The Colony of Chinese Hamsters of the C.H. Best Institute

A Review of Experimental Work

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Summary. Several investigators have used Chinese hamsters from our colony for experimental work, and results of various studies are reported. — The group of Dr. H.R. HAUSLER, who initiated the colony in Toronto, studied retinal changes in spontaneously diabetic animals and also in hamsters with the metahypophyseal type of diabetes, following serial injections of growth hormone and cortisone. Arteriolar and capillary aneurysms were found in about 30% of the animals with metahypophyseal diabetes, but only sporadically in spontaneously diabetic hamsters. — The group of Dr. K. SCHÖFFLING studied alterations of testicular tissue in biopsy material. Corresponding to the severity of the disease, spontaneously diabetic hamsters showed various degrees of inhibited spermatogenesis, resulting in a reduction of the thickness of the germinal epithelium and widening of the lumina of the seminiferous tubules. There was also a reduction in the number of Leydig cells. The overall histological picture resembled that of the testes of the diabetic human patient. — The group of Dr. J. CAMPBELL measured concentrations of serum free fatty acids (FFA) in nondiabetic Chinese hamsters and found the levels 4 to 9 times higher (3 to 5 mEq/l) than in dogs, rats and man, but similar to FFA levels of Syrian hamsters. Epididymal fat pads of rats and Chinese hamsters showed similar lipid contents, but the latter released more FFA into the incubation medium. Nonglycosuric hamsters were susceptible to the diabetogenic effect of cortisone, but not to ovine growth hormone. Cortisone was most effective in producing a high blood sugar and elevated serum insulin and FFA concentrations. — In our own laboratory, the concentration of glycoproteins in plasma and the mucopolysaccharide content of the skin were determined in nondiabetic, occasionally glycosuric and frankly diabetic hamsters. There was no difference amongst the three groups of animals with regard to either the immunoelectrophoretic pattern or the concentration of individual carbohydrate components of plasma proteins. Skins of diabetic hamsters contained more mucopolysaccharide in their ground substance than skins of nondiabetic animals of comparable age. Hamsters with a mean fasting blood sugar value of 60 mg% showed a uronic acid content of 579 μ g per g of dry tissue, whereas animals with a mean fasting blood sugar of 107 mg% had 822 μ g.

La colonie de hamsters chinois du C.H. Best Institute: revue des travaux expérimentaux.

Résumé. Plusieurs chercheurs ont utilisé les hamsters chinois de notre colonie pour leurs observations expérimentales et nous rapportons ici les résultats de plusieurs études. — Le groupe du Dr. H.R. HAUSLER, initiateur de la colonie à Toronto, a étudié les altérations de la rétine chez les animaux présentant un diabète spontané ou un diabète de type métahypophysaire résultant de l'injection d'hormone de croissance et de cortisone. Des anévrismes artériolaires et capillaires sont observés chez quelque 30% des animaux avec diabète métahypophysaire, mais sporadiquement seulement chez les hamsters avec diabète spontané. — Le groupe du Dr. K. SCHÖFFLING a étudié les biopsies du tissu testiculaire. Proportionnellement à la gravité du diabète, on trouve chez les hamsters présentant un diabète spontané une inhibition de la spermatogénèse,

avec réduction de l'épaisseur de l'épithélium germinal et élargissement de la lumière des tubules séminifères. Les cellules de Leydig sont également réduites en nombre. L'apparence histologique ressemble à celle du tissu testiculaire de diabétiques humains. — Le groupe du Dr. J. CAMPBELL a mesuré les taux d'acides gras libres (AGL) du sérum de hamsters chinois non-diabétiques. Ceux-ci dépassant de 4 à 9 fois (3 à 5 mEq/l) les taux observés chez le chien, le rat ou l'homme, des taux semblables ayant pourtant été mesurés chez le hamster doré. Le tissu adipeux épидидymaire de hamsters chinois ne diffère pas de celui du rat par son contenu en lipides, mais il libère davantage de AGL dans le milieu d'incubation. Les hamsters chinois non-glucosuriques sont sensibles à l'action diabétogène de la cortisone, mais non pas à celle de l'hormone de croissance de mouton. La cortisone s'est montrée très active quant à son action hyperglycémique, accompagnée d'une élévation des taux d'insuline sérique et des taux d'AGL. — Dans notre propre laboratoire, nous avons mesuré les concentrations en glycoprotéines du plasma et le contenu en mucopolysaccharides de la peau de hamsters non-diabétiques, avec glycosurie intermittente, ou franchement diabétiques. Nous n'avons pas observé de différences entre ces trois groupes d'animaux quant au profil immuno-électrophorétique, ou la concentration en composantes hydrocarbonées des protéines plasmatiques. La substance basale de la peau de hamster diabétique contient davantage de mucopolysaccharides que celle des animaux non-diabétiques du même âge. C'est ainsi que le contenu en acides uroniques de la peau d'un groupe de hamsters, avec un sucre sanguin à jeun de 60 mg% en moyenne, était de 579 μ g par g de tissu sec, alors qu'il était de 822 μ g pour un groupe d'animaux avec un sucre sanguin à jeun de 107 mg% en moyenne.

Die Kolonie chinesischer Hamster des C.H. Best Institutes — eine Übersicht der experimentellen Untersuchungen.

Zusammenfassung. Mehrere Forscher haben mit Tieren aus unserer Kolonie gearbeitet, und dieser Bericht betrifft eine Reihe von verschiedenen Resultaten. — Dr. H.R. HAUSLER und Mitarbeiter haben die Kolonie in Toronto begonnen und sich hauptsächlich mit Veränderungen in der Retina von spontandibetischen Tieren befaßt. Sie haben ebenfalls Beobachtungen gemacht bei Tieren mit Diabetes von metahypophysärem Typ, also nach Injektion von Somatotropin und Cortison. Aneurysmata der Arteriolen und Kapillaren wurden in etwa 30% der Tiere mit metahypophysärem Diabetes beobachtet, nur vereinzelt aber bei spontandibetischen Hamstern. — Dr. K. SCHÖFFLING und Mitarbeiter haben die Veränderungen in Hodenbiopsien geprüft. In direktem Zusammenhang mit der Schwere des Diabetes findet man bei spontandibetischen Hamstern verschiedene ausgeprägte Hinweise auf inhibierte Spermatogenese, aus Verminderung der Zellzahl des germinalen Epitheliums und Erweiterung der Lumina der Tubuli seminiferi. Auch die Zahl der Leydig-Zellen ist vermindert, und das histologische Bild ist allgemein demjenigen beim Diabetiker ähnlich. — Dr. J. CAMPBELL und Mitarbeiter haben bei nicht-diabetischen chinesischen Hamstern freie Fettsäuren (FFS) gemessen und haben beobachtet, daß deren Konzentration im Serum 4 bis 9 mal höher liegt (3 bis 5 mV/l) als beim Hund,

bei der Ratte und beim Menschen. Ähnlich erhöhte Werte findet man aber auch beim Goldhamster. Epididymales Fettgewebe von Ratten und chinesischen Hamstern enthielt etwa gleich viel Fett, aber das Fettgewebe chinesischer Hamster mobilisierte *in vitro* mehr FFS. Bei nicht-diabetischen Hamstern war Cortison diabetogen, nicht aber Somatotropin vom Schaf. Die Verabreichung von Cortison führte zu erhöhten Zuckerwerten, zu erhöhtem Seruminsulin und zu sehr hohen FFS-Konzentrationen. — In unserem eigenen Laboratorium haben wir den Gehalt an Glucoproteinen im Plasma und an Mucopolysacchariden in der Haut bestimmt, und zwar in nicht-diabetischen, in nur zeitweise glucosurischen, und in klinisch deutlich diabetischen Hamstern. Zwischen diesen drei Gruppen von Tieren ließen sich keine Unterschiede be-

züglich auf immunoelektrophoretische Verteilung oder auf Zuckergehalt einzelner Komponenten der Plasmabestandteile nachweisen. Die Haut diabetischer Hamster enthielt aber mehr Mucopolysaccharide als diejenige nicht-diabetischer Hamster gleichen Alters. Hamster, deren Nüchternblutzuckerwerte bei 60mg% lagen, wiesen einen Uronsäuregehalt von 579 μg per g Trockengewebe auf, während der entsprechende Wert bei Tieren mit durchschnittlich 107 mg% Blutzuckernüchternwert 822 μg war.

Key-words: Spontaneous diabetes, Chinese hamster, *Cricetulus griseus*, Retina anomalies, Testicular function, Free fatty acids, Glycoproteins, Mucopolysaccharides.

The occurrence of a disturbance in carbohydrate metabolism as a hereditary trait in the Chinese hamster (*Cricetulus griseus*) was first described by MEIER and YERGANIAN [7, 8, 9]. Our colony, which is housed in the C.H. Best Institute, was started in 1961 when 10 male and 20 female animals were obtained from Dr. YERGANIAN of the Children's Cancer Research Foundation in Boston. The initiative was taken by Dr. H.R. HAUSLER, of the Department of Physiology and the Department of Ophthalmology, University of Toronto. Dr. HAUSLER was anxious to start a colony of Chinese hamsters anticipating that the spontaneously diabetic animals may develop retinal lesions more frequently than do alloxanized or surgically pancreatectomized animals. The efforts of Miss BARBARA STACHOWSKA from Dr. HAUSLER's staff were instrumental in starting and later developing the colony. Over the past 5 years more than 2500 offspring were obtained from the original stock of 30 hamsters. When the colony with its own staff became established at the C.H. Best Institute and several investigators were interested in conducting research in the Chinese hamster, it was necessary to coordinate matters, and it was decided that our group should be in charge of operations. A breeding scheme was developed by Dr. L. BUTLER from the Zoology Department, University of Toronto, based on brother-sister and father-daughter matings. While I have no intention to infringe on Dr. BUTLER's presentation of the genetic studies in relation to the incidence and severity of diabetes, some comments ought to be made now, since they have some bearing on the design and results of experiments I will subsequently report on. Firstly, it became obvious already in the early phases of the breeding experiments that any prediction which animal would become diabetic and which would remain normal, could not be made. It became impossible, therefore, to study the "prediabetic" phase of the disease on a large scale. Secondly, we deliberately limited the use of diabetic animals for experimentation in an attempt to provide sufficient selection of animals for designing a meaningful breeding scheme. In other words, the use of animals in general and those with glycosuria in particular was limited to tissue biopsies and withdrawal of blood by

puncturing the orbital sinus of the medial canthus of the eye. Although the intentions were good, by doing so we lost the valuable opportunity of extensive metabolic studies in diabetic hamsters. This leads up to the third point I wish to make, namely, that some of our lines are now in the 13th generation and the animals have no diabetes. Many of the diabetic animals of former generations did not reproduce. In a number of hamsters the type of diabetes changed from a severe, insulin-dependent form to a mild, intermittent glycosuria. At the present time our colony is reduced to about 325 animals from its peak of some 900 hamsters three years ago, and we have no severely diabetic animals. It appears, then, that our colony is inbred to an extent that the animals do not reproduce or develop diabetes.

I have mentioned before that Dr. HAUSLER has been interested for some time in diabetic retinopathies, and I should like to present now some of the results which were obtained by him, Dr. T.M. SIBAY and B. STACHOWSKA in the Chinese hamster. Routine ophthalmoscopic examinations of the eyeground of nonglycosuric and diabetic hamsters revealed that only about 3% of the diabetic animals showed signs of arteriolar and capillary aneurysms. Although the vascular changes did not resemble those seen in human diabetic patients, the fact that vascular lesions were observed can be considered as meaningful in that the Chinese hamster has a retinal circulatory system which is similar to that of the human. The incidence of this type of angiopathy with aneurysms is greatly increased, up to about 30%, in animals that were rendered permanently diabetic by injections of cortisone and growth hormone. Injections of 12 mg of cortisone and 4 mg ovine growth hormone for 10 to 14 consecutive days produced in less than one third of the animals permanent signs and symptoms of diabetes. Animals with metahypophyseal diabetes did not require exogenous insulin for survival, and about one third of 92 animals developed retinal changes. Figures 1 and 2 are trypsin-digested retinal preparations that were mounted on microscopic slides and stained with hematoxylin-eosin [6]. The pathologic lesions most frequently encountered were aneurysmal dilatations

of arterioles with degeneration of the smooth muscle cells, such as seen in Fig. 1. In general, they were three times as frequent as capillary aneurysms. The latter is shown in Fig. 2. Gross abnormalities, such as hemorrhages, exudations or proliferation, were not observed.

Fig. 1. Trypsin-digested retinal flat preparation of a diabetic Chinese hamster. Degenerative changes are seen in smooth muscle cells surrounding arteriolar aneurysms. Hematoxylin-eosin stain. Magnification $\times 220$

Fig. 2. Trypsin-digested retinal flat preparation of a diabetic Chinese hamster showing a capillary aneurysm. Hematoxylin-eosin stain. Magnification $\times 220$

The changes did not resemble those of human retinopathies, but the animals when sacrificed were usually diabetic for six months only, and it is possible that longer duration may eventually have produced other types of lesions as well. It is interesting that the incidence of angiopathies was so much higher in animals treated with growth hormone and cortisone than in spontaneously diabetic hamsters. In this connection it may not be out of context to think of the improvements sometimes encountered in diabetic retinopathies upon surgical or radiological hypophysectomy in man.

I should like to turn next to the work of Dr. SCHÖFFLING and Drs. E. F. PFEIFFER, K. FEDERLIN and

W. SCHMITT, of the Section for Clinical Endocrinology of the Department of Medicine of the University of Frankfurt, Germany. In the past, Dr. SCHÖFFLING spent six months as a Lilly Fellow on our team and became interested in the diabetic Chinese hamster. Having had clinical experience with sexual disorders in human diabetic males [12], he was wondering whether the testicles of diabetic Chinese hamsters were histologically showing similar atrophic changes as seen in the human. There was a reason to assume this, since many diabetic male hamsters displayed less sexual interest than nonglycosuric males of similar chronological age. Testicular tissue biopsies were obtained from six nonglycosuric and 11 spontaneously diabetic Chinese hamsters, of which 5 required insulin and 6 animals did not. All the hamsters were somewhat less than one year old, and those with diabetes showed the first clinical signs before their 13th week of life. Following fixation in Bouin's solution, the tissues were embedded in paraffin. From each paraffin block eight slides were cut at random and stained with hematoxylin-eosin or by the so-called HOPA technique of Tonutti and Muschke (*Hemalum*, *Orange-G*-solution, *Phosphomolybdic acid*, *Aniline blue*) [13]. Fig. 3 shows normal testicular tissue of a mature Chinese hamster. The microphotograph represents a 250-times magnification of a hematoxylin-eosin stained paraffin section. In studying the developmental phases of spermatogenesis, the investigators limited themselves to the differentiation into spermatogonia, primary and secondary spermatocytes, spermatids, and immature and mature spermatozoa. The germinal epithelium is thick and the centre of the tubule is filled with mature sperms. Fig. 4 shows testicular tissue of a diabetic hamster in a 250-times magnification, HOPA stain. The thickness of the germinal epithelium is markedly reduced and, consequently, the lumina seem enlarged. There are still some primary and secondary spermatocytes, but no spermatids or sperms.

The histological changes were also expressed in quantitative terms by using the Zeiss integrating eyepiece I. This ocular has a network of 35 points, and the tissue element under a grid point is counted as a "hit". The sum of "hits" counted for each tissue component was expressed as percentage of total "hits". The latter amounted in each case to 1000 and were counted at random from 8 sections of each block. The investigators differentiated between germinal epithelium, lumen and interstitial tissue. This histometric analysis of data revealed that 77% of the total counts were represented by germinal epithelium in normal hamsters, whereas in diabetic hamsters the value decreased to an average of 67%, being slightly less for severely diabetic and slightly more for mildly diabetic animals. Lumen represented 5% in normals and 13% in diabetic animals. There was no significant difference in the volume of interstitial tissue, but the number of Leydig cells was reduced. The testicular changes were related mainly to the severity of the disease and to a lesser

extent to duration. The investigators concluded that the testicular changes observed in the Chinese hamsters were similar to those observed in human diabetic males and, therefore, worthy of further investigation.

hamster [3]. This is shown in Table 1. The Chinese hamster maintained on a commercial type of food containing some 10% fat derived mainly from corn oil, had in the hands of Dr. CAMPBELL's group 3 to

Fig. 3. Normal testicular tissue of an adult Chinese hamster. Hematoxylin-eosin stain. Magnification $\times 160$

Fig. 4. Testicular tissue of a diabetic Chinese hamster. HOPA stain. Magnification $\times 160$. Note the cessation of spermatogenesis and thin germinal epithelium

I should like to turn next to the metabolic experiments carried out by Drs. J. CAMPBELL, K.S. RASTOGI, and Mr. G.R. GREEN, partly in collaboration with Dr. HAUSLER's group in our Department of Physiology. Measuring the free fatty acid concentrations in serum of nonglycosuric Chinese hamsters, it was found that the levels are 4 to 9 times greater than in rats, dogs or man, but similar to those found in the Syrian

5 mEq/l of FFA, and the values were supposed to be even higher in fasted animals. This is certainly in striking contrast with the figures listed in the table for other animals, with the exception of the Syrian hamster. In our own limited experience the FFA concentrations, when determined by the same modified method of DOLE and MEINERTZ as used by Dr. CAMPBELL, were in nonglycosuric hamsters after a 10-hour

Table 1. Free fatty acids in serum and epididymal adipose tissue of animals under various conditions

Animals	Diet ¹	Conditions	Final body mass, range (g)	Free fatty acids		Glucose in serum (mg/100 ml)
				Adipose tissue (meq/g of dry, fat-free residue)	Serum (meq/liter)	
Chinese hamsters (10) ²	R	Fed	34—40		5.11 ³	86 ± 7 ⁴
	R	Fed	34—43	0.41 ± 0.07 ⁵	3.62 ± 0.05	84 ± 5 ⁴
Syrian hamsters (5)	R	Fed	62—84	0.27 ± 0.02	2.16 ± 0.07	
	R	Fasted 24 hours	66—81	0.32 ± 0.03	3.15 ± 0.09	
Rats	(12)	M	Fed	0.25 ± 0.05	0.59 ± 0.10	132 ± 5
	(6)	M	Fasted 24 hours	0.93 ± 0.06	1.14 ± 0.02	103 ± 2
	(5)	M	Diet reduced, 1 day	0.72 ± 0.10	1.10 ± 0.15	113 ± 3
	(6)	M	Diet reduced, 14 days	0.61 ± 0.05	1.02 ± 0.06	95 ± 3
	(11)	M	Alloxan-diabetic, fed	0.55 ± 0.03	2.18 ± 0.28	678 ± 65
Dogs	(4)	C	Fed		0.59 ± 0.07	82 ± 2

¹ Rockland Mouse Breeder Diet (R); Master Laboratory Cubes (M); Dog Chow, Ralston Purina Co., Clarkson, Ontario (C), provided *ad libitum*.

² Numbers of animals for calculation of the mean values.

³ Determination on pooled sera, therefore no statistical evaluation.

⁴ Values in whole blood.

⁵ Means ± S.E.

Fig. 5. The effect of cortisone and growth hormone on various parameters of study in the Chinese hamster [4]

fast considerably lower, namely, in the order of 2 mEq/l. However, our figures do basically agree with Dr. CAMPBELL's figures in the sense that they are much higher than in the normal rat, dog, and man. The possibility was considered by Dr. CAMPBELL that high FFA levels may be one factor that predisposes these animals to develop a disturbance in carbohydrate metabolism. It is an interesting thought, but has not received so far experimental evidence in its support. In Dr. CAMPBELL's experiments, adipose tissue of nonglycosuric hamsters *in vitro* released more FFA into the incubation medium than did adipose tissue of normal rats, but there was no evidence of a reduced sensitivity of this tissue to glucose and insulin in terms of lipolysis and glucose uptake.

The effects of cortisone and growth hormone [4] on serum FFA, as well as on other parameters of study, are shown in figure 5. Groups of 10 animals were each injected with saline and cortisone-suspension vehicle, with 4 mg of ovine growth hormone (NIH, lot No. S3) and cortisone-suspension vehicle, with saline and 12.5 mg of cortisone, and also with both hormones. All injections were given subcutaneously over a period of 14 days. In the group given cortisone only, serum insulin (immunoassay), blood glucose and sugar excretion rose considerably, but the range of values was very wide. Actually, only 3 animals out of 10 were definitely glycosuric. Corresponding to the rise in serum insulin levels was a reduction in the acid-alcohol extractable insulin content of the pancreas. There was also a marked effect on serum FFA (no standard error given because determinations were carried out on a pooled sample) and total liver fat. Growth hormone alone was less diabetogenic by far than cortisone, and

the combined treatment with the two hormones did not potentiate the steroid effect. Only one animal became severely diabetic in the latter series.

Essentially the same results were obtained in another series of experiments in which the dose of cortisone was reduced first to 3 and then to 1 mg, and that of growth hormone to 1 and 0.4 mg per day. Serum insulin levels rose in some instances to 1.25 mU/ml on cortisone (3 mg/day), and serum FFA rose to values well above 10 mEq/l. As in the previous series, not all animals became hyperglycemic and glycosuric. Whether treated with cortisone alone or with the combination of the two hormones, not more than 3 out of 8 animals of each group showed definite signs and symptoms of diabetes. As a rule, cortisone produced a glycosuria only transient in nature, but cortisone and growth hormone caused permanent metahypophyseal diabetes in susceptible animals [6, 4].

The resistance of the Chinese hamster to the diabetogenic effect of ovine growth hormone is an interesting phenomenon. Whether or not one is dealing with a diminished response due to species specificity remains to be determined. It is also noteworthy that only a small number of animals responded to cortisone, albeit with a severe hyperglycemia and glycosuria. It is possible that some animals were "genetically" but not as yet "metabolically" diabetic, i.e. in the symptom-less "prediabetic" phase, and injections of cortisone precipitated the transition into the overtly diabetic phase.

This sort of reasoning is touching on a very fundamental problem regarding normal control animals for metabolic studies. Our colony was started from an already inbred strain of Dr. YERGANIAN's colony, and upon further inbreeding some of the animals became diabetic. I have mentioned earlier that no definite genetic pattern was found according to which it would be possible to design a breeding scheme, permitting a reasonable prediction which animals would or would not develop diabetes. It is possible that our whole colony consists of "prediabetic" animals, and by a combination of factors of which inbreeding is one, some animals became diabetic and others did not.

In order to study this question more systematically, we investigated the serum protein pattern of nonglycosuric (to avoid the controversial issue of "normal"), occasionally glycosuric and frankly diabetic, insulin-dependent hamsters. Theoretically there was justification for a search of a "marker" for potential diabetes, since disease states are frequently associated with derangements of plasma proteins. We have screened a large number of nonglycosuric and glycosuric animals at different ages from 3 to 24 months by carrying out paper electrophoresis, immunoelectrophoresis and estimations of the monosaccharidic components of serum proteins, employing methods described elsewhere [14, 15]. Tables 2 and 3 contain results obtained from nonglycosuric hamsters. Although the values were determined on single pools of

5 animals each, the figures are, in our experience, representative of adult Chinese hamsters in general. I have limited myself to representative figures of nonglycosuric animals, because determinations of the same constituents in diabetic animals gave identical results. Next we turned to measurements of the carbohydrate moiety of serum proteins, and the results obtained from the two pooled serum samples

Table 2. *Blood chemistry of nonglycosuric Chinese hamsters*

Constituent	Male ¹	Female ¹
hematocrit (% formed elements)	46	49
Serum glucose (mg/100 ml)	111	119
Serum cholesterol (mg/100 ml)	115	118
Total protein (g/100 ml)	5.25	5.25

¹ Pooled blood of 5 nonfasting animals.

Table 3. *Serum protein fractions¹ of nonglycosuric Chinese hamsters*

Constituent	Male ²	Female ²
Albumin (g/100 ml)	3.24	3.68
Alpha-one globulin (g/100 ml)	0.290	0.245
Alpha-two globulin (g/100 ml)	0.343	0.368
Beta-one globulin (g/100 ml)	0.106	0.098
Beta-two globulin (g/100 ml)	0.738	0.588
Gamma globulin (g/100 ml)	0.527	0.270

¹ Paper electrophoresis in veronal buffer, pH 8.6, σ 0.1. Conventional nomenclature.

² Pooled blood of 5 nonfasting animals.

Table 4. *Serum glycoproteins of nonglycosuric Chinese hamsters*

Constituent	Male ¹	Female ¹
Hexose (mg/100 ml)	91.0	85.1
Seromucoid hexose (mg/100 ml)	6.7	6.5
Hexosamine (mg/100 ml)	93.4	85.1
Sialic acid (mg/100 ml)	45.4	42.2

¹ Pooled blood of 5 nonfasting animals.

Table 5. *Sialic acid concentrations in Chinese hamsters*

Nonglycosuric	Mildly Glycosuric	Severely Diabetic
50.1 $\pm$ 7.9 ¹	45.4 $\pm$ 9.0	47.9 $\pm$ 6.4
n = 25	n = 24	n = 25

¹ Mean $\pm$ S.D. mg/100 ml serum.

are presented in Table 4. Since the amounts of carbohydrate are relatively small in comparison with the protein moiety, we hoped to detect some subtle differences in diabetic, potentially diabetic and "normal" animals by determining their serum protein-bound hexose, hexosamine and sialic acid concentrations. Unfortunately, this was not the case. Table 5 gives the results of a series of sialic acid determinations in

individual serum samples of nonglycosuric and diabetic hamsters. Although a tendency toward higher values was reported for the prediabetic and diabetic human patient by CAMERINI-DAVALOS [2], there appears to be no such tendency in the Chinese hamster.

Finally we turned to immunoelectrophoresis for optimal resolution of individual serum proteins. We were fortunate to produce a potent antiserum against hamster serum in rabbits, and Fig. 6 presents a bromophenol blue stain of typical patterns. In the upper well was placed 5 μ l serum from a nonglycosuric hamster and in the lower well 5 μ l of serum from a diabetic animal. Upon separation of proteins by electrophoresis, 50 μ l of antiserum was applied to the central trough, and precipitates of individual serum proteins were permitted to form in the agar gel. We have analyzed many immunoelectrophoretic tracings without finding any changes characteristic for diabetes.

Fig. 7 shows benzidine stains of some of our protein patterns. The upper tracing in each microscope slide is from the serum of a nonglycosuric animal and the lower from a diabetic animal. It is readily seen that the number of benzidine-positive arcs varies in glycosuric as well as in nonglycosuric Chinese hamsters and this finding, therefore, bears no relation to the diabetic state of the animals. A variability in serum protein patterns unrelated to diabetes has also been reported by GERRITSEN and DULIN, using electrophoresis in acrylamide gels [5].

It is conceivable that either there is no characteristic "marker" in the serum protein pattern, or all of our animals were potentially diabetic. We have, therefore, abandoned the field and turned our attention to the composition of the ground substance of skins in terms of uronic acid containing glycosaminoglycans or acid mucopolysaccharides. Miss M. LOUGHNEY and Dr. H. MACKEY in our laboratory have been interested for some time in the hormonal control of connective tissue, especially vascular tissue of normal, diabetic and other endocrinologically deficient animals. Because of the small size of the Chinese hamster, we had to use skin as the most abundant tissue for our mucopolysaccharide studies. We employed the method of SCHILLER et al. [11]. In this procedure the extraction of mucopolysaccharides is dependent upon solubilization of acetone-defatted dry skin with papain, followed by further deproteinization with trypsin and trichloroacetic acid. Separation of the mucopolysac-

charides from the partially purified preparation is based on the differential solubility of their cetylpyridinium (CPC) complexes. Complete precipitation of the mucopolysaccharides is effected by CPC in the presence of 0.035 M NaCl, leaving glycoproteins and other

Fig. 6. Immunoelectrophoretic pattern of serum proteins. Bromo-phenol blue stain. Upper tracing: Normal Chinese hamster. Lower tracing: Diabetic Chinese hamster

Fig. 7. Immunoelectrophoretic patterns of serum proteins. Benzidine stain. Upper tracings: Normal Chinese hamsters. Lower tracings: Diabetic Chinese hamsters

substances in the supernatant. Hyaluronic acid is solubilized by 0.4 M NaCl, chondroitinsulfuric acid by 1.2 M NaCl and heparin by 2.1 M NaCl.

Table 6 contains the results obtained in 3 groups of 13 months old animals. The division of nonglycosuric animals into Groups I and II was admittedly arbitrary, but there was no need to pool more than 9 skins. Since exactly 9 animals had nearly identical fasting serum glucose levels, namely 83 mg%, they were considered as one group, and the remaining nonglycosuric animals formed the other group with a mean serum glucose of 60 mg%. It is interesting that animals with the

lowest fasting serum sugar also showed the lowest uronic acid content, and those with a relatively high blood sugar had the highest uronic acid content per g of dry skin. Uronic acid was determined by the carbazole method of BITTER and MUIR [1]. It has been reported by SCHILLER and DORFMAN [10] that skins of alloxan-diabetic rats had a low mucopolysaccharide

with insulin, the fasting serum sugar was close to 200 mg%. The results in the dog follow the same trend as reported for the alloxanized rat, in that the insulin-deficient animal shows a considerable reduction of the two major components of the amorphous ground substance, namely hyaluronic acid and chondroitin-sulfuric acid.

Our results are of a preliminary nature, but they do indicate that the low or high acid mucopolysaccharide content of skin and possibly other tissues is primarily dependent on the amounts of available insulin. Thus, the animal can be hyperglycemic and glycosuric, but as long as the insulin levels are normal or high, the mucopolysaccharide content in skin will remain normal or become elevated. High insulin levels are found in many patients in the early phases of their disease, but not in those who have diabetes of long duration. The metabolism of mucopolysaccharides is likely to be also insulin-dependent in the human but this, as well as the possible relationship to the vascular lesions, remains to be studied.

The mildly diabetic Chinese hamster goes through a phase of high serum insulin levels that is quite comparable with the early stages of this disease in man. It is truly a unique phase, since it cannot be reproduced in animals rendered diabetic by pancreatotomy or alloxan. By concentrating our efforts on a study of various metabolic parameters during this phase of the disease, valuable insight may be gained into the earliest stages of development of diabetes mellitus.

Table 6. Concentration of glycosaminoglycans in skin of Chinese hamsters

Constituent	Nonglycosuric		Mildly Glycosuric
	Group I	Group II	
Uronic acid ¹ (μ g/lg dry skin)	579	801	822
Serum glucose ² (mg/100 ml)	60 $\pm$ 4 (9)	83 $\pm$ 1 (9)	107 $\pm$ 4 (12)
Serum insulin ¹ (μ U/l ml)	45	31	78

¹ Skins and sera were pooled from 9, 9, and 12 animals respectively.

² Mean $\pm$ S. E. (No. of animals).

content, which could be normalized by insulin treatment. Though our results in diabetic skins are seemingly in contradiction, in reality they are in agreement with those of SCHILLER and DORFMAN, because our mildly glycosuric animals showed not only the highest uronic acid values in their skins, but also the highest insulin levels in blood (immunoassay).

The individual components of mucopolysaccharides, including the figure for interfering substances, all

Table 7. Concentration of glycosaminoglycans in skin of Chinese hamsters

Constituent	Nonglycosuric ¹		Mildly ¹ glycosuric	Dog Skin	
	Group I	Group II		Normal	Depancreatized
Total uronic acid (μ g/l g dry skin)	579	801	822	1280	984
Hyaluronic acid	219	239	397	733	556
Chondroitinsulfuric acid	185	224	213	375	251
Heparin	20	8	23	47	6
Uronic acid-like material (Glycoproteins)	120	96	111	94	83
Recovery %	94	71	91	98	91

¹ Skins were pooled from 9, 9, and 12 animals respectively.

expressed in terms of uronic acid, are presented in Table 7. It can be seen that the group of mildly glycosuric animals (with the highest insulin level) had the highest hyaluronic acid content per g of skin, while the chondroitinsulfuric acid content was not greatly different from the nonglycosuric groups of animals. Since we have no severely diabetic hamsters in our colony, I have included in the table the results of an identical analysis on dog skin for comparison. The pancreatectomized dog was poorly controlled

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