Spontaneous Hereditary Diabetes Mellitus in the Chinese Hamster (*Cricetulus Griseus*)

III. Maintenance of a Diabetic Hamster Colony With the Aid of Hypoglycemic Therapy

Hans Meier, D.V.M., Ph.D., and George Yerganian, Ph.D., Boston

Spontaneous diabetes mellitus, as observed in Chinese hamsters, has recently been described.[1] Apart from the fact that spontaneous diabetes is unusual in a rodent species, the similarity of the pathological findings to certain human changes and (alloxan-induced) diabetes in experimental animals is of particular interest. The disease is primarily pancreatogenic, the pathological changes consisting of severe damage, vacuolar ("hydropic") degeneration, and deficiency of beta cells; this is borne out by insulin assays.[2] A concurrent pathological finding is diffuse intercapillary glomerulosclerosis associated with deposition of PAS-positive staining material, and leading to uremia in some animals.[1] Polyuria and polydipsia are usually the most prominent clinical signs; some hamsters excrete up to 70 ml. of urine in twenty-four hours.

Considerable evidence has accumulated to indicate that the disease is hereditary. Diabetes arose spontaneously during the course of inbreeding at a time when many of the sublines of four major families approached the fourth generation of continuous brother-sister mating. The average frequency of genic homozygosity was estimated to be 65 per cent at that time. Attempts at maintaining an extensive breeding program for diabetic hamsters have, so far, been successful. To date, four generations of diabetic hamsters have been obtained with the incidence of diabetes increasing sharply from the fourth to eighth generation, up to 90 per cent of the offspring (e.g., JBY family) becoming diabetic later in life. The degree of penetrance, severity of the disease and age of onset vary greatly among families.[3] Maintenance of fertility of diabetic parents was an ever present obstacle. Also, there were losses of up to 20 per cent of the animals during pregnancy (abortions, resorption) and liveborn animals (death soon after delivery, cannibalism). Repeated daily mating tests and treatment considerably improved the number of pregnancies and successful deliveries since the estrus cycles of each female varied greatly. Many animals are totally dependent upon careful control of diabetes for continued existence and maintenance of fertility.

The present report deals with preliminary pharmacologic studies of three hypoglycemic agents, NPH insulin, phenformin, and tolbutamide in both normal and diabetic hamsters. Special emphasis will be placed on experience gained in maintaining a colony of diabetic hamsters for the study of their genetic background. (In addition, other potentially hypoglycemic agents, e.g., thioglycolic acid[4] are being studied.)

EXPERIMENTAL

A. *Laboratory Tests.* Clinical biochemical tests were originally restricted to basic diagnostic determinations, such as blood and urine glucose, NPN, urine ketone bodies and specific gravity. Blood glucose levels for normal hamsters average 110 ± 6 mg. per 100 ml.; for diabetic hamsters between 200 and 800 mg. per 100 ml. The specific gravity of urine is greatly elevated in the glycosuric animals, from 1,018 to abnormal, 1,029++. In hamsters showing signs of uremia, blood NPN levels over 100 mg. per cent are found, normal being 45 ± 3 mg. per cent. In attempts to determine whether or not an animal is diabetic, rapid qualitative or semiquantitative tests (test sticks*) are conducted routinely. These prove especially helpful in early detection of diabetes and also in evaluating hypoglycemic therapy and establishing effective drug dosages.

B. *Methods.* Phenformin† (N-beta-phenethylformamidinyliminourea hydrochloride) was given orally (by stomach tube), subcutaneously and intraperitoneally. Tolbutamide, 1-butyl-3-p-tolylsulfonylurea (Na-Orinase‡) was administered both subcutaneously and intra-

From The Children's Cancer Research Foundation and the Departments of Pathology, The Children's Hospital and Harvard Medical School. Dr. Meier's present address is Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

* Ames Company, Inc., Elkhart, Indiana.

† Generously supplied by Dr. H. S. Sadow, U.S. Vitamin & Pharmaceutical Corporation, New York, N.Y.

‡ Obtained through the courtesy of Dr. C. J. O'Donovan, The Upjohn Co., Kalamazoo, Michigan.

peritoneally. NPH* (aqueous) insulin was used by the subcutaneous route. All hamsters received single injections either at one or two sites. When compound solutions intended for gastric tube feedings exceeded 1.5 cc., they were administered in two divided doses within fifteen minutes. The animals were maintained on Purina laboratory chow with supplementary wheat germ flakes and water ad libitum.

A total of 116 *normal* adult hamsters were employed to establish maximum tolerated doses of each compound and to evaluate *drug toxicity*. Two to four animals (males and females) were used for each dose level of the drugs and each route of administration.

Evaluation of drug toxicity was difficult because many animals died of hypoglycemia before any toxic effects could be observed histologically. Clinical evidence of toxicity, however, occurred both with phenformin and tolbutamide, depending upon the route of inoculation, even before hypoglycemic shock resulted. The interval between drug administration and death, from either hypoglycemia or other causes, was timed routinely.

In *diabetic* hamsters, comparative studies were done on the relative effectiveness of the agents in reducing elevated blood sugar levels. The least toxic route of administration was chosen for *therapy*.

RESULTS

A. *Toxicity in Normal Hamsters.* When phenformin was given subcutaneously, drug induced hypoglycemia reached its maximum in about five hours. Animals given 450 mg./kg. developed reversible hypoglycemic shock; at 540 mg./kg. (LD_{50}) half, and at 630 mg./kg. (LD_{100}) all hamsters died from hypoglycemia. Clinical signs were: fast and spasmodic breathing, hyperactivity, and terminal convulsions; blood sugar levels were as low as 20 to 40 mg. per 100 ml. The majority of animals, however, died an unexplained death almost immediately after injection, usually within five to thirty minutes and before lowering of the blood sugar level. Similarly, upon *intraperitoneal* injection of phenformin, most of the animals died without overt symptoms within one to fifteen minutes; when 180 mg./kg. (LD_{50}) was given, half of the animals living beyond the first hour died from hypoglycemia between five and fifteen hours. Little toxicity was encountered upon *oral* administration, except for one death occurring within five minutes at 180 mg./kg. Irreversible hypoglycemia was induced via this route in approximately half of the animals with 1,620 mg./kg. (LD_{50}).

Tolbutamide given *subcutaneously* produced a hypoglycemic effect within three to four hours. At a dose of 178.5 mg./kg. (LD_{50}), half of the animals died from hypoglycemia between five and sixteen hours.

When tolbutamide was administered *intraperitoneally*, deaths from hypoglycemia occurred in half of the animals between five and sixteen hours at 178.5 mg./kg. (LD_{50}). Early and unexplained deaths, occurring within five to thirty minutes without hypoglycemia, resulted from doses of 2,412 mg./kg. to 4,824 mg./kg.

NPH insulin caused hypoglycemia within five to eight hours and later, with doses ranging from 16 to as many as 88 units per hamster (average weight 32 gm.).

B. *Therapy in Diabetic Hamsters.* In mildly diabetic hamsters (blood glucose between about 150 to 300 mg. per 100 ml.) it was possible to reduce elevated blood sugar levels to normal by appropriate doses of each of the drugs. The correct dosage was reached by gradual increases in daily amount. The starting dose depended upon the degree of diabetes, but was roughly one which lowered the blood glucose level in both healthy animals to about half normal. Normal blood sugar levels in severely diabetic animals, excreting 10 to 15 per cent or more urinary sugar per diem, could be maintained only with NPH insulin; both phenformin and tolbutamide were ineffective. In a few instances, diabetic hamsters died soon after the administration of any of the three compounds, before a normal sugar level could be reached. Some animals required increasingly higher drug levels over a period of weeks, indicative either of resistance or progressive worsening of the condition.

Since some deaths were attributed to the daily handling and transfer of animals from one cage to another for breeding purposes, it was decided to supply phenformin or tolbutamide in the drinking water. This proved helpful and time-saving as the number of diabetic hamsters increased. Only animals totally dependent upon insulin were injected daily. Phenformin was given at 30 mg./100 cc., and tolbutamide at 134 mg./100 cc. in water bottles. The bottles were renewed daily. The dilutions were more or less arbitrary, but daily water intake and relative effectiveness of the compounds were taken into consideration. Most animals maintained themselves at moderately hyperglycemic levels, the mortality rate was considerably reduced from about 30 per cent to 5 per cent over a half-year period, and fertility and the number of complete pregnancies increased. Quantitative data on survival are not yet at hand. In the course of therapy it became obvious that the sensitivity of diabetic animals to hypoglycemic drugs was greater than that of normal animals; comparatively less compound was required to lower elevated sugar levels than to induce hypoglycemia in normal hamsters. For instance, 2 to

*Eli Lilly and Company, Indianapolis, Indiana.

40 units of NPH insulin per hamster (30-33 gm.) would (often when 70 to 100 U. or more was required) reduce elevated blood sugar levels to normal, even in severe diabetics; in order to induce hypoglycemic shock in normal controls 40 to 90 U. were required.

DISCUSSION

In mild to moderately severe cases of diabetes, there was an almost linear relationship between reduction of the urine sugar excretion and dosage of NPH insulin. In severely diabetic hamsters, insulin was the only effective drug. It is, therefore, doubted, contrary to the reports of others, that phenformin can act in *complete* absence of insulin.[5] It was estimated that phenformin replaced roughly one fourth of the insulin required for adequate therapy in mild to severely diabetic hamsters. Diabetic animals were, within limits, comparatively more sensitive to the hypoglycemic action of phenformin than normal animals.

Although LD_{50}'s had not been determined in other animal species at the time of Ungar's report in 1957,[5] semiquantitative data on the action of phenformin on the blood sugar in various normal animals would suggest that the species most sensitive to subcutaneous inoculation of phenformin is the rhesus monkey. The species most resistant was found to be the rat. Normal Chinese hamsters were approximately ten times more resistant than the rat and fifty times more resistant than the rhesus monkeys to phenformin given subcutaneously. This observation was made by comparing the LD_{100}'s of our normal hamsters with those determined and published for other animals.[5,6] The subcutaneous administration, however, proved somewhat erratic, since many animals died almost immediately following injection, or within fifteen minutes thereafter. The oral route of administration caused fewest toxic deaths.

Tolbutamide, administered subcutaneously and intraperitoneally, was about as effective in normal hamsters as was oral or intravenous administration in normal rats.[6]

The doses of subcutaneous NPH insulin (about 500 U./kg.) required to lower the blood sugar levels to about half of normal were larger for normal hamsters than for any other species.

From the data presented in this report and those available in the literature it may be extrapolated that on a weight basis, normal hamsters were approximately ten to twelve times more resistant to tolbutamide, eight times to phenformin, and about 150 times to NPH insulin, than man. The exact reasons for these and the underlying metabolic species-differences are obscure.

SUMMARY

Methods for successful maintenance of Chinese hamsters suffering from a spontaneous hereditary diabetes are described. Therapy with either phenformin, tolbutamide and/or NPH insulin increased fertility, improved the number of successful pregnancies, and reduced the losses of liveborn animals. Toxicity studies on normal hamsters proved them to be more resistant than other species, including man, to all three agents. Spontaneous hereditary diabetes mellitus in Chinese hamsters provides an excellent tool for the screening and study of potentially hypoglycemic agents.

SUMMARIO IN INTERLINGUA

Spontanee Diabete Mellite Hereditari in Hamsters Chinese (Cricetulus griseus).

III. Le Mantenentia de un Colonia de Hamsters Diabetic con le Adjuta de un Therapia Hypoglycemic

Es describite provate methodos pro le mantenentia de hamsters chinese con spontanee diabete hereditari. Therapia con phenformina o tolbutamida e/o insulina NPH augmentava le fertilitate, augmentava le numero del pregnantias successose, e reduceva le perditas subsequente de vivenatos. Studios de toxicitate in hamsters normal demonstrava que illos es plus resistente a omne le tres agentes que altere species, incluse le homine.

ACKNOWLEDGMENT

This investigation was supported in part by a grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service, No. CY 3335, and from The Damon Runyon Foundation and The National Science Foundation.

The assistance of Messrs. Henry Gagnon, John Hedges, Wendell A. Staples, and the Misses Barbara J. McNeil and Marietta Vogt is gratefully appreciated.

REFERENCES

[1] Meier, H., and Yerganian, G.: Spontaneous hereditary diabetes mellitus in the Chinese hamster (Cricetulus griseus). I. Pathological findings. Proc. Soc. Exper. Biol. & Med. *100*:810.

[2] Zahnd, G. R., Christophe, J., and Yerganian, G.: Hepatic glucose metabolism in two forms of spontaneous hyperglycemia (in press).

[3] Yerganian, G., and Meier, H.: Spontaneous hereditary diabetes mellitus in the Chinese hamster (Cricetulus griseus). A. Preliminary report on clinico-pathological findings, genetic aspects, breeding and responses to hypoglycemic drugs. Fed. Proc. *18(1)*:514, 1959.

[4] Freeman, M. V., Draize, J. H., and Smith, P. K.: Some aspects of the mechanism of toxicity of thioglycolate. J. Pharm. and Exper. Ther. *118*:296-303, 1956.

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

[6] Houssay, B. A., Penhos, J. C., Teodosio, N., Bowkett, J., and Apelbaum, J.: Action of the hypoglycemic sulfonyl compounds in hypophysectomized, adrenalectomized and depancreatized animals. Ann. New York Acad. Sc. *71*:12, 1957.