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NATURAL ESTROUS CYCLE IN NORMAL AND DIABETIC BITCHES IN RELATION TO GLUCOSE AND INSULIN TESTS

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Summary The influence of spontaneous «sex seasons» on blood sugar (BS) and serum insulin levels was studied in bitches with natural diabetes mellitus (DM) and normal controls, in the basal condition and during glucose and insulin tests, was studied. DM increased basal BS, reduced glucose tolerance, distribution space (DS) and clearance from blood, and induced resistance to insulin hypoglycemic action. In normals, occurrence of «seasons», inconsistently modified basal BS, increased glucose tolerance and DS; during estrogenic phase (EP), these variables were above those during luteal phase (LP). In diabetics at LP, BS found in fasting condition and during glucose test were higher than in diabetic bitches at EP (respective values at anestrous (A) in between) and glucose DS was smaller. Rate of glucose clearance from blood remained unaffected by «seasons» in both dog groups. Basal serum IRI was not modified by DM or «seasons». In normals, serum IRI response to glucose load was nonsignificant during A and increased during the «seasons»; either insulin DS or the rate of insulin clearance from blood stream remained unchanged under the circumstances, the increase being mediated by insulin secretion. During EP, the increase was particularly intense and mean insulinogenic index (MII) rose. During LP, MII returned to A value, whereby diabetic states might be manifest. Serum IRI profiles during insulin test were not modified by «seasons» in normal bitches; such response in diabetic bitches was intense during A, then decreased (EP) or was later abolished (LP). Either in normal or diabetic bitches, the sensitivity to exogenous insulin hypoglycemic action remained unchanged in spite of «seasons». In diabetic bitches at A, serum IRI after glucose challenge peaked higher than in respective normal controls (insulin clearance and insulin DS were similar): they exhibited relative insulin shortage and resistance to insulin hypolgycemic action partly compensated by promoted insulin secretion. Along with «season», abolished serum IRI response to glucose load in diabetics was observed. During EP, extrapancreatic factors regulating serum IRI concentration and MII did not change in respect to A, whereby abolishment appears mediated by depressed insulin secretion. During LP, insulin antagonism in conjunction with 1) absolute insulin deficiency and 2) intense decrease in MII appears as a powerful factor exposing diabetic bitches to a severe or fatal derangement in diabetic disease.

Key words: diabetes, estrous cycle, metabolism in bitches

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The spontaneous activation of hypothalamus-pituitary-ovarian axis has been reported to induce glucose intolerance and increased serum immunoreactive insulin (IRI) responses to glucose challenge in ovariectomized rats[1] and normal bitches[2].

The canine species shows spontaneous diabetes mellitus (DM), which is seldomly pancreatic in origin but frequently secondary to other marked endocrine changes. For instance, in bitch, there is a great body of evidence supporting the influence of the activation of gonadal axis on onset or deterioration of diabetic states[3-8], which may be observed during the estrogenic phase of estrous cycle (EP)[8], but becomes highly conspicuous or fatal in aged intact bitches during the course of their luteal phases (LP)[3-8]. Although these observations have been well documented, the pathogenesis of the disorder remains unknown. According to Eigenmann et al[3-5, 7], the high protracted hyperprogesteronemia characterizing the long-lasting canine metadiestrous might be responsible for the elevated serum growth hormone (GH) levels observed in diabetic and in some normal bitches at LP; as time elapses, in certain predis-ponsed animals, this disorder would result in Langerhans islet histological lesions, exhaustion or deterioration of pancreatic insulin stores and settlement of ketose-prone, insulin-dependent diabetic states, similar to those exhibited by normal dogs under GH treatment for several days[9].

According to results obtained in our laboratory[2,10-13], we decided to check Eigenmann's suggestion from an ampler standpoint. This appeared pertinent because the involvement of menstrual cycle in outset or deterioration of a diabetic state in women still remains unclear. Therefore, we studied the influence of spontaneous estrous cycle (also referred to as «sex season») upon some pancreatic and extrapancreatic mechanisms regulating blood sugar (BS) and serum IRI levels in bitches with naturally occurring DM and in normal controls, fasting and during the course of glucose and insulin tests. Thus, BS and serum IRI were measured and then we calculated either glucose or insulin distribution spaces in body tissues and clearance rates from blood stream and also respective mean insulinogenic index. The results obtained are discussed in relation to the pathogenesis of outset or deterioration of a diabetic state on the estrous cycle in the canine species.

Material and methods

Animals. Thirty female dogs, of unknown ages, weighing 8-28 kg, were used in the experiments. Fifteen of them were mixed-breed mongrel normal controls which were disinfested, kept in individual kennels, and fed on dog chow pellets and «ad libitum» water, for a 3 month-period before the tests performance. The remaining 15 bitches were privately owned, ambulatory patients attending to Dr. Ernesto Cánepa Small Animal Hospital, Veterinary Sciences Faculty, Buenos Aires University, for naturally occurring DM care; most of them had never received insulin therapy; only 2 were injected N.P.H. insulin for a few days till the 5th day preceding the tests performance: they then received only chrystalline insulin for 1 day, which was later interrupted until the tests were over.

Every bitch was checked for estrous cycle through exfoliative vaginal cytology[13]. They were randomly used for these experiments as they reached anestrous (A), E (eosinophillic index = 20-100%) and LP (early metadiestrous). Continuous A for 3 months was required by us to include a bitch in the A group. The bitches that were to be tested on the following day fasted overnight (18-22 h fast); meanwhile, they had free access to water.

Tests. Every dog was tested for glucose (IVGTT) and insulin (ITT) tolerances on consecutive days; glucose tests were performed first. Glucose (dose 1 g/kg body wt.) was dissolved in distillated water (conc. 20 g/dl). Glucagon free chrystalline ox insulin, potency 27.5 IU/mg protein, was dissolved (conc. 1 mg/ml) in 0.005N HCl, pH 2.4, and was further diluted with saline so that the total dose per animal (0.25 IU/kg body wt.) was contained in a final volume of 5.0 ml. Every dog received quick-, alternatively one of these solutions into a peripheral (external saphenous, median or radial) vein. Blood samples were taken from these veins (except that used for injection) basally, 5, 15, 25, 45, and 60 min after glucose challenge (IVGTT) and at 15,20, 25, 30, 35, and 40 min from insulin load (ITT).

Assays. All blood samples were assayed for BS in a Technicon Autoanalyzer[14]; some NaF was added to a small portion of every sample to prevent coagulation, and then it was diluted (5 per cent) in distillated water for the assay. The remaining portion of the samples was allowed to clot for 2 h at room temperature, it was then centrifuged at 2500 r.p.m. for 5 min at the same temperature, its serum was separated and stored at -25°C. Serum IRI was later measured by using a commercial kit (C.N.E.A., Argentina); pork insulin standard and guinea pig anti-(pork insulin) serum were used in the assay, for there is a reasonable cross-reaction rate between dog and pork insulins[15]. In a basal serum sample from every bitch, beta-estradiol[16] and progesterone[17] levels were measured.

Statistical evaluation. All missing values were estimated first[18]. As the influence of sex stages (Phase=P) in normal and diabetic bitches (Group=G) on BS and serum IRI levels, fasting and at several time intervals from

glucose and insulin load (Time = T) was to be studied, in analysis of variance (ANOVA) of three factor experiments (factors: G,P.T) with repeated measures on one factor (T), was applied[19]; the original data had been submitted to log transformation for homogeneity of variance; mean comparisons by « a posteriori» one-tailed Dunnett test[19] or Tuckey test[18] were carried out; whenever not indicated, the latter was used.

Equations for the log-linear regressions representing both glucose[20] and insulin[21] disappearance from blood stream in every dog during glucose and insulin test were respectively calculated. The equations' general form was $Y = \log_e V = \log_e a - kt$, where Y = variable response, V = variable concentration, $\log_e a$ = constant (Y axis intercept), k = constant, and t = time elapsed from glucose and insulin loads, respectively; the significance of regression line deviations from linearity was tested[22]. Either glucose or insulin, half-life time in blood stream ($t_{1/2} = 0.69/k$ min) and distribution space (DS) in body tissues per dog were calculated; aware of Y_0 value by extrapolating the mean regression line for t = 0, glucose or insulin total dose injected and dog body weight, the respective distribution space was calculated.

The insulinogenic index (II) at every time during glucose test per dog from the following formula was calculated.

II = Serum IRI (µU/ml)/Blood sugar (mg/dl)

MII was then estimated by calculating the respective area according to trapezoid method.

$$MII = \frac{1}{60} \int_0^{60} II(t).dt$$

To study the influence of sex stages (P) in normal and diabetic bitches (G) on both glucose and insulin, $t_{1/2}$ and DS, and also on MII, an ANOVA of two factor (G,P) experiments with 5 observations per cell was applied[19]; $t_{1/2}$ values first underwent inverse transformation, and DS and MII data were submitted to log transformation for variance homogeneity; mean comparisons were then performed (Tuckey test)[18].

In this study, a mean difference was considered significant as equal or as $P > 0.05$.

Results

1. *Influence of diabetes mellitus and estrous cycle on several variables in bitches*

DM did not modify the mean basal serum concentrations of 17 beta - estradiol and progesterone in bitches at the EP and LP of their estrous cycles, respectively (Table 1, Fig. 1). The diabetic

TABLE 1.— *Variables studies in this paper. Mean values and respective S.E.M. are given; in the last 5 variables, only mean values are shown.*

	Normal controls			Diabetic dogs		
	Anestrous	Estrogenic Phase	Luteal Phase	Anestrous	Estrogenic Phase	Luteal Phase
Estradiol, pg/ml	-	112±13	-	-	161±65	-
Progesterone, ng/ml	-	-	2.1±0.7	-	-	2.1±1.3
Fasting glycemia, mg/100ml	81±7	28±3▼	45±9▼	232±24##	191±60##▼	355±45##▼
Fasting serum insulin levels, µU/ml	14±5	15±5	7±3	2±1	23±3	8±2
Glucose distribution space, % body wt.	32.8	87.04▼▼	58.5▼▼	20.1##	25.4##	16.4## *
Glucose $t_{1/2}$, min	35.4	26.4	32.6	92,7##	65,0##	110.6## **
Insulin distribution space, % body wt.	15.2	22.4	36.5	38.4	20.1	743.0▼▼ **
Insulin $t_{1/2}$, min	12.2	10.3	12.7	14.4	7.3	39.7**
Insulinogenic index, µU/ml x 100	0.15	1.20▼	0.48	0.10	0.12	0.01*

$P < 0.01$ Levels of significance of comparisons in respect to respective normal controls.
▼,▼▼ $P < 0.05$, $P < 0.01$ Levels of significance of comparisons in respect to respective anestrous.
*,** $P < 0,05$, $P < 0.01$ Levels of significance of comparisons in respect to respective estrogenic phase.

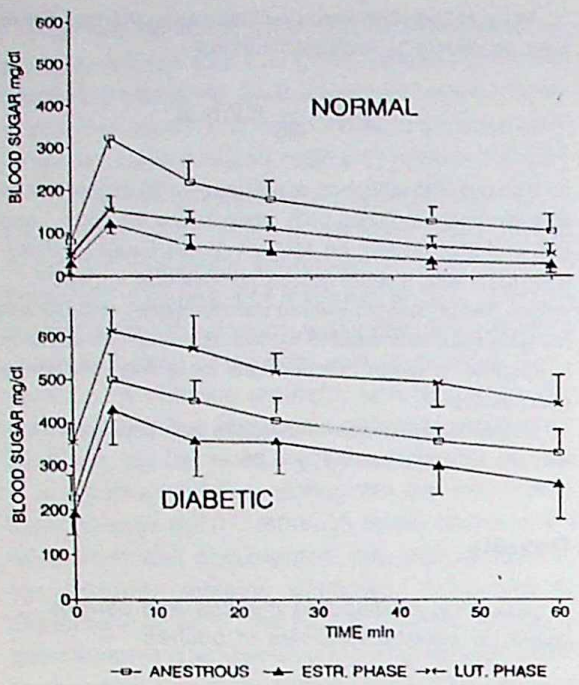

Fig. 1.— *Blood sugar levels in normal and diabetic bitches at anestrous and during the estrogenic and luteal phases of estrous cycle over intravenous glucose test.* Glucose dose: 1 g/Kg body wt.; it was injected at 0 time into a peripheral vein. Systemic venous blood withdrawn in the basal condition and over the test. blood sugar assay in Technicon Autoanalyzer. Means of 5 animals for group are shown.

condition significantly increased either the mean basal BS level or glucose $t_{1/2}$ and diminished glucose distribution space in body tissues (Tabla 1 Fig. 3). DM failed to affect the mean fasting serum IRI concentration, insulin $t_{1/2}$ as well as the mean of insulinogenic index.

The occurrence of estrous cycle modified these variables in the diabetic dogs and in the normal controls, except for the basal serum IRI level. Thus, in the latter, the cycles decreased the mean basal BS level and increased their glucose distribution spaces in body tissues. In the diabetic dogs, the fasting BS concentration decreased during EP in respect to A but increased during the LP, and just a small decrease in glucose distribution space as estrous cycle progressed from EP to LP was observed. In the normal controls, estrous cycle failed to modify glucose $t_{1/2}$ whereas in the diabetic dogs during LP this variable intensely rose as compared with A (Table 1, Fig. 3).

The occurrence of estrous cycle modified the mean fasting serum IRI level in neither dog group except for the diabetic bitches at EP, in which a moderate increase in relation to respective A was observed. As for insulin distribution space in body tissues and insulin $t_{1/2}$ in blood stream of normal bitches, they were very small and remained unaffected by estrous cycle. Either were these variables in the diabetic bitches at A and EP, whereas during the LP, such variables were both quite increased. In the normal group, the insulinogenic index intensely peaked during the EP whereas, in the diabetic dogs, it significantly decreased only during the LP as compared with the respective controls at A (Table 1, Fig. 3).

2. *Blood sugar*

A. *Glucose test.* DM influenced the mean BS concentration during the test ($P < 0.01$); this level was increased. The mean BS level varied with time elapsed after glucose challenge ($P < 0.01$); hyperglycemia was observed. The mean BS profiles in normal and diabetic bitches differ ($P < 0.01$); in the normal controls, hyperglycemia between 5 and 45 min from glucose load was observed ($P < 0.01$) and base line was again reached at 60 min; in the diabetic group, hyperglycemia throughout this test was found ($P < 0.01$) (Dunnett test) (Fig. 1, Table 2).

The sex stages influenced the mean BS levels in normal and diabetic bitches, either combined ($P < 0.01$) or separately considered ($P < 0.01$). Thus, in the normal ones at every sex stage, the mean BS concentrations during glucose test were different ($P < 0.01$); a relative maximum at A –and a minimum during EP– was observed. In the diabetic group, only EP and LP affected these levels differently ($P < 0.01$); a relative maximum during the course of LP –and a minimum during EP– was found. On the other hand, the effects of DM on the mean BS profiles during this test in bitches at every sex stage were significant ($P < 0.01$); at every sex stage, DM raised them. (Fig. 1, Table 2).

B. *Insulin test.* DM influenced the mean BS levels during this test ($P < 0.01$); which were increased. The mean BS concentration varied with time after glucose challenge ($P < 0.01$); hypoglycemia was observed. The mean BS curves in normal and diabetic bitches differed ($P < 0.05$);

TABLE 2.— *ANOVA of blood sugar levels in normal and diabetic bitches (Group), at anestrous and during natural estrous cycle (both phases) (Phase), basally and during the course of intravenous glucose and insulin tests (Time). Respective mean results shown in Fig. 1 and 2. Missing data were estimated; log transformation of original data; three factor ANOVA with repeated measures on one factor (conservative test for insulin test only); d.f.: degrees of freedom; MS mean square; F: Fisher value.*

Source of variation	Glucose test ↓			Insulin test ↓		
	d.f.	MS	F	d.f.	MS	F
Group (G)	1	18.0753	167.49**	1	33.4065	151.93**
Phase (P)	2	1.6927	15.68**	2	0.6183	2.81
G x P	2	0.7132	6.61**	2	0.8033	3.65*
Subj. within G	24	0.1079		22	0.2199	
Time (T)	5	0.8930	107.20**	6	0.1763	16.86**
G x T	5	0.1269	15.24**	6	0.0545	5.21*
P x T	10	0.0110	1.32	12	0.0246	2.35
G x P x T	10	0.0039	0.46	12	0.0128	1.22
T x Subj. within G	120	0.0083		136	0.0105	

* ** Levels of significance of F ($P < 0.05$ and $P < 0.01$ respectively).

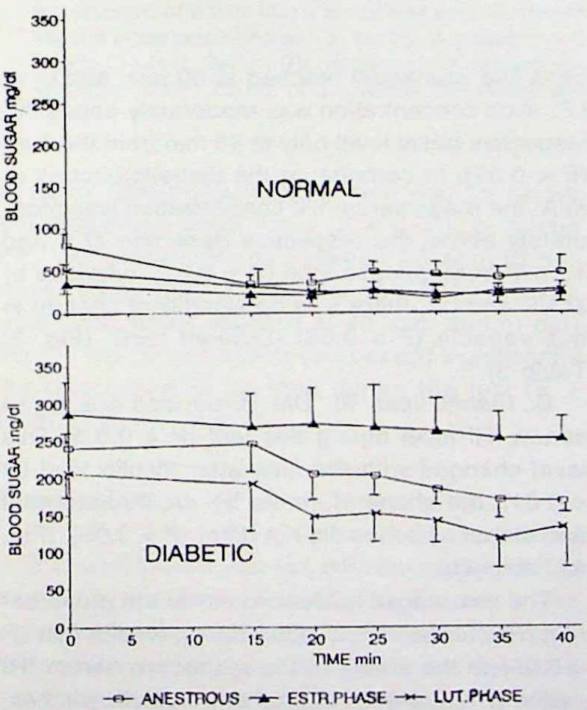

Fig. 2.— *Blood sugar levels in normal and diabetic bitches at anestrous and during the estrogenic and luteal phases of estrous cycle over intravenous insulin test.* Insulin dose: 0.25 IU/Kg body wt; it was injected at 0 time into a peripheral vein. Systemic venous blood was withdrawn in the basal condition and during the test. Blood sugar assay in Technicon Autoanalyzer. Means of 5 animals per group are shown.

in the normal group, hypoglycemia at every time throughout this test was found ($P < 0.05$) whereas in the diabetic bitches the mean BS levels reached the respective base line from 25 min till the test was over ($P < 0.05$) (Dunnett test) (Fig. 2, Table 2). The effect of sex stages on the mean BS level of every group during this test was non-significant and therefore they were not compared in particular.

3. *Serum immunoreactive insulin*

A. *Glucose test.* DM did affect the mean serum IRI concentration during the course of this test ($P < 0.05$). The mean serum IRI level varied with the time after glucose challenge ($P < 0.01$), and the shape of serum IRI curves obtained in normal and diabetic bitches differed ($P < 0.05$). (Fig. 3, Table 3).

The effect of sex stages on the mean serum IRI concentrations during this test was significant ($P < 0.01$). In normal and diabetic bitches at every sex stage, the shapes of the mean serum IRI profiles during the test differed ($P < 0.05$). Thus, in the normal bitches a) at A, the mean serum IRI concentration did not change significantly ($P > 0.05$), b) at EP, this concentration intensely rose above the respective base line at 5, 15, 25 and 45 min from glucose load ($P < 0.05$):

TABLE 3.— *ANOVA of serum immunoreactive insulin levels in normal and diabetic bitches (Group), at anestrous and during natural estrous cycle (both phases) (Phase), basally and during the course of intravenous glucose and insulin tests (Time). Respective mean results shown in Fig. 3 and 4. Missing data were estimated; log transformation of original data; ANOVA of three factor experiment with repeated measures on one factor: d.f.: degrees of freedom; MS mean square; F: Fisher value.*

Source of variation	Glucose test			Insulin test		
	d.f.	MS	F	d.f.	MS	F
Group (G)	1	7.1469	7.38*	1	10.2231	8.36**
Phase (P)	2	5.5897	5.78**	2	5.3551	4.38*
G x P	2	0.5018	0.52	2	1.3041	1.07
Subj. within G	24	0.9678		22	1.2230	
Time (T)	5	0.8809	6.45**	6	2.6709	62.64**
G x T	5	0.3327	2.43*	6	0.0353	0.83
P x T	10	0.2609	1.91	12	0.1465	3.44**
G x P x T	10	0.2649	1.94*	12	0.1009	2.37**
T x Subj. within G	120	0.1367		141	0.0426	

* ** Levels of significance of F ($P < 0.05$ and $P < 0.01$ respectively)

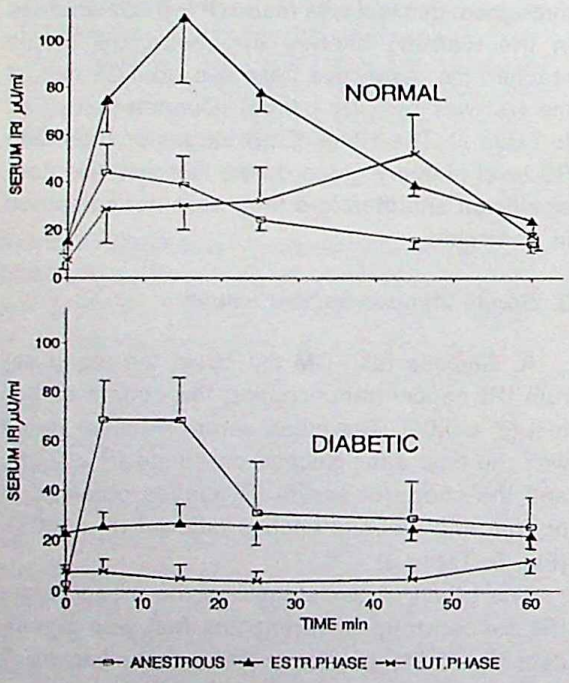

Fig. 3.— *Serum immunoreactive insulin (IRI) levels in normal and diabetic bitches at anestrous and during the estrogenic and luteal phases of estrous cycle over intravenous glucose test.* Glucose dose: 1 g/Kg body wt.; it was injected at 0 time into a peripheral vein. Systemic venous blood was withdrawn in the fasting condition and during the test. Serum insulin measured by radioimmunoassay. Means of 5 animals per group are shown.

base line was again reached at 60 min, and c) at LP, such concentration was moderately above the respective basal level only at 45 min from the load ($P < 0.01$). In contrast, in the diabetic bitches a) at A, the mean serum IRI concentration was moderately above the respective base line at 5 and 15 min from glucose load ($P < 0.01$), whereas b) at EP and LP, there was no significant change in this variable ($P > 0.05$) (Dunnett test). (Fig. 3, Table 3).

B. *Insulin test.* III. DM influenced the mean serum IRI level during this test ($P < 0.01$). This level changed with the time after insulin load ($P < 0.01$); the shape of serum IRI profile in normal and diabetic bitches did not differ ($P > 0.05$). (Fig. 4, Table 3).

The sex stages influenced either the mean serum IRI concentration found during insulin test ($P < 0.05$) or the shape of the respective serum IRI profile ($P < 0.01$). In normal and diabetic bitches, this shape was differently affected by sex stages ($P < 0.01$). Thus, in normal bitches at every sex stage, these profiles coincided, and they were above base line throughout the test ($P < 0.01$, Dunnett test). In contrast, in the diabetic group at every sex stage, the profiles differed ($P < 0.01$); thus, a) at A, the mean serum IRI concentration

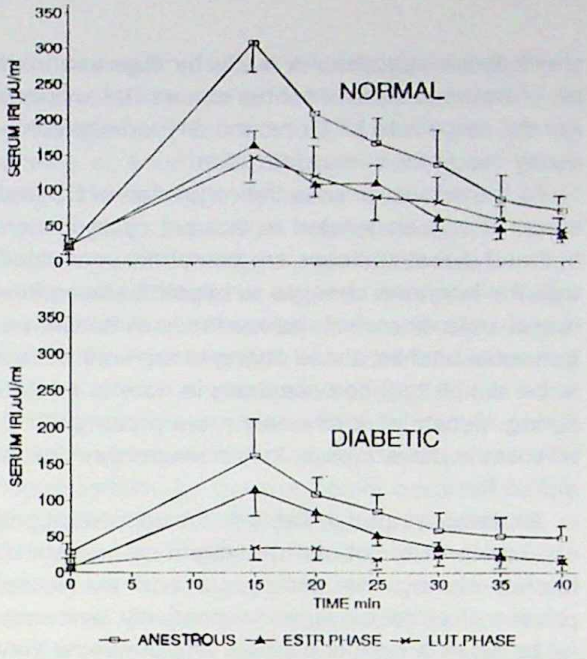

Fig. 4.— *Serum immunoreactive insulin (IRI) levels in normal and diabetic bitches at anestrous and during the estrogenic and luteal phases of estrous cycle over intravenous insulin test.* Insulin dose: 0.25 IU/Kg body wt.; injected at 0 time into a peripheral vein. Systemic venous blood was withdrawn in the basal condition and during the test. Serum IRI measured by radioimmunoassay. Mean values of 5 animals per group are shown.

at every time throughout the test was far above the respective basal value ($P < 0.05$), b) during EP, the concentrations between 15 and 35 min were moderately above this value ($P < 0.01$), which was again reached at 40 min, and c) during LP, the concentration overpassed significantly the basal level at no time during the test ($P > 0.05$). (Fig. 4, Table 3).

Discussion

It is well known that the intense hypoglycemia evoked by insulin load during insulin test in the normal animals is primarily counteracted through a dissipation of the injected insulin; some quick enhancement in the blood concentration of insulin antagonists usually occurs as well[23-27]. It is likely that the same did occur in the bitches studied herein; we have demonstrated that at least insulin, epinephrine and cortisol participate in this concern in the canine species[28]. On the other hand, glucose challenge during glucose test provoked hyperglycemia and hyperinsulinemia in many groups of bitches studied herein, and other investigators have demonstrated that a depression in GH and in glucagon secretion occurs[6]. Later, hyperinsulinemia exerts its anabolic action as observed in several species of mammals including dogs[29], and a compensation of the excessively brisk fall of the BS level by various insulin antagonistic hormones is usually developed[9, 24, 30], GH appearing excluded in this respect[20]. Although glucose is a major pancreas stimulus for insulin secretion in canines[2, 10, 11, 13, 30-32], the normal bitches at A exhibited nonsignificant serum IRI responses to hyperglycemia, thereby confirming our previous reports[2, 10, 12], on account of a marked sex dimorphism in this variable behavior during glucose test[11].

As expected, the diabetic bitches showed in general altered regulation in BS levels certainly related to their relative or absolute insulin deficiency. During A, this deficiency was only relative; apparently, the «in vivo» insulin secretion in these animals was more intense than in the respective normal controls; their major extrapancreatic factors normally regulating serum IRI levels, viz. insulin distribution space in body tissues and insulin clearance rate from blood stream, were within their respective normal ranges. On the other hand, the diabetic state induced in the bitches at A a resistance to insulin hypoglycemic action. It is impossible for the diabetic bitches to have developed insulin antibodies as a consequence of insulin therapy because most of them had never been submitted to such therapy or had eventually been given the hormone just for a few days so as to insure survival during the severe acute diabetic crises characterizing the «seasons», mainly LP. Such resistance appears rather related to a deranged insulin-antiinsulin balance in their body tissues. Although the intrinsec nature of the insulin antagonist(s) provoking the disorder remains unknown, its occurrence is in keeping with observations made in humans. Thus, type I or II diabetic subjects exhibit basal hyperglucagonemia and lack of glucagon suppressibility by hyperglycemia[24]; hyperglucagonemia potentiates epinephrine hyperglycemic action in both insulin-dependent humans and dogs[23, 24]; type I diabetic patients show also basal GH overproduction and poor blood GH suppressibility by hyperglycemia[33], but in type II this suppressibility is adequate[9]. Apparently, at least glucagon, epinephrine and the

permissive action of cortisol can be blamed for the quick insulin resistance observed in the diabetic bitches at A; GH share in this respect appears remote[6]. In agreement with results shown here, the literature reports that the insulin resistance exhibited by diabetic human subjects appears lower as studied during insulin test. Thus, in recent-onset type I DM, glucagon secretory response to hypoglycemia decreases being only in part transiently compensated by epinephrine, GH and cortisol[23, 33]; in type II diabetic humans this response is normal.

The increased serum IRI response to glucose challenge observed in the normal bitches «in season» (either phase) was in keeping with our previous findings[2], and both glucose, tolerance and distribution space in body tissues varied accordingly. This increase did not result from a favorable stimulation by the BS level nor was it mediated by any change in the extrapancreatic factors normally regulating serum IRI concentration (see above); it seems more conceivably related to enhanced «in vivo» insulin secretion. In contrast, serum IRI response to stimulation by glucose load was abolished in the diabetic bitches «in season». The mechanism whereby this absolute insulin shortage occurs remains obscure, but it is quite clear that the abolishment starts during EP, as expected from clinical studies[8]. The shortage is definitely not related to a deficient pancreas stimulation for insulin secretion by glucose because in these animals the BS peak was certainly above (LP) or just hardly below (EP) that observed in the diabetic controls at A. During EP, one might expect that an intensely depressed «in vivo» insulin secretion provoked the shortage, because the major extrapancreatic factors regulating serum IRI (see above) remained unchanged despite that this phase was triggered. As for LP, inferring the state of insulin secretion from "in vivo" insulin response to glucose challenge, it is unfortunately unattainable because of the huge share of the tremendously broadened insulin distribution space in body tissues —an extrapancreatic factor— in the development of the insulin shortage. However, either the basal GH overproduction, lack of serum GH suppressibility by hyperglycemia or Langerhans islet histological lesions observed by well known investigators in diabetic bitches at LP[2-7] strongly suggest their pancreas to be exhausted for insulin secretion. Unfortunately, we could not check these suggestions made by Eigenmann et al[6, 7, 9] because there is neither canine GH antibody nor the respective kit for canine GH radioimmunoassay ready for comercialization.

All the disturbances in the regulation of BS and serum IRI levels related to estrous cycle in normal and diabetic bitches are somehow connected with the hormone changes in hypothalamus-pituitary-ovarian axis which induce the «sex seasons». In normal bitches, these changes are well known to be similar to those occurring in normal women during menstrual cycle only more prolonged[34-36], whereas in diabetic dogs they have not been studied so far.

As demonstrated in Table 1, the concentrations of 17 beta-estradiol and progesterone in diabetic bitches during the estrogenic and the luteal phases of estrous cycles, respectively, are similar to those in normal controls and therefore they do not seem to account for these disturbances. The respective FSH, LH and GnRH levels could not be measured (unavailable dog specific RIA kits), but since the «seasons» in the bitches with DM used herein progressed normally as proved by exfoliative vaginal cytology, these levels are expected to have been within the normal range at the time of the experiments. Nevertheless, it seems interesting to remark that, like in diabetic women and rats, sex cycles occurrence in some diabetic bitches was difficult (luteinization being most frequently impaired). On the other hand, at present is is quite clear for us that the ovarian hormones physiologically inducing either phase of the sex cycles should not be blamed for any stimulatory action[6, 25, 37-45] on serum IRI response to hyperglycemia in the normal group[35]; prolactin mediation can be ruled out as well[46]; in contrast, FSH and LH are major factors evoking this stimulation during the EP[10] and likely over the LP. As found by us, neither do the ovarian hormones[12] nor prolactin[46] account for the improved glucose tolerance observed in the normal dogs «in season»; we observed also that combined FSH and LH nonsignificantly account for it[10], which might suggest that GnRH —whose role(s) in the regulation of BS and serum IRI levels is still ignored— is responsible for these variables displayed by the activation of the gonadal axis.

In the normal bitches, the progress of naturally triggered estrous cycles from EP to LP is mildly diabetogenic. Thus, during the LP, although glu-

cose distribution space in body tissues and glucose clearance rate from blood stream remained unchanged with respect to EP, either glucose tolerance or serum IRI response to glucose challenge moderately decreased, and the mean insulinogenic index then approached its A value. These results strongly suggest that most normal bitches at LP shall not become diabetic; in keeping with this observation, clinical studies report that only a minor fraction of intact bitches develop DM[3, 4, 47], which is manifested during LP, i.e. when 1) progesterone synthesis is maximal[4, 6], and 2) basal hypersomatotrophinemia and lack of GH suppressibility by hyperglycemia occur[6, 7, 48]. The dog, like other carnivores, is highly sensitive to GH diabetogenic action[6]. Although the diabetic symptoms and hypersomatotrophinemia were described to reverse after ovariohysterectomy[3, 6, 47] in bitches at LP, the role played by progesterone alone in the pathogenesis of DM under these circumstances is still controversial. Apparently, there is no cause-effect relationship of progesteronemia to GH levels; thus, some normal bitches at LP showing signs of intense glucose intolerance (with or without acromegaly) exhibit normal serum progesterone concentrations but elevated serum GH levels[6]. GH production was then proposed to be paradoxically controlled by normal blood progesterone levels[6] through an unclear mechanism, which develops only in older age[6]; in this context, it was interestingly remarked that dog, in contrast to other species, exhibits very high postestrous progesterone levels for about two months whether pregnant or not; futhermore, in bitch, the reproductive cycles do not cease in older age. Whether such life-long exposure to high blood progesterone somehow favours the development of DM remains unclear but is possible[3-7]. It has been suggested that progesterone alone cannot account for the precipitation of the disease in normal bitches, but it is apparent that in conjunction with a genetically determined predisposition or another progesterone-controlled diabetogenic factor it must be responsible for the induction of the disease[6]. This key role played by progesterone appears to deserve some comments because this possibility, based upon the strongly diabetogenic action of synthetic progestagens in canine females[4, 5], does not fully match our results reported herein and elsewhere[12]. Thus, although it is true that progesterone is diabetogenic when administered alone at physiological doses to normal bitches at A, this effect is lost when the hormone is injected according to the normal estrogen-progesterone sequence[12]. Furthermore, the normal bitches at LP studied herein, in spite of their high progesterone levels[35, 36] showed better glucose tolerances as compared with respective controls at A, expected to exhibit neglectable blood progesterone[34-36]. Moreover, since apparently just diabetic bitches at LP and some normal controls at undetailed sex state were studied for serum GH levels by Eigenmann's group[3, 4], one might wonder whether such hypersomatotrophinemia could be only a characteristic of the diabetic state itself, as recently observed in humans with type I DM[33]. Therefore, our recent findings[2, 10, 12, 41] suggest a more complicated view of the pathogenesis of DM as triggered by estrous cycle in bitch, not exclusively based upon the possibilities outlined by Eigenmann et al.[3-7].

As far as is known today, we might suggest that, in normal bitches «in season», both moderate insulin-resistance and pancreas overstimulation for insulin secretion induced by high serum FSH and LH from the very beginning of the «season»[10], predispose them for a diabetic onset in the long run, viz. as soon as 1) a pancreas failure for compensation and/or 2) GH overproduction[3-7] (if any) will occur. It is apparent that these mechanisms most frequently occur in aged animals (more exposure to diabetogenic hormones of gonadal axis, in time and periodicity) and/or in bitches exhibiting a genetically determined predisposition for insulin deficiency.

As for the diabetic bitches, during A either their glucose intolerance or the resistance to insulin hypoglycemic action were at least partly compensated by a moderate augmentation in the «in vivo» insulin secretion, whereby the mean insulinogenic index was normal and they showed a poor prospective tendency to exhibit severe ketose-prone acidotic diabetic crises, such as found in clinical studies[3-8]. Unfortunately, as «sex seasons» happened to be spontaneously triggered in these animals, their serum IRI responses to glucose stimulation were abolished and the outset of diabetic crises could occur, albeit seldom during EP[8], most surely during LP[3-8]. Our observations explain these findings, partly at least. Thus, during EP, despite the insulin secretion blockade, glucose tolerance and the insulinogenic index remained nonsignificantly

altered in respect to A: it is likely that some adaptation in the insulin antagonism must have occurred. In contrast, estrous cycle progress from EP to LP in the diabetic bitches resulted in a marked deterioration in their disease. Thus, in the diabetic group at LP, exhibiting a decreased glucose clearance rate from blood stream already from respective A and also a blocked serum IRI response to stimulation from EP, the deterioration was at least in part related to 1) a reduction in glucose distribution space in body tissues most probably depending upon their absolute insulin deficiency, and 2) a tremendous enlargement in the insulin distribution space in body fluids which, despite the modest reduction in the insulin clearance rate from blood stream, continuously contibutes to a decrease in serum IRI level which insulin secretion is absolutely unable to overcome. The progressive pancreas histological lesions, such as Langerhans islet vacuolization, B-cell hydropic degeneration, and islet hypoplasia observed in diabetic bitches at this phase[48] appear to explain this unability. The combined actions of all these diabetogenic factors provoke in these animals the worst glucose tolerance found in this study, resulting in turn in a dramatic decrease of the mean insulinogenic index with the subsequent aggravation in their sickness. As far as is known it is apparent that, in the diabetic bitches at LP, the synergy of high levels of various insulin antagonists, such as combined FSH and LH[10, 34-36], and GH[3-7] and the absolute insulin shortage, is a powerful mechanism which, without excluding other(s), induces the severe or fatal derangement in their diabetic condition which is frequently observed in veterinary clinical medicine[3-7].

In brief, the foregoing study leads us to conclude that the occurrence of spontaneous estrous cycles in normal and diabetic bitches modifies their glucose metabolism and their serum IRI responses to stimulation which —mainly during LP— can result in outset or aggravation of diabetic states thereby explaining the results of various clinical observations made in canines, as reported in the literature.

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Resumen

Ciclo estral espontáneo en perras normales y diabéticas en relación con pruebas de glucosa e insulina

Se estudió la influencia de los ciclos sexuales espontáneos sobre la glucemia (G) y niveles de insulina sérica inmunoactiva (IRI) en perras con diabetes mellitus (DM) espontánea y en controles normales, en la condición basal y durante las pruebas de glucosa e insulina. DM aumentó la G basal, redujo la tolerancia, espacio de distribución (ED) y el índice de aclaramiento de la glucosa, induciendo resistencia a la acción hipoglucemiante de la insulina. En normales, las «estaciones sexuales» modificación inconsistentemente la G basal, aumentaron la tolerancia a la glucosa y su ED; durante la fase estrogénica (FE), estas variables estuvieron por encima que las de la fase luteal (FI). En diabéticas en FL, la glucemia basal o durante la prueba de glucosa estuvieron por encima de las halladas en diabéticas en FE, con los valores de anestro (A) entre ellas y el ED de la glucosa fue menor. El índice de aclaramiento de glucosa de la sangre no fue afectado por el ciclo en ambos grupos de perros. La IRI sérica basal no se modificó por DM o ciclos. En normales, la respuesta de IRI sérica a la sobrecarga de glucosa fue no significativa durante el A y aumentó durante los ciclos; tanto el ED de insulina como el aclaramiento de insulina de la corriente sanguínea quedaron no afectadas en esas circunstancias; el aumento es mediado por la secreción de insulina. Durante la FE, el aumento fue particularmente intenso y el índice insulinogénico medio (IIM) aumentó. Durante la fase luteal, el IIM retornó al valor de A, pudiéndose presentar crisis diabéticas. Los perfiles de IRI sérica durante la prueba de insulina no se modificaron por los ciclos en perras normales; dicha respuesta en perras diabéticas fue intensa durante A, luego decreció (FE) y fue más tarde abolida (FL). Tanto en perras normales como diabéticas, la sensibilidad a la acción hipolucemiante de la insulina exógena no cambió a pesar de los ciclos. En perras diabéticas en A, el pico de IRI sérica después de la sobrecarga de glucosa fue más alto que en controles normales respectivos (aclaramiento y ED de insulina no variaron): ellas mostraban una privación parcial de insulina y resistencia a la acción hipoglucemiante de esta hormona parcialmente compensada por aumentada secreción de insulina. A lo largo del ciclo, se observó abolición de la respuesta de IRI sérica a la glucosa exógena. Durante la FE, factores extrapancreáticos que regulan el nivel de IRI sérica y

el IIM no cambiaron respecto de A, por lo que la abolición parece mediada por depresión en la secreción de insulina. Durante la FL, el antagonismo insulínico junto con 1) deficiencia absoluta de insulina, y 2) intensa disminución en el IIM parecen factores poderosos que exponen a las perras diabéticas a un severo o fatal empeoramiento de su enfermedad diabética.

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