrate was added

* These studies were performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York, and were supported in part by a research grant from the Medical Research and Development Board Office of the Surgeon General Department of the Army under Contract No. DA-49-007-MD-451.

** Each unit is equivalent to $2.2 \cdot 10^4$ disintegrations/minute.

TABLE I

DIRECT EFFECTS OF INSULIN ON CARBOHYDRATE BALANCE AND LIPOGENESIS IN THE ISOLATED PERFUSED RAT LIVER

Expt. No.	*Liver donor at operation*			*Units insulin during perfusion*	*mg change in total carbohydrate**	*Activity recovered in liver fatty acids***
	State	*Blood sugar*	*Urine ketones*			
139	Normal fed			0	N.M.***	35.0
173	Normal fed			0	N.M.***	40.0
274	Normal fed			0	— 50	119.0
300	Normal fed			0	— 29	62.0
143	Diabetic fed	450	0	0	N.M.	10.0
197	Diabetic fed	620	0	0	+331	2.6
308	Diabetic fed	480	0	0	+255	9.0
160	Diabetic fed	370	++++	0	N.M.	4.6
315	Diabetic fed	408	++++	0	+320	4.0
148	Diabetic fed	490	0	8.5	N.M.	65.0
310	Diabetic fed	445	0	8.5	— 85	28.0
322	Diabetic fed	609	0	8.5	+ 43	88.0
336	Diabetic fed	543	++	35.0	+103	49.0
319	Diabetic fed	374	++++	8.5	+300	3.8
343	Diabetic fed	408	++++	35.0	+264	7.0

* △ (circulating blood glucose) + △ (liver glycogen).
** Arbitrary units per liver. *** No measurement.

to the circulating blood at 0 time. In the others it was infused at a constant rate during the first 2½ hours. The insulin, wherever used, was glucagon-free (generously supplied by the Eli Lilly Co.) and was added by continuous infusion throughout the experiment. Perfusions No. 139, 143, 148 and 160 continued for six hours; the others were terminated at four hours.

The data are corrected to an arbitrary standard 300 cm^2 body surface area, calculation having been made from body weight and a regression formula derived from data of LEE[12].

Inspection of the table shows that insulin has a profound effect on the isolated *perfused* diabetic liver with respect to both lipogenesis and carbohydrate balance. Fatty acid synthesis is restored to the normal range within four hours. It is therefore quite unnecessary to postulate an indirect effect of insulin on diabetic liver mediated in some way by the extra-hepatic tissues[2,3,5].

Failure to elicit an insulin effect occurred only when the liver donor was severely ketotic (Experiments 319 and 343). The role of insulin in ketosis is to be studied further.

A more detailed report of these and other liver perfusions is now in preparation.

Department of Radiation Biology and Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. (U.S.A.)

DAVID E. HAFT*
LEON L. MILLER

[1] R. O. BRADY AND S. GURIN, *J. Biol. Chem.*, 187 (1950) 589.
[2] A. E. RENOLD, A. B. HASTINGS, F. B. NESBETT AND J. ASHMORE, *J. Biol. Chem.*, 213 (1955) 135.
[3] N. A. NIELSEN, *Skand. Arch. Physiol.*, 66 (1933) 19.
[4] E. LUNDSGAARD, *Johns Hopkins Hosp. Bull.*, 63 (1938) 90.
[5] E. LUNDSGAARD, *Josiah Macy Jr. Foundation Twelfth Conf. on Liver. Inj.* (publ. 1954) 11.
[6] L. L. MILLER, C. G. BLY, M. L. WATSON AND W. F. BALE, *J. Exptl. Med.*, 94 (1951) 431.
[7] N. ALLEGRETTI AND V. FISTER, *Arch. Intern. Physiol.*, 61 (1953) 41.
[8] L. L. MILLER, W. T. BURKE AND D. E. HAFT, *Federation Proc.*, 14 (1955) 707.
[9] C. A. GOOD, H. KRAMER AND M. SOMOGYI, *J. Biol. Chem.*, 100 (1933) 485.
[10] N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.
[11] W. F. BALE, unpublished.
[12] M. O. LEE, *Am. J. Physiol.*, 89 (1929) 24.

Received November 18th, 1955

* Post-doctoral Fellow of the National Institute of Arthritis and Metabolic Diseases, U.S Public Health Service.