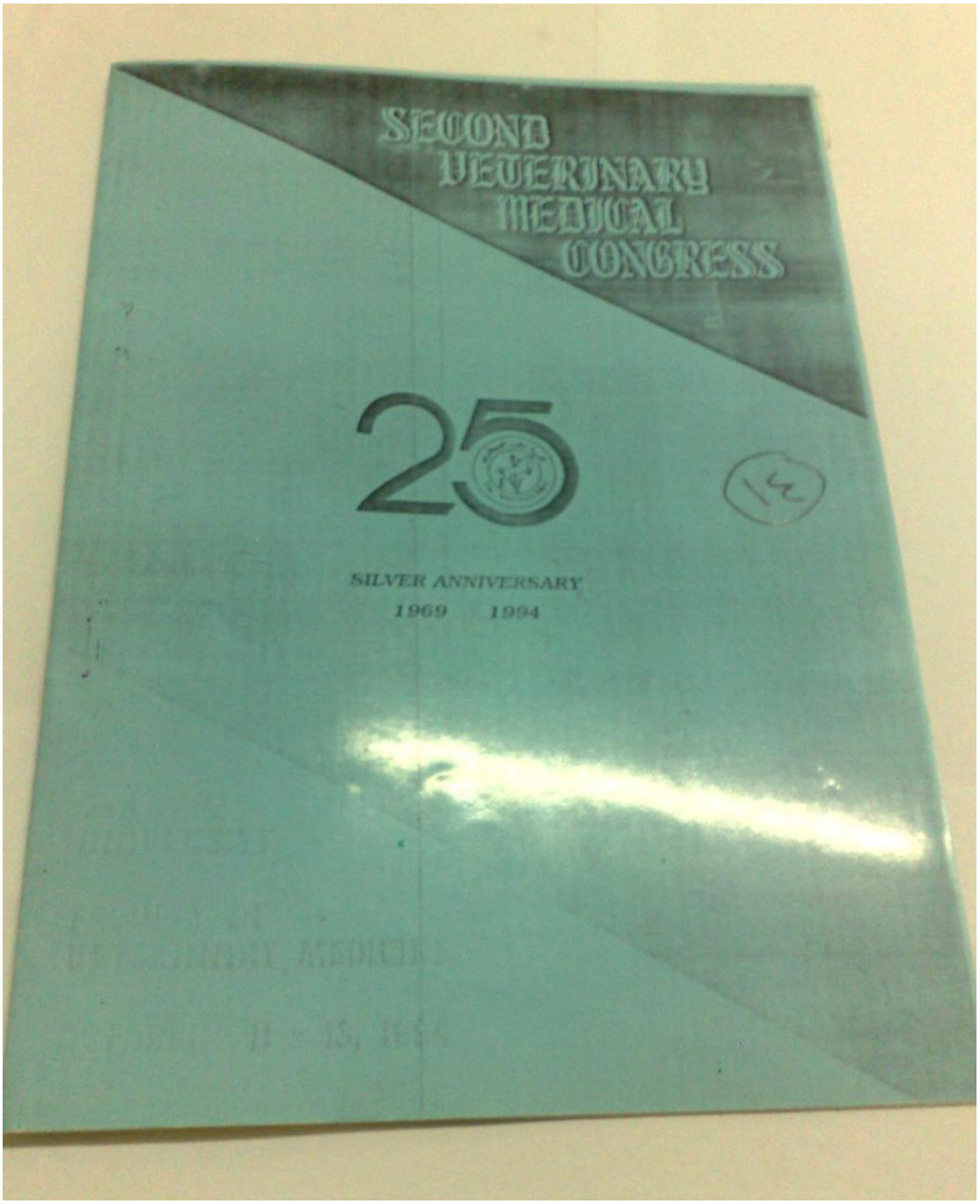
SECOND
VETERINARY
MEDICAL
CONGRESS
25
SILVER ANNIVERSARY
1969 1994

SPLENECTOMY IN THE CAMELUS DROMEDARIUS
4- POSTOPERATIVE IMMUNE RESPONSE

S.A. Al-Tuwaijri, K. A. Al-Nagedan*; I.M., Ibrahim; H.A. Abdel-Rahman; S.A. Hussein ** and S.A. El-Mougy
Dept. Vet. Med., Faculty of Agriculture and Veterinary Medicine, King Saud University, And * Bureidah Municipality (Dept. Env. Health).

** Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Moshtohor, (benha Branch) Zagazig University, Egypt.

ABSTRACT

The effect of splenectomy on the total and differential leucocytic counts, protein electrophoresis and chemiluminescence (CL) were studied in camel. There was a significant difference between the intervals days of operation (Pr. and 3,6,12,20,28,47 and 77 d.PO.) for all previous parameters. There was a tendency toward leucocytosis after the operation till 12 d.Po. with a fluctuating tendency toward a lymphocytosis (28-77 d.Po) and eosinophilia (47-77 d.Po.). Neutrophils (28-77 d.Po.) and monocytes (47-77 d.Po.) showed a decrease in the number relative to the Pr. levels. There was a decrease in the gamma globulins (47 d.Po.) and total globulins (77 d.Po.) with an increase in the total protein, albumin (77 d.Po), alpha 1 & 2 and beta globulins (47 d.Po.) relative to the Pr. levels. The A/G ratio severley decreased after the operation and returned to its Pr. level after 77 d.Po. The CL in the splenectomized camel was significantly higher than the normal camel either with PMA or OPZ.

INTRODUCTION

Granulocytes and monocytes represent the major component of the phagocyte system of the blood. The phagocyte system is considered to be the first line of defence against a variety of microorganisms that invade the host. Several techniques have been used to study the physiology of phagocyte cells. Chemiluminescence (CL) has become an important tool to assess the bacteriocidal activity of various phagocyte cells in experimental and clinical studies (Allen, 1977; Barbour et al., 1980; Welch, 1980 and Tono-Oka et al., 1983). CL has been used to examine the nature of oxidative metabolism of phagocytosis which apparently correlates well with the microbiocidal activity (Trush et al., 1978; Horan et al., 1982). In addition, the production of toxic-reactive metabolites as measured by CL was found to be closely related to

microbiocidal activity of phagocyte cells (**Ballart et al., 1978**). Phorbol myristate acetate (PMA-soluble agent) and opsonised (particulate antigen) yeast were used as phagocytic stimulant to produce a high CL response.

Much effort has been expended in attempting to unravel the way in which the spleen and lymph nodes take up foreign antigens and initiate the immune process. Studies in experimental animals clarify the importance of the spleen in clearing and trapping cellular antigens (**Likhite, 1976**). Splenectomized animals and congenitally a splenic mice reflect defective production of immunoglobulins that may parallel the inefficient thymus, bone marrow synergism observed in these animals (**Battisto et al., 1969**). Splenectomy also results in impairment of the immune response toward blood born particulate antigens (**Motohashi, 1968**).

The purpose of this study was to throw light on the effect of splenectomy on some immne responses of camel.

MATERIALS AND METHODS

Animals:

Three healthy one-humped male camles ranging from 2 to 4 years old and one male splenectomized camel (2 years) bred in the farm of the Faculty of Agriculture and Veterinary Medicine, Buriedah (King Saud University) were used in this study.

Blood collection:

Jugular blood samples were collected into tubes containing [EDTA or heparine (20 units /ml blood)] and also serum samples were obtained for protein electrophoresis. Two ml of blood from each tube (EDTA or heparin) were stored in an ice bath for the analysis of whole blood CL which was used within 3 hr. of collection. CL were carried out for splenectomized camel when neutrophil % were fixed (at 28, 47 & 77 day after operation).

Blood from the splenectomized camel were collected preoperative (Pr.) and post operative (Po.) at 3,6,12,20,28,47 and 77 days. Three samples with an hour interval for each day were obtained for statistical analysis.

Total and differential leucocytic counts: Were performed by the method of **Doxey (1971).**

Serum protein electrophoresis: Fractionation of serum proteins was carried out according to the method of **Bierer (1969)** on cellulose polyacetate strips (Gelman** Instrument Co., Ann Arbor, Michigan, U.S.A.) in an electrophoresis system (Sigma Chemical Co., St. Louis, Missori, U.S.A.) using a freshly prepared high resolution tris barbital sodium buffer (pH 8.8, ionic strength 0.05).The current was applied for 15 min. at a constant voltage 225 V. Strips containing the stained serum fractions were processed on a DCD 16 digital computing densitometer (**).

*** Chemiluminescence (CL) measurement:**

The principle of the CL assay is the oxidation of luminal, 5-amino-2,3-dihydro- 1,4-phthalzinedione (Boehringer Mannheim), by reactive oxygen species produced during phagocytosis in phagocytes. Luminol (10^{-5} M) enhance the amount of light generated by PMNs (polymorphonuclear leukocytes) upon stimulation with a soluble stimulus PMA (phorbol myristate acetate -2 ng/ml) and OPZ (opsonised zymosan, 1,25 ng/ml). Luminometer (LKB- Wallace 1251) with a constant temperature (37C°) monitor was used to measure CL. It records light emission in mV. on a digital display. The luminometer was set on the 60 seconds after addition of each stimulus (PMA & POZ) to the blood to allow the long period before the onset of CL. The reaction mixture consisted of 100 ul (also, tested 200 µl for comparison of effect of amount of blood on CL) whole blood and 900 µl medium containing 10^{-5} M luminol. The results were recorded and presented as CL maximum peak light (mV).

$$CI\ inhibition\ \% = \frac{Control\ (PBS)\ CL - Blood\ of\ camel\ CI}{Control\ CL} \times 100$$

PBS = phosphate buffer solution (0.14 M NaCl, 2.7 mM KCl, 12 mM Na_2HPO_4, 0.9 mM $CaCl_2$ in distilled water)

Opsonization of zymosan:

Zymosan A (Sigma) was opsonized (OPZ) by suspending 50 mg in 3ml fresh pooled camel serum and 1 ml PBS, incubated for 30 min. at 37C° and then centrifuged at 1500 rpm for 10 min. at ambient temperature. The supernatant was then removed and the pellet was washed twice with buffer. After a second centrifugation, the pellet was resuspended in PBS at concentration of 1.25 mg/ml and stored in the freezer until use.

Statistical analysis:

Results were expressed as the mean ± standard deviations (SD). Data were evaluated by analysis of variance for leucocytic count and protein electrophoresis in between intervals days (I.d) of operation and the differences between I.d. determined by the method of least significant differences. Also, student's T-test for CL in between normal and splenectomized camel were done.

RESULTS

The mean values for plasma proteins and their fractions are presented in Table 1. The table indicates a significant differences in between I.d. of operation. The plasma proteins and protein fraction were significantly decreased at 3 d.Po. with the exception of alpha 1 and gamma globulin which increased. Relative to the pre-operative level, there was a decrease in the gamma globulins (47 d.Po.) and total globulins (77 d.Po.) with an increase in total protein, albumin (77 d. Po.), alpha 1 and 2 and beta globulins (47 d.Po.). The A/G ratio severly decreased after operation and returned to pre-operative level after 77 d.Po.

Table 2 reveals a tendency toward leucocytosis after splenectomy till 12 d.Po. The post-operative differential leucocytic count was characterized by a flactuating tendency toward lymphocytosis (28-77 d.Po.) and eosinophilia (47 - 77 d.Po.). Neutrophils (28-77 d.Po.) and monocytes (47 - 77 d.Po.) showed a decrease in the number relative to the Pr. numbers.

Table -3 indicates that the CL in the splenectomized camel to be significantly higher than the normal camels either with PMA or OPZ. Moreover, table 3 showed that the CL were decreased with addation of EDTA either in normal or splenectomized camels. Also, from table 3 & 4 the CL was significantly increased when 100 µl blood with heparin was used with OPZ as stimulant.

DISCUSSION

The recorded results in the total proteins of splenectomized camel agree with the previous results of **Moore and Van Den Hend (1978)** in horse and **Bolbal et al. (1982)** in sheep. Splenectomy has no profound effect on immunological responsiveness. Nevertheless the spleen is an important source of antibodies (**Myerson, 1957**) and its removal

delays the peak of antibody response in young birds, and depresses antibody production in older birds (**Freeman, 1983**). It is of particular interest that the production of antibody proteins was not inhibited in splenectomized persons who had been immunized with tetanus toxoid (**Myerson, 1957**), whears inhibition in antibody protein production occurred in splenectomized cattle infected with anaplasmosis (**Ristic et al., 1958**).

The A/G ratio in the present study returned to its preoperative level at 77d. Po. which is of importance in maintaining the osmotic pressure balance of the serum proteins (**Dimopoullose et al., 1959**). Since albumin contributes more to osmotic pressure than do the globulins (**Cohn, 1945**), a compensatory increase in this fraction may still be required after surgical shock, loss of blood and recovery. The possibility also exists that hypertrophic non-splenic lymphoid tissues produced this increased proportion of albumin to globulin.

The leucocytosis occuring in the present study after splenectomy is docomented by the findings of (**Lipson et al., 1959; McBride et al., 1968**) (human) and **Said et al.**, in press (donkies). The observed increase in lymphocyte is in agreement with the results of **Crosby (1963)** who suggested that after removal of the spleen, its functions are to some extent taken over by other lymphoid organs. **McBride et al., (1968)** reported that the spleen plays some part in the regulation of lymphocyte count.

The recorded decrease in neutrophil in the present study is in agreement with **Said et al.** (in press) in donkeys. This results disagrees with that in human. The neutropenia of hypersplenism has been attributed to selective removal of neutrophils by the spleen. It is associated with neutrophilic hyperplasia of the marrow and is corrected by splenectomy (**Henry, 1983**).

Several techniques have been used to study the physiology of phagocyte cells. The intravenous clearance of radioactive colloids and carbon colloids are, commonly, used in both clinical and experimental studies to evaluate the phagocytosis in vivo. In addition, serum lysozyme level was used as an indication for phagocytosis (**Koskoshis and**

Diluzio, 1979). On the otherhand, there are several in vitro techniques which have been well established e.g. red oil emulsion, and nitroblue tetrazolium. Furthermore, chemiluminescence has become an important tool to assess the bacteriocidal activity of various phagocyte cells in experimental and clinical studies (**Welch, 1980 and Tono-Oka et al., 1983**). The observed decrease in CL with addition of EDTA in the present study, agrees with **Ross et al., (1981)** in human and **Nagahata et al. (1991)** in dogs. The authors demonstrated that the binding of C_3-Coated zymosan and C_3+ IgG - coated zymosan to neutrophils was completely inhibited by the addition of EDTA. In present study, the CL in splenectomized camel were significantly higher than normal camel CL., which agrees with the study of **Nagahata et al., (1991)** in dogs (without removal of spleen). Who, found that the enhanced CL responses may be associated with activation of neutrophils by inflammatory conditions.

REFERENCES

Allen, R.C.; Stjerhholm, R.L.; Heed, M.A.; Harper, T.B.; Gupta, S.; Steele, R.H. and Waring, W.W. (1977): Correlation of metabolic and chemiluminescence responses of granulocytes from three female siblings with chronic granulomatous disease. J. infect. Dis. 136, 510-518.

Ballart, I.J. Estevez, M.E.; Diez, R.A. and Sen, L. (1978): Comparison of Candida killing activity measured by chemiluminescence and cytomorphological methods in human phagocytes. J. Immunol. Methods 97 263-268.

Barbour, A.G.; Craig, D.A.; Stolberg, C.O. and Hille; H.R. (1980): Chemiluminescence by polymorphonuclear leukocytes from patients with active bacterial infection. J. infect. 141, 14-26.

Battisto, I.R.; Cantor, I.C. and Borek, F. (1969): Immunoglobulin synthesis in hereditary spleenless mice. Nature, 222: 1196-1198.

Bierer, B.W. (1969): Electrophoretic analysis of blood serum and plasma proteins of normal horses. Am. J. Vet. Res. 30, 2237-2240.

Bolobol, A.E.; Hassan, M.S.; Hassan, N.K. and Misk, N.A. (in press): Long term splenectomy in sheep.

Cohn, E.J., (1945): The chemical separation and the clinical appraisal of the components of the blood. Medicine, 24: 333-338.

Crosby, W.H. (1963): Hyposplenism an inquiry to normal functions of the spleen. Am. Rev. Med. 14, 349.

Dimopoullos, G.T.; Foote, L.E. and Schrader, G.T. (1959): Electrophoretic studies of bovine serum. I. Changes in the serum proteins after splenectomy. Am. J. Vet. Res. 20: 270-272.

Doxey, D.L. (1971): Veterinary clinical pathology. Billiere Findall and Cassell Ltd. London.

Freeman, P.M. (1983): Physiology and biochemistery of the domestic fowl. A cademic press. USA.

Henry, J.B. (1984): Clinical diagnosis and management by laboratory methods. 17th Ed. W.B. Saunders Company, Philadelphia, USA, London.

Horan, T.D.; English, D. and Mcpherson, R.A. (1982): Association of neutrophile chemiluminescence with microbial activity. Clin. Immunol. Immunopathol. 22, 259-269.

Koskoshis, P.L. and Diluzio, N.R. (1979): Serum lysozome: an index of macrophage function. J. Reticuloendothel. Soc. 25, 85-89.

Likhite, U.U. (1976): Immunological impairment and susceptibility to infection after splenectomy. J.A.M.A. 236, 1376-1377.

Lipson, R.L.; Bayrd, E.D. and Watkins, C.H. (1959): The post splenectomy blood picture. Am. J. Clinic. Path., 32, 526-532.

McBride, J.A.; Dacie, J.V. and Shapley, R. (1968): The effect of splenectomy on the leucocytic count. Brit. J. of Haematology, 14, 225-231.

Moor, A.D., Van Den Hende, C. and Moens, Y., (1978): Influence of promazine on the venous haematocrit and plasma protein concentration in the horse. Zbl. Vet. Med. A, 25, 189-197.

Motohashi, S.J. (1968): The effect of splenectomy on the production of antibodies. Med. Research. 43, 473-485.

Myerson, R.M., Stout, R. and Havens, W.P., (1957): The production of antibody by splenectomized persons. Am. J. M. Sci., 234: 297-300.

Nagahata, H.; Sako, T.; Reiter, J.A.; Dibartola, S.P.; Couto, G.G. and Kociba, G.J. (1991): Neutrophil adherence, phagocytic - Nitroblue tetrazolium reduction and chemiluminescence in canine whole blood. Veterinary research communications. 15, 181-188.

Ristic, M., White, F.H., Creen, J.H. and Sanders, D.A., (1958): Effect of cortisone on the mechanism of anaplasma immunity in experimentally infected calves. I. Hematological and immunoserological studies. Am. J. Vet. Res., 19: 37-43.

Roos, D.; Bot, A.A.M.; Van Schsik, M.L.J.; DeBoer, M. and Daha, M.R. (1981): Interaction between human neutrophils and zymosan particles: The role of opsonins and divalent cations. Journal of Immunology, 126, 433-440.

Said, A.H.; Gergis, S.M.; Youssef, A.H. and Mahfouz, M.F. (in press): Splenectomy in donkeys with reference to the postoperative immune response and phagocytic acivity of neutrophils.

Tono-Oka, T.; Matsumoto, T.; Yashiki, N., and Matsumoto, S. (1983): Chemiluminescence of whole blood 11. Application to clinical examination of phagocytic functions of whole blood from various types of diseases. Clin. Immunopathol., 29, 333-340.

Trush, M. A.; Wilson, M.E. and Van Dyke, R. (1978): The generation of the chemiluminescence by phagocytic cells. Methods Enzymol. 57, 462.

Welch, W.D. (1980): Correlation between measurements of luminol-de-pendent chemiluminescence response and bacterial susceptibility to phagocytosis. Infect. Immunity 30, 370-374.

Table (1): Electrophoretic pattern of serum proteins in splenectomized camel.

Intervals from splenectomy (Days =d)	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G ratio	Globulin fractions (%)			
					Alpha 1	Alpha 2	Beta	Gamma
Pr.	5.79b ± 0.10	2.58b ± 0.02	3.21d ± 0.11	0.80a ± 0.03	1.00d ± 0.05	10.50e ± 0.20	15.50e ± 0.17	22.55d ± 0.20
3 d.po.	4.46e ± 0.03	1.80d ± 0.03	2.66e ± 0.02	0.68b ± 0.02	1.50c ± 0.13	6.50f ± 0.19	14.55f ± 0.13	23.00c ± 0.21
6 d.po.	5.32c ± 0.06	1.93c ± 0.04	3.39c ± 0.12	0.57c ± 0.01	2.00b ± 0.20	12.00d ± 0.15	20.00d ± 0.28	16.50f ± 0.27
12 d.po.	5.80b ± 0.10	1.93c ± 0.13	3.87a ± 0.11	0.50c ± 0.04	1.50c ± 0.11	13.00c ± 0.30	20.00d ± 0.35	25.01b ± 0.44
20 d.po.	5.21c ± 0.12	1.43e ± 0.05	3.78b ± 0.13	0.38e ± 0.00	1.50c ± 0.01	14.00b ± 0.21	22.00c ± 0.30	28.00a ± 0.17
28 d.po.	4.76d ± 0.21	1.18f ± 0.01	3.58b ± 0.14	0.33f ± 0.00	2.00b ± 0.03	12.00d ± 0.40	28.00a ± 0.21	23.00c ± 0.18
47 d.po.	5.39c ± 0.11	1.70d ± 0.00	3.69b ± 0.10	0.46b ± 0.01	3.00a ± 0.05	15.00a ± 0.22	25.00b ± 0.25	19.00e ± 0.09
77 d.po.	6.01a ± 0.14	2.73a ± 0.09	3.30d ± 0.21	0.80a ± 0.15	±	±	±	±
LSD	0.2073	0.1069	0.2203	0.0979	0.1813	0.4396	0.4407	0.4191

Mean ± SD Pr: Pre- operative Po: Post - operative. LSD : Least significant differences.
a,b,c,d,e,f,g,.....is the arrangement of highly significant values to less one.

Table (2): Mean values of total and differential leucocytic cell counts in splenectomized camel blood.

Intervals from splenectomy (Days ±d)	Total leucocytic count 10^3/cu.mm.	Differential leucocytic counts (%)				
		Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Pr.	4.00c ± 0.51	66.00c ± 0.21	20.00g ± 0.31	10.00b ± 0.81	4.00d ± 0.24	0.00 -
3 d.po.	23.00b ± 0.73	60.00c ± 0.01	30.00f ± 0.80	6.00d ± 0.60	4.00d ± 0.20	0.00 -
6 d.po.	27.00a ± 0.25	8.00h ± 0.14	80.00a ± 1.24	10.00b ± 0.82	2.00e ± 0.10	0.00 -
12.d.po.	19.50c ± 0.34	75.00a ± 0.15	15.00h ± 0.25	8.00c ± 0.71	2.00e ± 0.30	0.00 -
20 d.po.	8.00d ± 0.15	26.00g ± 0.20	54.00b ± 0.31	6.00d ± 0.35	14.00a ± 0.50	0.00 -
28 d.po.	8.50d ± 0.20	47.00e ± 0.40	36.00e ± 0.41	15.00a ± 1.35	2.00e ± 0.20	0.00 -
47 d.po.	8.90d ± 0.13	43.00f ± 0.21	40.00e ± 0.90	3.00e ± 0.21	8.00b ± 0.10	0.00 -
77 d.po	18.65c ± 1.50	50.00d ± 0.99	38.00d ± 0.76	5.00d ± 1.00	7.00c ± 1.15	0.00 -
LSD	1.1422	0.7006	1.4748	1.3930	0.8256	

pr : pre - operative Po: Post -operative.
LSD : Least significant differences.
a,b,c,d,e,f,g,h,.....is the arrangement of highly significant values to less one.
Means with the same letter are not significantly different (P<0.0001).

Table (3): Effect of splenectomy on the chemiluminescence (CL) response of camel's whole blood stimulated with PMA and OPZ using different anticoagulents.

		PMA		% inhibition		OPZ		% inhibition	
		Heparine	EDTA	Heparine	EDTA	Heparine	EDTA	Heparine	EDTA
Normal	Base Line (mV)	0.771 ± 0.084	0.583 ± 0.013	0.00	0.00	0.771 ± 0.084	0.582 ± 0.013	0.00	0.0
	Peak CL (mV)	2.519 ± 0.488	1.423** ± 0.234	0.00	0.00	15.027 ± 3.470	0.614 ± 0.072	0.00	0.00
Splenectomized	Base Line (mV)	0.843 ± 0.050	0.525 ± 0.031	0.00	0.00	1.333 ± 0.897	0.705 ± 0.186	0.00	0.00
	Peak CL (mV)	3.459** ± 0.185	0.725 ± 0.025	0.00	0.00	33.60** ± 4.038	3.068** ± 0.349	0.00	0.00

Mean ± SD Highly significant (** $P < 0.01$) 2 ng/ml PMA , 1.25 mg/ml OPZ and 10^{-5} M luminol were used at 37°C

Table (4): Chemiluminescence (CL) response of camel's whole blood stimulated with OPZ using different blood volume.

	100 ul Blood	200 ul Blood
Base line (mV)	1.143 ± 0.039	0.537 ± 0.34
Peak CL (mV)	38.251 ± 0.047 **	9.358 ± 0.042

Mean ± S.D. (** $P < 0.01$) 1,25 mg/ml opsonized zymosan (POZ) and 10^{-5} M.luminol were used at 37° C.

استئصال الطحال فى الجمال

٤- رد الفعل المناعى بعد العمليه

صالح عبد العزيز التويجرى ، خالد النقيدان * ، ابراهيم محمد ابراهيم ،
حسن عبد الرحمن عبد الرحمن، سامى على حسين * * ، سامى احمد الموجى
قسم الطب البيطرى بكليه الزراعه و الطب البيطرى - جامعة الملك سعود وبلديه مدينه بريده - اداره صحة البيئه *
* * قسم الفسيولوجيا والكيمياء الحيوية والفارما كولوجيا - كليه الطب البيطرى بمشتهر - جامعة الزقازيق - فرع بنها

تأثير إستئصال الطحال على العدد الكلى والنوعى لكرات الدم البيضاء والفصل الكهربى للبروتينات واللمعان الكميائى قد تم دراسته على الجمال وقد لوحظ ان هناك فروق معنويه قبل العمليه وبعد العمليه بـ ٣ ، ٦ ، ١٢، ٢٠ ، ٢٨ ، ٤٧، ٧٧ . يوم لاغلب النقاط التى سبق ذكرها . وقد وجد ان هناك ميل لزيادة عددكرات الدم البيضاء حتى اليوم ١٢ بعد العمليه مع زياده ملحوظه فى الخلايا الليمفاويه (من اليوم ٢٨- ٧٧ بعد العمليه) وكذلك الخلايا الحمضيه (٤٧- ٧٧ يوم بعد العمليه - بينما لوحظ نقص فى عدد كل من الخلايا المتعادله (٢٨ - ٧٧ يوم بعد العمليه) والخلايا وحيده النواه (٤٧ - ٧٧ يوم بعد العمليه) بالمقارنه بالعد قبل العمليه . كما وجدنا ان تركيزات كل من جاما جلوبيولين (٤٧ يو م بعد العمليه) والجلوبينا ت الكليه (٧٧ يوم بعد العمليه قد نقصت بينما مستويات البروتينات الكليه والالبيومين (٧٧ يوم بعد العمليه) والفا ٢,١ وبيتا جلوبيولين (٤٧ يوم بعد العمليه) قد ازدات بالمقارنه بـ المستويات قبل العمليه . ونسبه A/G قد نقصت بصوره شديده بعد العمليه واستعادت مستوياتها قبل العمليه بعد ٧٧ يوم بعد العمليه كما وجد ان اللمعان الكيميائى عند استئصال الطحال قد سجل فرق معنوى عالى بمقارنته بالجمال الطبيعيه (بدون استئصال الطحال) سواء مع PMA أو OPZ .