o,p'-DDT preparations, by virtue of the open positions on the ring bearing the *o*-Cl, is rapidly converted to hydroxy and methoxy metabolites that are rapidly excreted in the feces (*11*). In contrast, *p,p'*-DDT, the minor impurity, is relatively inert metabolically and accumulates in the lipid of the animal body.

Thus, this differential metabolic behavior leads to the simultaneous disappearance of the major component of relatively pure samples of *o,p'*-DDT, with the concomitant appearance of the minor constituent, the *p,p'*-DDT impurity. There is no existing chemical or biological information or data to support the idea that *o,p'*-DDT is converted to the *p,p'*-DDT isomer.

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Methamphetamine-Induced Insulin Release

Abstract. *Administration of methamphetamine or amphetamine to rats and mice produces a rapid increase in the level of immunoassayable plasma insulin not attributable to hyperglycemia. While in the mouse this release of insulin is followed consistently by a profound hypoglycemia, in the rat this response is variable. Studies in vitro demonstrate that insulin is released by a direct effect of methamphetamine on the pancreas.*

Toxicity of *d*-amphetamine in rats and mice is manifested by enhanced motor activity and excitement, followed by depression. The latter state is particularly noted in acutely aggregated mice rather than in animals caged individually (*1*), and it is during this stage of depression that many of the animals die. This terminal lethargy resembles the manifestations of hypoglycemia, and indeed Moore *et al.* noted that such mice showed a marked decrease in blood glucose after intraperitoneal injection of *d*-amphetamine (*1*). These authors attributed the hypoglycemia to stress factors and a syndrome of experimental shock.

Amphetamine and its derivatives, however, are closely related structurally to tranylcypromine, a monoamine oxidase inhibitor that has been demonstrated to cause insulin release and hypoglycemia in the mouse (*2*). This similarity suggested that amphetamine may also produce hypoglycemia by releasing endogenous insulin, and the present studies were designed to evaluate this possibility by examining the effect of methamphetamine and amphetamine on insulin and glucose levels in mice and rats.

Initial studies were carried out on male Sprague-Dawley rats weighing 180 ± 10 g, which were housed in cages 9 by 8 by 6 inches (1 inch = 2.54 cm), two or three per cage; they were allowed free access to food and water. Four hours prior to drug administration, food was removed and animals were aggregated seven to eight per plastic cage (18 by 10 by 6 inches). Methamphetamine hydrochloride (30 mg/kg) in 0.5 ml of normal saline or saline alone was injected intraperitoneally. Swiss-Webster mice weighing 24 to 26 g were used in other studies. Similarly, food was removed 4 hours prior to injection, and the mice were injected intravenously with either methamphetamine (7.5 mg/kg) in 0.2 ml of saline or saline alone. They were then housed four animals per cage (11 by 6 by 5 inches). At appropriate intervals, rats and mice were killed by decapitation, and blood from the carotid arteries was collected in heparinized beakers. Concentrations of blood glucose in barium hydroxide–zinc sulfate filtrates and of plasma glucose were measured by a glucose oxidase method (*3*). Plasma insulin was measured against mouse insulin standards by a modification of a radioimmunoassay method in which the separation of bound and free insulin was performed by precipitating the bound insulin with a goat antiserum to guinea pig gamma globulin (*4*). Methamphetamine had no effect on the measurement of insulin in this system.

Studies of insulin release in vitro were carried out with mouse pancreas tissue by using a slight modification of a previously described incubation system (*5*). In the present studies, after an initial 15-minute preincubation, each piece of mouse pancreas underwent two sequential 30-minute incubations, and portions of each incubation medium were assayed for insulin. In all studies, glucose and insulin values for animals treated with drugs were compared with control animals injected simultaneously with saline, by use of Student's *t*-test.

Figure 1 illustrates the effect of methamphetamine (30 mg/kg, intraperitoneally) on insulin release in the rat. After methamphetamine injection, plasma insulin values rose rapidly, increasing three- to fourfold over control

Table 1. Time relation of insulin and glucose levels in mice. Swiss-Webster mice were injected intravenously with methamphetamine (7.5 mg/kg) in 0.2 ml of saline or an equal volume of saline alone as described in the text. After decapitation, blood from the carotid arteries was assayed for glucose and insulin as described. Each value represents the mean ± standard error of the mean (S.E.M.) of six observations and is compared (Student's *t*-test) with a control group killed at the same time.

Experimental group	Time after injection (minutes)			
	0	2.5	5	10
	Insulin (μunit/ml)			
Control (saline)	25 ± 6	14 ± 3	21 ± 6	27 ± 6
Methamphetamine	25 ± 6	44 ± 7*	85 ± 24†	36 ± 7‡
	Glucose (% zero time control)			
Control (saline)	100	90 ± 10	99 ± 8	95 ± 5
Methamphetamine	100	101 ± 4‡	92 ± 5‡	94 ± 6‡

* $P < .01$, different from control. † $P < .05$, different from control. ‡ Not significantly different from control.

levels at 15 minutes. This high insulin level fell off rapidly during the next 15 minutes but remained elevated significantly above control levels until 60 minutes after injection. Concentrations of plasma glucose in the rat during the same period are shown in the lower section of Fig. 1. Animals treated with methamphetamine showed a slight but significant hyperglycemia when compared with controls during the first hour after injection, a pattern paralleling the insulin values. The possibility existed that this insulin release could be secondary to hyperglycemia. Consequently, a study to determine the time relation of insulin release and concentration of glucose was carried out in mice that were injected intravenously with methamphetamine. Results of this study (see Table 1) demonstrate that in the 10 minutes immediately following methamphetamine injection there was no significant difference in glucose levels between mice treated with methamphetamine and control mice. Insulin levels, however, were significantly higher in mice treated with methamphetamine by 2.5 minutes after injection, reached a peak at 5 minutes, and declined by 10 minutes. Additional experiments were conducted in rats in which intraperitoneal injections of glucose were used to duplicate the plasma glucose elevation seen after administration of methamphetamine. These experiments showed that insulin release following injection of methamphetamine was significantly greater than that following hyperglycemia produced by injection of glucose.

To determine whether a direct effect on the pancreas might be part of the mechanism of methamphetamine-induced release of insulin, studies were carried out by incubating sections of mouse pancreas in modified Krebs-Ringer bicarbonate medium containing 0.6 mg of glucose per milliliter, as previously described (*5*). Insulin release did not differ in the two groups during the initial 30-minute incubation in basal medium alone. Addition of methamphetamine ($2.5 \times 10^{-3} M$), however, produced a marked increase in insulin release during the subsequent 30-minute experimental incubation (120 ± 26 μunit per 100 mg of pancreas) when compared with the control group (32 ± 10 μunit per 100 mg of pancreas) which was again incubated in basal medium alone ($n = 12$; $P < .02$).

These data suggest that methamphetamine releases insulin by a mechanism not dependent on hyperglycemia and that at least part of its activity is due to a direct effect on the pancreas. Further studies in the rat have demonstrated that *d*-amphetamine and meth-

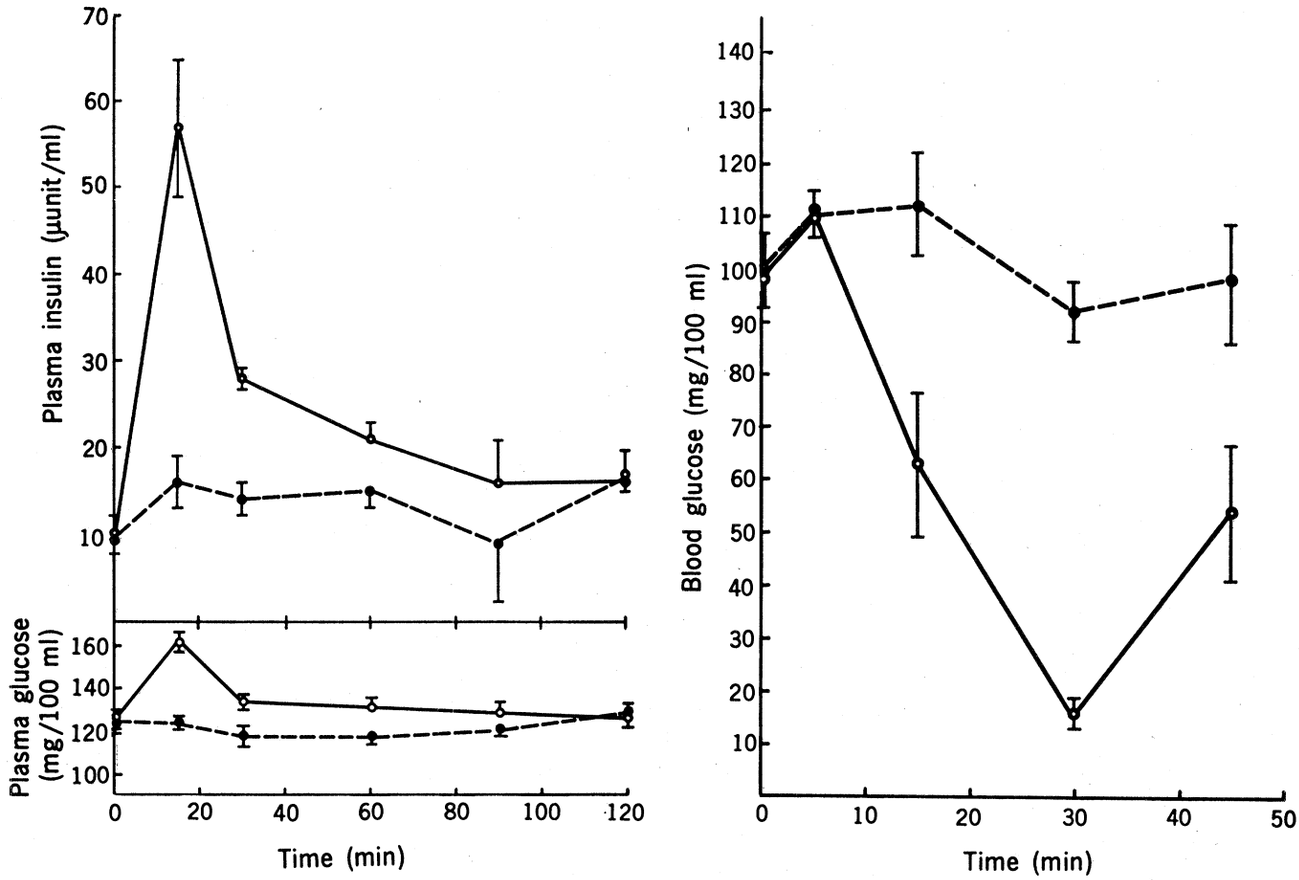

Fig. 1 (left). Effect of methamphetamine on plasma insulin and glucose in the rat. Each value represents the mean ± S.E.M. of at least eight animals, except the 90-minute control group, which contained four animals. Insulin values are expressed as microunits of insulin per milliliter of plasma; glucose levels are expressed as milligrams of glucose per 100 ml of plasma. Plasma glucose and insulin levels are significantly different from controls at 15 ($P < .001$), 30 ($P < .01$), and 60 ($P < .05$) minutes after injection. The methamphetamine groups received 30 mg/kg intraperitoneally in 0.5 ml of normal saline, while controls received an equal volume of saline. Solid lines indicate the group treated with methamphetamine; dashed lines indicate controls. Fig. 2 (right). Hypoglycemic effect of methamphetamine in the mouse. Each value represents the mean ± S.E.M. of five observations. Mice treated with methamphetamine received 7.5 mg/kg intravenously in 0.2 ml of normal saline, while controls received an equal volume of saline. Blood glucose concentrations in animals treated with methamphetamine are significantly lower than controls at 15 ($P < .05$), 30 ($P < .001$), and 45 ($P < .05$) minutes. Solid lines indicate animals treated with methamphetamine; dashed lines indicate controls.

amphetamine (30 mg/kg) are equipotent in releasing insulin in vivo (62 ± 1 versus 66 ± 15 μunit/ml at 15 minutes) and that methamphetamine (10 mg/kg) is effective in releasing insulin, with a three- to fourfold increase over control values at 15 minutes. Although the studies presented here demonstrate that methamphetamine can cause insulin release through a direct effect on the pancreas, additional mechanisms are certainly not ruled out. The possibility that the release of insulin by methamphetamine is partly mediated through a neural mechanism should be investigated.

The hypoglycemic effect of amphetamine described by Moore *et al.* (*1*) was also noted in our studies. As shown in Fig. 2, no significant change in blood glucose concentrations was seen until 15 minutes after intravenous injection of methamphetamine, at which time a significant decline of 40 percent was seen in the experimental animals when compared with their controls injected with saline. This hypoglycemia was even more marked at 30 minutes after injection and was still present at 45 minutes.

While methamphetamine administered to mice was followed consistently by severe hypoglycemia, this response in the rat was observed only in certain experiments. The factors precipitating such a reaction are unclear at this time, but recent data from our laboratory (*6*) suggest that the hyperinsulinemia combined with certain hormonal and environmental conditions may produce severe hypoglycemia in animals treated with methamphetamine.

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Kinetic Path of Genes Undergoing Selection

Abstract. *As natural populations approach genetic equilibrium, the various genes in the population are capable of assuming intermediate distributions that might not be anticipated from either the rate of the process or the final distribution of the genes. Since it is possible that many populations have not reached genetic equilibrium, the distribution of genes in natural populations may be a reflection of the kinetic path by which the genes approach equilibrium. Attention to kinetic path provides an explanation for an apparent discrepancy in recent studies of selection in man.*

Each large, natural population usually includes individuals of dissimilar genetic makeup. Two questions arise concerning the genetic characteristics of the population: (i) What will be the ultimate frequency of the various genes? and (ii) How rapidly will the change, if any, occur? The answer to either question may have considerable practical importance. Soon after Mendelian principles of heredity began to receive general recognition, an assertion was made that genetically dominant traits, such as the hereditary hand deformity known as brachydactyly, will increase in prevalence until a majority of the population has the dominant characteristic. To refute this assertion, the mathematician G. H. Hardy introduced calculations to show that the frequency of either of two alternative genes (alleles) at a single genetic locus remains unchanged when the population consists of a large number of randomly mating individuals who have neither a selective advantage nor disadvantage. Moreover, within one generation, the alleles distribute themselves in a predictable ratio among homozygotes and heterozygotes (*1*). His calculations serve as a basis for the Hardy-Weinberg equilibrium, one of the fundamental principles of genetics. Similarly, the successful medical treatment of rare, recessive disorders has generated recent concern that the prevalence of these disorders will increase with each generation until they become a major problem. Available prediction equations (*2*) and techniques of computer simulation (*3*) lead to the conclusion that the frequency of the abnormal genes may, indeed, increase in the population to some extent, but only after a lag of many millennia. These two aspects of genetic systems, equilibrium distribution and the rate of approach to equilibrium, are similar to those encountered in thermodynamics and chemical kinetics, fields in which both the extent and rapidity of a reaction are significant.

I now wish to call attention to the importance of a third characteristic of genetic systems: intermediate gene distribution. As genes approach equilibrium, they can distribute themselves in a manner which may not be anticipated from either the rate of the process or the ultimate fate of the genes. The intermediate distribution of the genes may be characteristic of the system and yet dissimilar to the ultimate distribution they will have when the system reaches equilibrium or fixation. Intermediate distributions of genes have received attention in numerous earlier studies of genetic systems. Examples are Lewontin and White's analysis of the adaptive surface for inversion in the grasshopper *Moraba scurra* (*4*) and Livingstone's use of the computer to simulate the approach to equilibrium of human populations with abnormalities of hemoglobin or deficiencies of glucose-6-phosphate dehydrogenase (*3*). The following is an example of a genetic process in which attention to the intermediate distribution of the genes provides an explanation for an apparent discrepancy in studies of selection in man.

Geographic (*5, 6*) and recent cellular (*7*) studies suggest that hemoglobin S (Hb S), hemoglobin C (Hb C), and β-thalassemia (β-th), which are determined by alleles at an autosomal locus, and sex-linked deficiency of human red cell glucose-6-phosphate dehydrogenase all provide a selective advantage against falciparum malaria. In support of this theory, a highly positive geographic correlation is found between the frequency of genes for glucose-6-phosphate dehydrogenase deficiency and those for either β-th or Hb S (*5*). When the frequency of Hb S in numerous villages is plotted against the frequency of either Hb C (*5*) or β-th (*8, 9*), however, a positive correlation is not seen (Fig. 1). The points tend to distribute themselves in a triangular area bounded by the major axes and a diagonal line of negative slope. This triangular area is much