Effect of Phenformin on Peripheral Glucose Utilization in Human Diabetic and Nondiabetic Subjects

Leonard L. Madison, M.D., and Roger H. Unger, M.D., Dallas

Neither the precise biochemical mechanisms whereby phenethylbiguanide (DBI) lowers blood glucose concentration in human subjects nor its site of action, whether in the peripheral tissues and/or the liver, are known. In the absence of a urinary loss of glucose or glucose intermediaries, hypoglycemia can result only from an increase in peripheral glucose utilization or a decrease in hepatic output of glucose. Although many factors may be involved in either increased peripheral utilization or decreased hepatic production of glucose, the localization of the site of action of DBI might offer some insight into its possible mechanism of action.

The present study was designed to determine the effect of DBI on peripheral glucose utilization in diabetic and normal human subjects. Changes in peripheral glucose utilization* were measured by following the changes in femoral arteriovenous glucose concentration difference after the administration of DBI.

METHODS

Fourteen normal and eleven diabetic subjects were studied. Blood glucose concentration was measured in all twenty-five subjects after the administration of DBI and changes in arteriovenous glucose difference were followed in five of the normal and seven of the diabetic subjects. The studies were performed in a constant temperature room with the subjects in the postabsorptive state. All the diabetic subjects were of the "maturity onset" or stable type and were controlled by diet alone or by diet plus tolbutamide, in which case the sulfonylurea therapy was stopped forty-eight to seventy-two hours prior to each experiment.

Blood samples were collected simultaneously from the femoral artery and the contralateral femoral vein by means of indwelling Cournand needles. After collecting two paired control blood samples, 150 to 350 mg. of DBI were given orally over a one- to two-hour period. Immediately after starting DBI administration blood samples were collected at twenty-minute intervals for five hours in the diabetic subjects; in normal subjects, blood specimens were obtained at thirty-minute intervals from the first to the fourth hour and at twenty-minute intervals for the ensuing two hours.

Glycolysis was minimized by collecting the blood in chilled tubes containing sodium fluoride. Immediately thereafter blood glucose was determined in duplicate by the Somogyi iodometric method[1,2] on 5 ml. of filtrate prepared from 2 ml. blood samples; in our laboratory duplicate determinations checked within 1 mg. per cent, thereby permitting as Somogyi has already indicated[3] the valid measurement of arteriovenous glucose concentration differences of as little as 2 mg. per cent.

RESULTS

Changes in femoral arterial and venous glucose concentrations and in arteriovenous glucose differences following the administration of DBI to normal and diabetic subjects are listed in tables 1 and 2 respectively. Examples of experiments in one normal subject and two diabetic subjects are shown in figures 1-3.

In the fourteen normal subjects, the acute administration of 150-300 mg. of DBI failed to lower blood glucose concentration significantly (table 1). In contrast, the administration of 200-350 mg. of DBI to eleven diabetic subjects produced a decline in blood glucose which was apparent within the first hour (table 2).

In both the five normal subjects and the seven dia-

*Peripheral glucose utilization is used to connote peripheral glucose disappearance which in turn may result from either increased oxidative metabolism, glycogen storage, lactic acid production or conversion to fat.

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 5, 1959.

From the Departments of Medicine, The University of Texas Southwestern Medical School and the Veterans Administration Hospital, Dallas, Texas.

TABLE 1

Effect of DBI on arterial and venous glucose concentrations in normal subjects

Subject	Age	Dose DBI mg.		Control 0-15	Control 0	Time after DBI administration 60	90	120	150	180	210	240	260	280	300	320	340	360
K.P.W.	28	225	Art.	87.0	85.6	86.5	85.1	84.5	84.0	82.6	86.2	93.2	88.7	89.0	86.2	82.6	83.4	82.3
59 kg.			Ven.	85.6	84.5	83.7	84.2	84.2	83.1	80.9	84.5	92.9	87.6	89.6	85.1	80.9	82.8	82.3
			A-V	1.4	1.1	2.8	0.9	0.3	0.9	1.7	1.7	0.3	1.1	—0.6	1.1	1.7	0.6	0.0
H.B.	23	200	Art.	87.8	87.3	85.8	86.1	85.3	87.8	84.4	84.1	85.0	86.3	87.2	90.3	91.0	91.5	92.1
82 kg.			Ven.	86.1	83.6	84.1	84.1	82.4	87.4	82.7	82.7	82.7	83.3	88.0	90.6	89.8	88.9	92.1
			A-V	1.7	3.7	1.7	2.0	2.9	0.4	1.7	1.4	2.3	3.0	—0.8	—0.3	1.2	2.6	0.0
B.K.	26	200	Art.	90.1	90.4	87.0	85.7	84.4	84.7	84.1	82.7	83.0	85.3	81.3	79.6	80.4	80.4	80.7
68 kg.			Ven.	87.0	87.3	85.3	83.9	82.4	82.4	83.8	80.4	81.6	83.0	79.9	77.9	79.9	79.0	78.4
			A-V	3.1	3.1	1.7	1.8	2.0	2.3	0.3	2.3	1.4	2.3	1.4	1.7	0.5	1.4	2.3
M.L.	26	175	Art.	83.8	84.4	84.4	84.1	83.8	81.9	80.1	77.3	78.7	78.4	83.6	83.6	82.4	82.7	82.4
60 kg.			Ven.	82.7	83.6	82.4	82.0	81.6	79.3	76.1	75.0	77.8	76.1	82.1	82.1	79.9	79.3	79.6
			A-V	1.1	0.8	2.0	2.1	2.2	2.6	4.0	2.3	0.9	2.3	1.5	1.5	2.5	3.4	2.8
J.H.	25	300	Art.	78.0	78.6	77.6	77.0	78.4	77.0	80.8	76.8	73.0	71.0	68.0	69.2	70.8	70.4	70.0
74 kg.			Ven.	76.8	78.0	76.0	74.0	77.6	76.0	78.8	75.6	71.6	68.4	67.2	68.4	68.0	68.8	68.4
			A-V	1.2	0.6	1.6	3.0	0.8	1.0	2.0	1.2	1.4	2.6	0.8	0.8	2.8	1.6	1.6
N.K.	27	200	Ven.		92.9	91.2		88.9		90.3		86.1			93.7			89.2
J.K.	26	200	Ven.		93.7	93.1		91.2		87.0		82.8			82.8			80.8
M.C.	30	200	Ven.		76.0	74.4		75.6		84.8		75.6			80.4			80.6
C.A.	24	150	Ven.		86.0	83.2		83.6		86.2		88.0			90.0			95.6
P.H.	31	200	Ven.		80.2	83.0		87.0		90.8		91.0			93.8			92.6
L.F.	40	200	Ven.		73.2	80.6		78.0		85.4		75.2			83.0			84.0
C.R.	38	150	Ven.		85.0	82.4		84.4		83.4		84.6			83.4			89.6
J.A.	27	150	Ven.		58.0	68.0		68.0		59.6		60.2			59.0			70.8
G.C.	35	200	Ven.		83.6	83.6		89.6		74.0		89.2			92.0			88.0

TABLE 2

Effect of DBI on arterial and venous glucose concentrations in diabetic subjects

Subject	Age	Dose DBI mg.		Control 0-10	Control 0	Time after DBI administration 20	40	60	80	100	120	140	160	180	200	220	240	260	280	300
M.S.	61	250	Art.	157.3	157.2	157.5	151.0	149.0	141.8	137.2	127.8	126.5	122.3	118.5	112.4	106.2	104.5	102.6	100.7	98.8
84 kg.			Ven.	155.3	155.0	158.4	152.0	145.5	140.8	135.0	131.0	125.5	122.3	119.5	111.8	106.8	104.8	100.3	101.2	98.5
			A-V	2.0	2.2	—0.9	—1.0	3.5	1.0	2.2	—3.2	1.0	0.0	−1.0	0.6	—0.6	—0.3	2.3	−0.5	0.3
F.L.	60	300	Art.	162.4	163.4	159.0	154.4	150.2	146.4	140.6	136.0	131.0	127.0	124.0	119.3	114.4	110.6	106.0	100.2	96.0
81kg.			Ven.	160.1	161.1	158.4	154.0	150.6	145.1	138.9	136.0	129.0	125.0	122.2	118.0	114.4	108.2	104.8	101.0	96.0
			A-V	2.3	2.3	0.6	0.4	—0.4	1.3	1.7	0.0	2.0	2.0	1.8	1.3	0.0	2.4	1.2	−0.8	0.0
M.F.	43	350	Art.	138.1	136.4	135.0	128.2	120.1	117.7	113.9	108.3	103.4	97.1	92.5	85.2	79.6	74.0	73.2	71.0	70.0
67 kg.			Ven.	136.6	133.8	134.0	126.1	118.9	118.0	110.9	105.8	102.2	96.8	90.6	81.4	76.9	72.0	72.0	68.4	68.0
			A-V	1.5	2.6	1.0	2.1	1.2	—0.3	3.0	2.5	1.2	0.3	1.9	3.8	2.7	2.0	1.2	2.6	2.0
B.D.	65	300	Art.	250.0	252.0	255.0	249.0	242.0	236.0	234.0	234.0	224.0	212.0	209.0	204.0					
73 kg.			Ven.	250.0	252.0	254.0	247.0	240.0	236.0	233.0	234.0	223.0	212.0	209.0	203.0					
			A-V	0.0	0.0	1.0	2.0	2.0	0.0	1.0	0.0	1.0	0.0	0.0	1.0					
V.B.	52	300	Art.	198.6	197.6	194.9	191.8	184.5	177.0	169.8	165.9	165.2	165.4	163.7	158.6	158.8	156.4	155.4	151.3	152.3
60 kg.			Ven.	194.7	197.1	195.2	188.1	182.6	177.0	171.8	165.7	162.3	161.3	160.0	157.1	159.8	155.4	157.4	152.0	149.1
			A-V	3.9	0.5	—0.3	3.7	1.9	0.0	—2.0	0.2	2.9	4.3	3.7	1.5	—1.0	1.0	—2.0	—0.7	3.2
D.J.	44	350	Art.	133.0	132.7	129.8	128.4	120.2	116.4	109.2	108.4	108.7	105.2	104.4	98.5	98.5	97.5	92.6	91.0	89.4
72 kg.			Ven.	132.2	131.4	127.6	124.7	119.1	116.7	109.5	107.9	108.4	104.9	101.5	99.1	98.5	96.1	92.6	92.4	89.2
			A-V	0.8	1.3	2.2	3.7	1.1	—0.3	—0.3	0.5	0.3	0.3	2.9	—0.6	0.0	1.4	0.0	—1.4	0.2
J.S.	58	300	Art.	102.0	98.5	98.0	94.4	87.6	84.6	83.0	84.9	80.4	80.1	80.4	77.7	75.3	75.0	74.5	74.5	76.1
70 kg.			Ven.	100.9	97.5	96.4	93.6	89.2	86.0	84.6	83.8	78.8	77.4	76.6	76.4	75.0	73.7	73.4	71.8	72.4
			A-V	1.1	1.0	1.6	0.8	—1.6	—1.4	—1.6	1.1	1.6	2.7	3.8	1.3	0.3	1.3	1.1	2.7	3.7
G.C.	47	200	Ven.		165			149			137			122			110			97
M.D.	58	200	Ven.		132			128			113			110			97			97
H.F.	35	200	Ven.		283			291			274			266			266			262
T.E.	40	250	Ven.		138			118			107			93			74			73

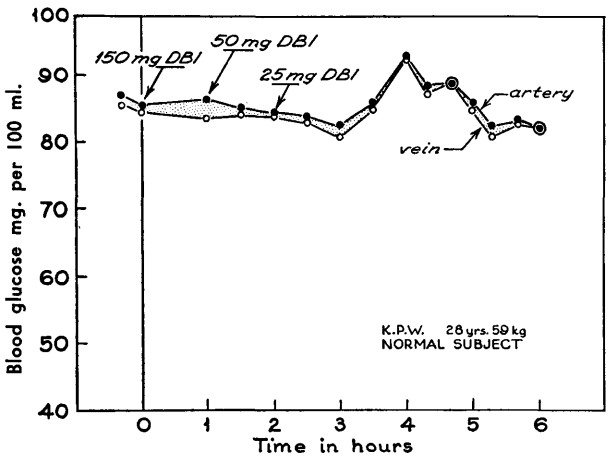

FIG. 1. The effect of DBI on arterial and venous glucose concentrations in a normal subject.

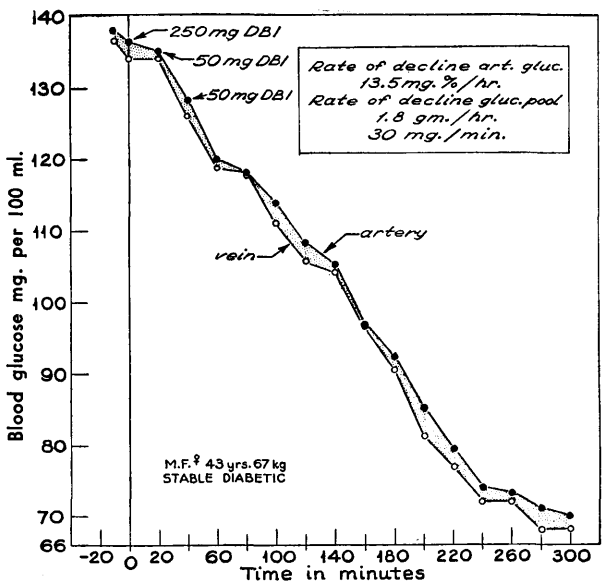

FIG. 2. The effect of DBI on arterial and venous glucose concentrations in a diabetic subject.

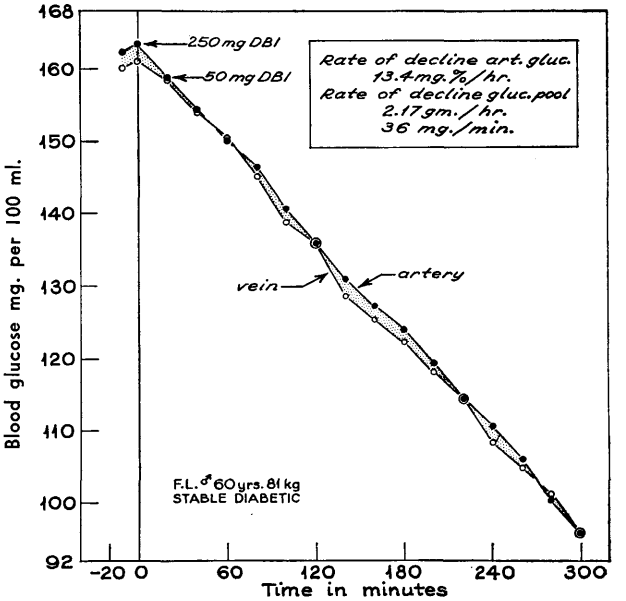

FIG. 3. The effect of DBI on arterial and venous glucose concentrations in a diabetic subject.

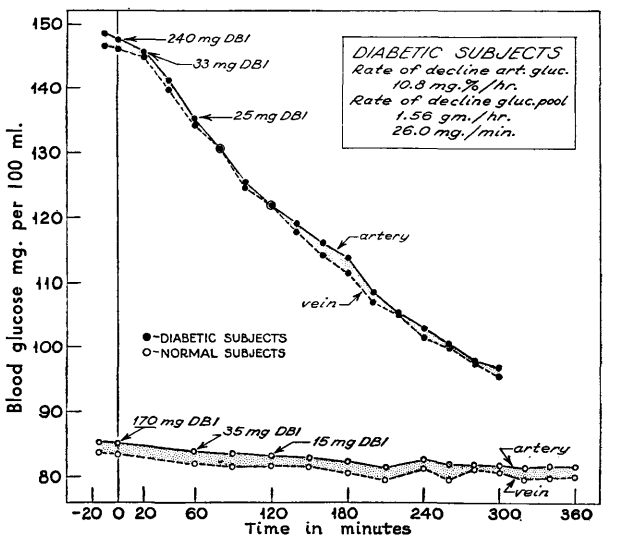

FIG. 4. Comparison of the effects of DBI on mean arterial and venous glucose concentrations in seven diabetic and five normal subjects.

betic subjects, arteriovenous glucose difference did not increase, despite the fall in arterial blood glucose in the diabetic subjects (tables 1 and 2, figures 1-4). Arteriovenous glucose difference during the control periods averaged 1.8 mg. per cent in the normal subjects and 1.7 mg. per cent in the diabetic subjects; after DBI administration arteriovenous glucose difference averaged 1.6 mg. per cent and 1.0 mg. per cent for the normal and diabetic subjects respectively (figure 4).

DISCUSSION

The failure of DBI to lower the blood glucose concentration in *normal human subjects* contrasts sharply with its effect in nondiabetic laboratory animals of several species including the rat, cat, guinea pig, rabbit, and monkey.[4] Although the dose administered to nondiabetic human subjects (> 3 mg./kg.) was less than that given to nondiabetic animals (5-20 mg./kg.),[4] this dose, nevertheless, reduced the blood glucose of diabetic human subjects. This difference in blood glucose response between nondiabetic human subjects and nondiabetic laboratory animals suggests that the biochemical changes[5-7] following administration of DBI,

which are said to be causally linked with the hypoglycemic effect in laboratory animals, may not be operative in human diabetic subjects.

The failure of DBI to lower blood glucose concentration in nondiabetic human subjects is of considerable interest but is not explained by these data. Although it may be related to the initial level of the blood glucose, it should be noted that a hypoglycemic effect occurred in diabetic subjects with normal and near normal fasting blood glucose concentrations (subjects J.S., M.F., D.J., table 2). Alternatively, the blood glucose lowering effect of DBI in diabetic but not in nondiabetic subjects suggests a specific metabolic defect in the diabetic group which is altered by DBI.

The failure to find any increase in femoral arteriovenous glucose difference associated with the fall in blood glucose concentration in the subjects with stable diabetes indicates that the hypoglycemic effect was either not the consequence of increased peripheral glucose utilization or that the rate of increased peripheral glucose utilization was too small to be measured by the technics of this study. The latter possibility seems unlikely in view of the magnitude of glucose utilization by muscle in human subjects in the basal state compared to the magnitude of decline in the glucose pool in the present experiments.

The rate of decline in the glucose pool, assuming that the glucose space is limited to the volume of distribution of the extracellular fluid, is shown in table 3. The decline in the glucose pool varied from 11.3 to 36 mg. per minute and averaged 26 mg. per minute. Despite the fact that the muscle mass constitutes approximately 40 per cent of body weight, in the basal state only about 22 mg. of glucose are utilized per minute by the entire muscle mass.[8] This rate of utilization by the muscles in the resting postabsorptive state is associated with a mean femoral arteriovenous glucose difference of about 1.8 mg. per cent in this and other studies.[8-10] If the mean rate of decline in glucose pool of 26 mg. per minute, found in this study after DBI (table 3), was entirely the result of increased glucose utilization by the muscles, a doubling of the femoral arteriovenous glucose difference would have been expected provided there was no alteration in peripheral blood flow.

It is possible, however, that an increase in peripheral glucose utilization occurred but because it was confined for the most part to the adipose tissue rather than to muscle, could not be measured by the technic used in this study; blood specimens were collected from the femoral rather than the saphenous vein in these experiments. They therefore most likely reflected changes in muscle rather than in adipose tissue metabolism.

TABLE 3

Effect of DBI on rate of glucose disappearance in diabetic subjects

Subject	Wt. kg.	Glucose pool liters	Fall blood glucose mg./100 ml./hr.	Decrease in glucose pool gm./hr.	Decrease in glucose pool mg./min.
M.S.	84	16.8	11.7	1.90	31.6
F.L.	81	16.2	13.4	2.17	36.1
M.F.	67	13.4	13.5	1.80	30.0
B.D.	73	14.6	14.0	2.04	34.0
V.B.	60	12.0	9.2	1.10	18.3
D.J.	72	14.4	8.7	1.25	20.9
J.S.	70	14.0	4.8	0.68	11.3
Mean	72	14.5	10.8	1.56	26.0

The *in vitro* evidence[6,7,11] that DBI lowers blood glucose primarily by decreasing oxidative metabolism, and, as a consequence of this, secondarily increasing anaerobic glycolysis is not supported by these data insofar as the peripheral muscles are concerned. The increased utilization of glucose by muscle, whether the result of increased oxidative metabolism, increased glycogen storage or increased conversion to lactate, should have resulted in an increased femoral arteriovenous glucose difference.

The failure in these studies on *human subjects* to ascribe the hypoglycemic effect of DBI to increased glucose utilization by the peripheral muscles, coupled with the failure of Tranquada et al.[12] to obtain evidence of decreased splanchnic glucose output suggests two possible alternate explanations for the hypoglycemic effect. One, the adipose tissue may be a major locus of action of DBI. Two, *more likely* the increase in the rate of glucose utilization of only 26 mg. per minute may have been divided between the liver and the peripheral tissues and therefore would be difficult to detect by measuring changes in either femoral arteriovenous glucose difference or in splanchnic glucose output.

SUMMARY

The changes in arterial blood glucose concentration following the administration of phenformin were followed in fourteen normal and eleven diabetic subjects. The effect of phenformin on peripheral glucose utilization was studied by following changes in femoral arteriovenous glucose difference in five of the normal and seven of the diabetic subjects. Phenformin failed to lower the arterial blood glucose or alter the arteriovenous glucose difference in all the normal subjects. In diabetic subjects despite a fall in mean arterial blood glucose from 148 to 94 mg. per cent, representing a mean decrease in the

glucose pool of 26 mg. per minute, there was no evidence of increased peripheral glucose utilization as manifest by the absence of any increase in femoral arteriovenous glucose concentration difference. The possibility that the hypoglycemic action of phenformin *in diabetic human subjects* was either the consequence of an effect of phenformin on adipose tissue or, more likely, the result of a simultaneous effect on both hepatic and peripheral tissues, was discussed.

SUMMARIO IN INTERLINGUA

Le Effecto de Phenformina Super le Utilisation Peripheric de Glucosa in Humanos Diabetic e Non-diabetic

Le alteration del concentration de glucosa in le sanguine arterial esseva observate post le administration de phenformina (DBI) in dece-quatro subjectos normal e in dece-un diabeticos. Le effecto de DBI super le utilisation peripheric de glucosa esseva studiate per observar alterationes occurrente in le differentia arteriovenose femoral de glucosa in cinque del normales e in septe del diabeticos. DBI non reduceva le glucosa del sanguine arterial e non alterava le differentia arteriovenose de glucosa in ulle del subjectos normal. In le subjectos diabetic — in despecto del reduction del nivello medie de glucosa in sanguine arterial ab 148 a 94 mg pro cento, lo que representa un reduction medie de glucosa in le reserva de 26 mg per minuta — nulle indication esseva trovate de un augmento del utilisation peripheric de glucosa, a judicar per le absentia de omne augmento in le differentia arterio-venose femoral del concentration de glucosa. Le possibilitate es discutite que le effecto hypoglycemic de DBI in patientes diabetic es (1) le consequentia de un effecto de DBI sur tissu adipose o (2), lo que es plus probabile, le resultato de un effecto simultanee sur le tissu hepatic e sur le tissu peripheric.

REFERENCES

[1] Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69, 1945.

[2] Somogyi, M.: Notes on sugar determination. J. Biol. Chem. *195*:191, 1952.

[3] Somogyi, M.: Studies of arteriovenous glucose differences in blood sugar. I. Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. J. Biol. Chem. *174*: 189, 1948.

[4] Ungar, G., Freedman, L., and Shapiro, S. L.: Pharmacological studies of a new oral hypoglycemic drug. Proc. Soc. Exper. Biol. & Med. *95*:190-92, 1957.

[5] Williams, R. H., and Steiner, D. F.: The effects of biguanide compounds upon respiratory enzymes. Clin. Res. 6: 55, 1958.

[6] Wick, A. N., Larson, E. R., and Serif, G. S.: A site of action of phenethylbiguanide, a hypoglycemic compound. J. Biol. Chem. *233*:296-98, 1958.

[7] Williams, R. H., Tyberghein, J. M., Hyde, P. M., and Nielsen, R. L.: Studies related to the hypoglycemic action of phenethylbiguanide metabolism. *6*:311-19, 1957.

[8] Andres, R., Cader, G., and Zierler, K. L.: The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. Clin. Invest. *35*:671-82, 1956.

[9] Bell, D. M.: Femoral arteriovenous sugar differences in fasting human beings. J. Lab. & Clin. Med. *40*:337, 1952.

[10] Madison, L. L., and Unger, R. H.: The physiologic significance of the secretion of endogenous insulin into the portal circulation. II. Comparison of the effects of glucagon-free insulin administered by rapid intravenous injection and slow intravenous infusion on its major site of action. Metabolism 9:97-108, 1960.

[11] Fajans, S. S., Moorehouse, J. A., Doorenbos, H., Louis, L. H., and Conn, J. W.: Metabolic effects of phenethylformamidinyliminourea (DBI) in normal subjects and in diabetic patients. Clin. Res. *6*:252, 1958.

[12] Tranquada, R., Kleeman, C., Brown, J.: The acute effects of DBI on human hepatic intermediary metabolism. Clin. Res. 7:110, 1959.

Physiological Genetics

The idea that genes control the properties of cells by determining the enzymes that are present goes back in a sense to the earliest years of the century, and the idea that genes act by imposing specific patterns in the process of synthesis of macromolecules, whether in their own duplication, in the production of antigenic specificity, or in that of the enzymes, is also far from recent. These conjectures had, however, little or no real impact on general physiology. Until very recently, the textbooks in that subject rarely made any mention of the gene and treated the cell as the ultimate unit of life.

The real breakthrough came recently when systematic exploration of the control of elementary processes of the mould *Neurospora* was made by the combined efforts of a geneticist and a biochemist, Beadle and Tatum. Now we have a rapid expansion of biochemical genetics or genetic biochemistry. What had been two almost airtight compartments of biology are now inextricably interwoven.

From "Genetics and the Hierarchy of Biochemical Sciences," by Sewall Wright, in *Science,* Vol. 130, No. 3381, pp. 959-65, Oct. 16, 1959.