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Pancreotoxicity of Chloroquine in the Rabbit.

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ABSTRACT

The Histologic and biochemical changes produced by chloroquine phosphate in doses 5, 10, and 15mg/kg over 30, 60, and 90 days in the albino (n = 10) and pigmented (n = 22) rabbits, with mean weight value of 1.46+ 0.44 kg and age mean value of 9 ± 0.25 months, were investigated in the college of medicine, University of Nigeria, Enugu Campus. Histopathology results showed chloroquine- induced lesions in the pancreas. Some pancreatic islet cells showed partial or total destruction. At day 90 the pancreas also showed fatty infiltration of the stroma, arterial attenuation in the peritoneum close to the pancreas as well as increased capilarization at dose 15mg/kg in some instances.

Biochemical analysis showed statistically significant ($P < 0.05$) increased mean values of tests over the control. This increase in sugar levels was dose and not time dependent at different periods of the study. This result has shown that the hyperglycaemia or diabetes mellitus was chloroquine induced suggesting that chloroquine is diabetogenic in experimentally induced rabbits. The histological findings in the present study were in agreement with biochemical observations. One should be wary of indiscriminate consumption of chloroquine.

Keywords: Pancreas, islet cells, diabetes mellitus, fatty infiltration, arterial Attenuation.

Chloroquine Phosphate is a valuable drug used in the control of malaria and the treatment of amoebiasis. The drug was developed in the United States during World War II for use as an anti malarial agent because of the shortage of quinine; the drug having been synthesized and studied as early as 1934 in Germany. It is currently being used for the treatment of malaria, amebiasis, rheumatoid arthritis, and for discoid and systemic erythematosus. Side reactions such as skin eruption, bleaching of the hair, retinopathy and blurred vision had previously been reported (Editorial, 1966). The usual dose is 0.5mg/day for antimalarial therapy; larger doses have been given for antiamebiasis and antirheumatoid arthritis therapy. Acute over dose of chloroquine, if untreated, will lead to circulatory failure, convulsions, respiratory and cardiac arrest and death (Mason et al, 1954; Kiel, 1954; Lobel et al, 1998).

Almost since bacteria were discovered, one theory of the etiology of rheumatoid disease has been that it is a systemic response to a chronic infection. It is tempting therefore to suggest that chloroquine acts by suppressing such a chronic infection, as it does malaria and amebiasis. Amebiasis itself, and pleuropneumonia-type organisms have each been incriminated by an enthusiastic investigator, but not supported by proof. It appears therefore that rheumatoid disease is a final common pathological pathway capable of being triggered by a variety of stresses, but it is unreasonable to suppose that it is invariably due to an elusive organism chemotherapeutically susceptible to chloroquine in a majority of instances. In addition to the progressive frontal fibrosing alopecia in all their patients on chloroquine medication, total loss or a marked decrease of the eyebrows was observed in 13 of 16 patients (Kossard et al, 1997). The authors concluded that the progressive frontal fibrosing alopecia was a clinically distinct variant of lichen planopilaris that affected in particular elderly women and frequently involved the eyebrows and that the basis for lichenoid tissue reaction targeting frontal scalp follicles and eyebrows was unknown. The embryotoxicity of chloroquine has been evaluated in vitro using the rat whole embryo culture system. Chloroquine was found to be embryotoxic and dysmorphogenic when added directly to the culture media containing gestational day (GD) 10 rat conceptuses
(Ambroso and Harris, 1993;Meyer, 1963). Histologic evaluation revealed that the cytoplasm of the visceral yolk sac (VYS) endoderm epithelium was distended

due to vacuolization produced by chloroquine exposure.

Cytoplasmic vacuolation and membranous inclusions also have been described in the leukocytes and pancreatic exocrine cells of rats fed chloroquine (Fedorko,1968). Because literature on chloroquine toxicity of the pancreas is scant, we decided to investigate its overall chronic histologic and biochemical effects in this organ using the human therapeutic dose regimen similar to that of Wilkinson and Davidson (Wilkinson and Davidson, 1996). In the current study, determination of the onset of toxicity and its relationship with dose was undertaken and the pattern of progression of chloroquine-induced histologic lesions with time was also established.

MATERIAL AND METHODS

Animals

Thirty-two gnotobiotically reared adult rabbits each approximately 9 months of age, 22 pigmented and 10 albino were procured from the Veterinary Research Institute, Vom, Nigeria and kept in the animal house of the college of Medicine, University of Nigeria, Enugu Campus for 4 weeks for acclimatization. They were housed in bottom-wired stainless metal cages and were allowed food and tap water ad libitum. Individual identification of the animals was by metal ear tags.

Experimental Design.

The experimental animals were divided into 4 groups comprising 8 sex-matched rabbits in each of the 4 groups. Those in groups 1, 2 and 3 constituted the test groups, while the 4th group acted as the control. The animals in group 1 were given doses of chloroquine 5mg/kg /day. The animals in group 2 received 10mg/kg (twice the dose), while the 3rd group received 15mg/kg (thrice the dose), all given intramuscularly dose daily for a period of 90 days. The 4th group received equal volume of normal saline daily.

Experimental Procedure:

Blood samples were taken from the marginal ear for biochemical parameters prior to drug administration. Thereafter, similar samples were collected at days 30, 60 and 90. Blood samples collected prior to drug administration produced biochemical values, which represented the baseline values. Two animals from each of the test groups and two from the control group were painlessly sacrificed after 30, 60 and 90 days. The rabbits were all living when the study terminated and were given intramuscular injection of 50mg/kg of sodium thiopentane and were exsanguinated. Necropsies were performed immediately. Representative samples of the pancreas were promptly fixed in 10% neutral formal saline, histologically processed using the Elliot automatic tissue processor, embedded in paraffin wax, and sectioned at 5μ thick using the Rotary microtome and stained by Haematoxylin and Eosin technique (Ehrlich, 1886).

The Pfizer Pharmaceutical Company Limited, Lagos, Nigeria, supplied the drugs used for the work.

Statistical Analysis:

The results were expressed as mean $\pm$ standard error of mean (SEM) and significance of differences between control and treated as well as before and following treatment for biochemical parameters were determined using paired student t-test. Statistical significance was set at $P < 0.05$.

Sample collection:

About 1.5 ml whole blood was collected in fluoride-oxalated tubes for glucose determination after fasting the animals in all the groups for 12 14hrs overnight.

Biochemical Analysis:

The method used for fasting blood sugar estimation was according to Trinder (Trinder, 1998)

RESULTS

Clinical Observation:

Clinical changes were observed in all animals receiving chloroquine phosphate. The most consistent clinical observations were sluggishness, suppressed appetite, especially in group 3 and poor general appearance.

Group I received chloroquine in the amount of 5mg/kg/day for 60 days with no apparent ill effect. Two animals in this group lost weight after the first 2 weeks of the chloroquine administration. The rest of the animals also maintained consistent slight weight loss till sacrifice. On the first day of the drug administration, one gravid animal in group 2 spontaneously aborted one fetus 3 hours later, whereas on the 9th day of treatment, 2 of 8 rabbits aborted 4 fetuses each in group 3. It was also observed that the sexual activity of all the animals sharply declined with time during treatment. The males became impotent

during the period of therapy. The obvious clinical changes in the rabbits of the groups were mild dermatitis, which affected more of the albino rabbits than the pigmented, and hyperpigmentation of the faeces.

Onset of dermatitis was at day 65 and involved entire body, which was found dry and scurfy with prominent loss of hair in most albinos and in some of the pigmented on which hair bleaching was observed. All the rabbits in all the groups survived the treatment regimen and experienced slight weight loss throughout the test period. The rabbits in the control group appeared in excellent health throughout the period of study.

Gross Anatomical Observations:

The pancreas obtained from the control group showed no differences in their normal gross anatomical features, i.e. size, color and consistency. It was noted that alopecia, dermatitis and hyperpigmentation of the faeces probably due to loss of blood from the stomach haemorrhages occurred in the rabbits in all the groups. Bullae were observed on the abdominal regions of the rabbits in all the groups, particularly 2 and 3. After some days the bullae were observed to have ruptured and the site ulcerated. Shortly after, they healed with scar tissue formation. At necropsy, all the animals in the treatment groups showed dark red shrunken pancreas.

Histologic findings in Pancreas:

The pancreas of the animals in the treated groups showed cytoplasmic vacuolation of the pancreatic acinar cells throughout the study period. In addition, vacuolization of the islet cells of Langerhan was more evident. The islet cells, particularly the cells were either partially or totally destroyed (Fig.1) Chloroquine had more predilection for the Beta cells than the alpha -cells as it has for cones than the rods in the retina. Inflammatory cell infiltrates were not evident in the pancreas. However, only those animals in the high dose class showed fatty infiltration of the pancreatic stroma (Fig. 2). The artery very dose to this organ and located in the peritoneum showed moderate attenuation, whereas 1 of 8, in the 15mg/kg dose category displayed increased stromal capillarization. The nuclei of islet cells were hyperchromatic and enlarged. Most cell cytoplasms in the treated groups were also ill defined and non-granulated. These changes were interpreted to be hydropic degeneration. Figs. 3 & 4 represent sections of liver stained by PAS-diastase technique for glycogen storage in the treated and control. Fig. 5 shows pancreas displaying normal architecture.

Biochemical findings:

Fig 6 shows the blood sugar levels at 5, 10 and 15 mg/kg doses at different periods of study. The baseline and test mean values were 5.26 $\pm$ 0.16 and 6.24 $\pm$ 022, 6.55 $\pm$ 027 and 6.84 $\pm$ 0.21 mmol/1 at days 30, 60 and 90 respectively. There were statistically significant ($P<0.05$) increases in mean values of test over the baseline at different periods of the study. As has been shown, the results showed that when the animals were dosed 5mg/kg over the periods of study, a statistically significant increase in blood sugar was noted and was dose and not time dependent.

In group 2, the baseline and test mean values were 4.81$\pm$ 015 and 6.03 $\pm$0,20, 6. 18$\pm$ 0.24 and 6.25 $\pm$ 0.21 mmol/1 at days 30, 60 and 90 respectively. Statistically significant ($P<0.05$) increases of test mean values over the baseline were observed at different periods of the study. Overall, the result showed a statistically significant increase in blood sugar level, which was dose and not time dependent. In group 3, the baseline and test mean values were 4.35 $\pm$ 0.32, and 7.59 $\pm$ 0.38, 7.70$\pm$ 0.79 and 8.05 $\pm$ 0.6 mmol/1 at days 30, 60 and 90 respectively. Statistically significant ($P<0.05$) elevations in test mean values over the baseline were observed over the period of estimation. These results showed that 5,10 and 15 mg/kg dose of CHQ produced significant elevation in blood sugar level in the rabbit over the period of study, and were only dose dependent. The result therefore showed that CHQ phosphate is hyperglycaemic and therefore diabetogenic at these doses and the same period of study.

DISCUSSION

It is rewarding to investigate the toxicity of chloroquine in the pancreas, as not much has been documented in literature on this important organ. Here in Nigeria, malaria, rheumatoid arthritis and other autoimmune diseases have caused increased mortality and morbidity.

In this series, the histology of the pancreas under the light microscopy revealed partial and or complete destruction or dissolution of the cells of islet of Langerhan. Some of the islet cells, the and -cells showed agranulocytosis and vacuolization.

Although agranulocytosis and dissolution of

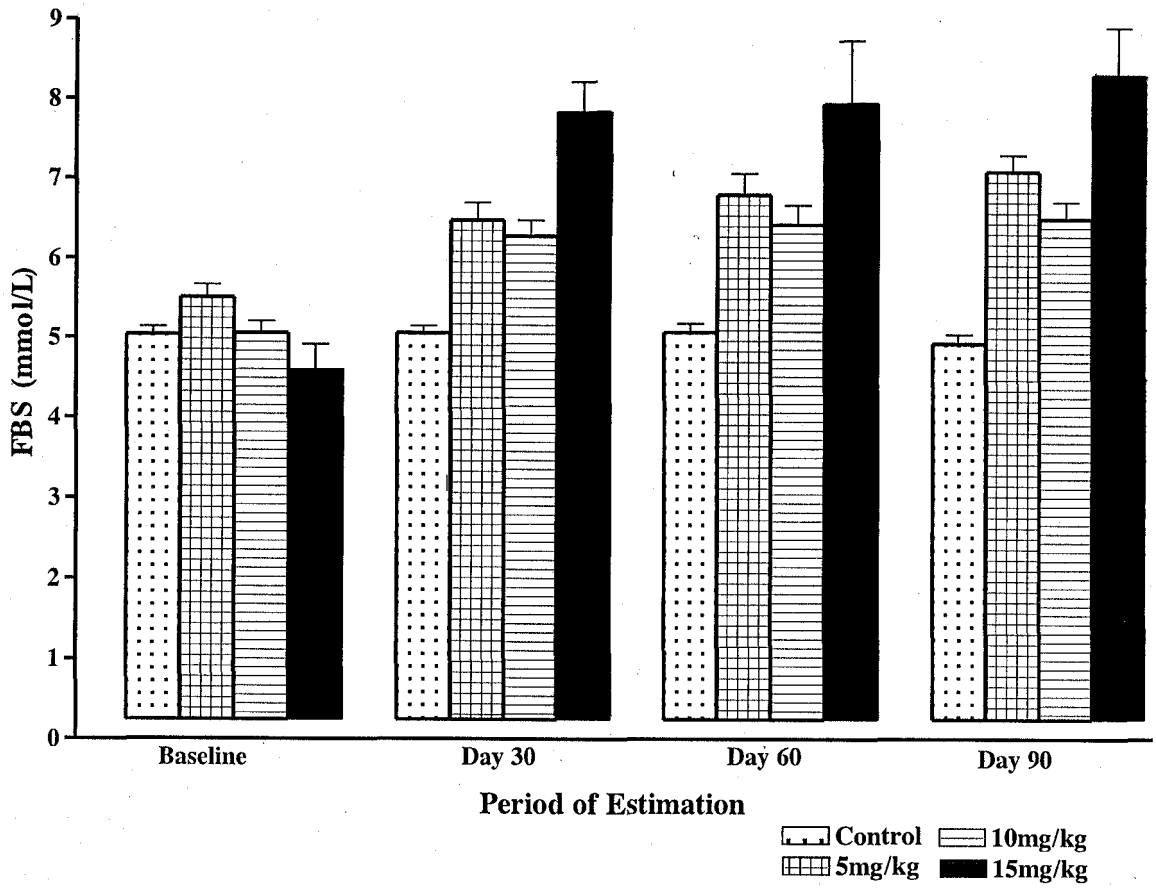

Fig.6 : Mean values of Fasting Blood Sugar(FBS)

zymogen granules has been well established (Fedorko, 1968), our finding of the destruction or dissolution of the islet cells has not been documented in literature, to the best of our knowledge. Although the pancreas of the chloroquine administrated rabbits in this study, contained focal lesions frequently around the islets, there was absence of inflammatory cells. However, the mechanism by which chloroquine causes islet cells' destruction is unknown, but it is believed that CHQ does this by causing necrosis of the cells, or its binding mechanism and possibly its inhibition of proinsulin synthesis. In the current investigation, the histologic changes observed in the pancreas included fatty infiltration of the organ at 15mg/kg dose at day 90. Only one animal of the six showed increased stromal capillarization, which reflects healing process in this particular case. Fasting blood sugar tests on the animals showed statistically significant ($P < 0.05$) increases over the control at day 30, 60 and 90 at 5, 10 15mg/kg doses of chloroquine administration. It was found that chloroquine-induced hyperglycaemia, as noted in our study, was both dose and not time dependent. The observed hyperglycaemia or experimentally induced diabetes mellitus was due to the histologic finding in the pancreas that CHQ caused destruction of the -cells. The -cells are insulin producing. Absence, destruction or loss of these cells causes an absolute deficiency leading to type 1 diabetes (IDDM). Insulin is essential in processing of carbohydrate, fat and protein. Insulin reduces blood glucose levels by allowing glucose to enter muscle cells and fat cells and by stimulating the conversion of glucose to glycogen as a carbohydrate store. In addition, it inhibits the release of stored glucose from liver glycogen and slows the breakdown of fat to triglycerides, free fatty acids, and ketones. It is not surprising therefore that fatty infiltration of the pancreatic stroma was noted in our case in view of the fact that absence of insulin causes increased breakdown of fats in the body. Furthermore insulin slows the breakdown of protein for glucose production (Lamb and Court, 2000). It was noted that there was an inverse relationship between blood glucose level and liver glycogen storage in our own series, which reflects diabetes mellitus.

The present study showed that chloroquine administration produced spontaneous abortion in the rabbits which consonnates well with the findings of Madati (Madati, 1971). Who noted that CHQ

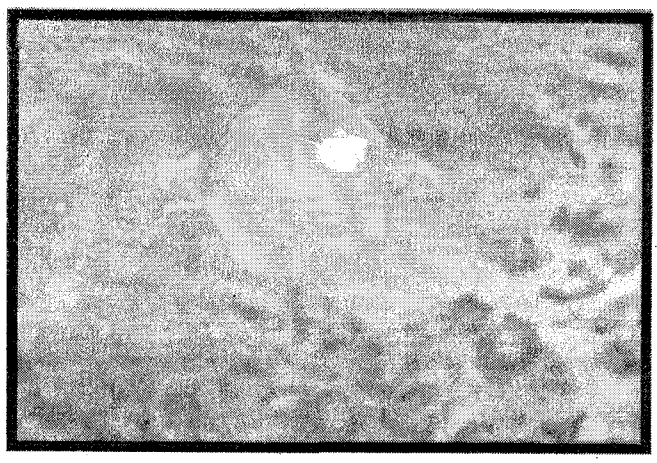

Fig. 1 shows section of pancreas with loss or destruction of the Islet cells.

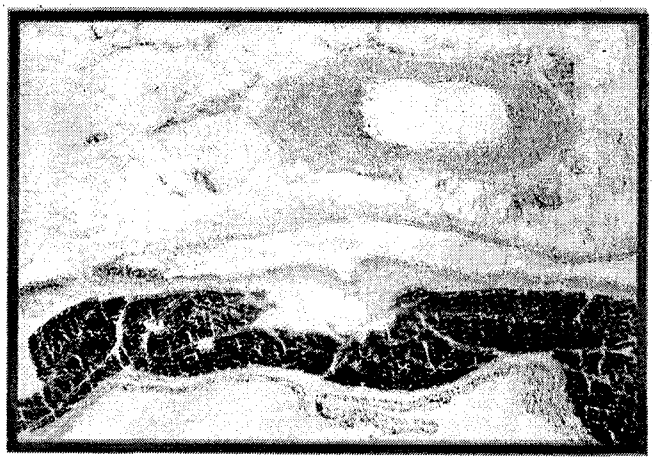

Fig 2 stromal fatty infiltration of the pancreas.

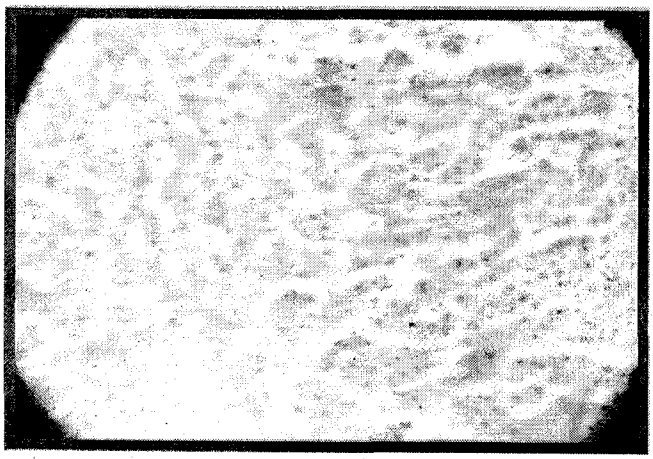

Fig 3 liver section showing liver glycogen storage as evidenced in the untreated animals.

Fig 4. Liver section from the treated rabbit showing depleted glycogen storage.

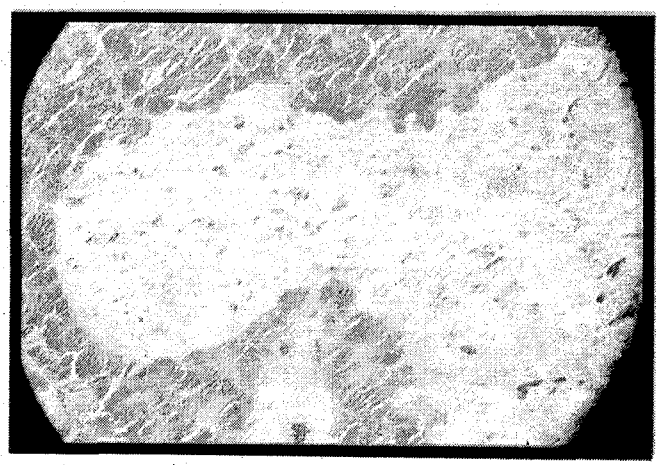

Fig 5. Section of control showing normal architecture of pancreas.

administration was abortifacient in the rat. It was also observed that during CHQ administration the sexual urge of the male rabbits totally declined and there was no more reproduction among the animals. This development could be ascribed to diabetes in them. Although B-cells destruction and subsequent insulin deficiency is the central event in the pathogenesis of insulin dependent diabetes mellitus, it is now well acknowledged that insulin dependent diabetes mellitus is also characterized by insulin resistance in peripheral tissues (De fronzo et al, 1982; Peterson and Neilson, 1987). Production dysfunction is a well recognized consequences of diabetes mellitus in men (Bartak et al, 1975; Fairburn, 1981) and experimentally induced (Paz et al, 1978 Murray et al, 1981) or spontaneous diabetic animals (Murray et al, 1981; Johnson and Sidman, 1970). Over two centuries back Rollo John Pollo (Rollo John Pollo, 1798), reported an association between diabetes and erectile failure. Naunyn regarded impotence as one of the commonest symptoms of diabetes (Naunyn,1906). However, in our series, it was observed that most of the islet cells of Langerhan were affected by chloroquine administration.

There is wisdom in avoiding indiscriminate intake of chloroquine in view of its toxic potential and patients of malaria and other autoimmune diseases should be periodically reviewed clinically to ascertain the extent of toxicity of the drugs in these patients.

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REFERENCES

Ambroso, J.L; Harris, C.(1993)chloroquine embryotoxicity in the postinplantation rat conceptus in vitro. Teratology; 48(3): 213-226.

Bartak, V.; Josifko, M; Hor adkora, (1975) Juvenile diabetes and human quality. Int. J. Fertil; 20: 30-32.

Chloroquine Retinopathy, editorial, JAMA 1966; 195: 154.

De Fronzo, R. A; Hendler, R; Simson, D. (1982) Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes;; 31: 795-801.

Ehrlich, P (1886) Demonstration of tissue structures. Z. Wiss Mikr.; 3: 150.

Fairburn, C. G. (1981) The sexual problem men. Br. J. Hos Med.; 25: 484 491.

Fedorko, M. E. (1968) Effect of chloroquine on morphology of leukocytes and pancreatic exocrine cells from the rat. Lab. Invest.; 18: 27.

Johnson, L.M; Sidman, R. L (1970) Reproductive endocrine profile in the diabetes (dh). Biol. Reprod; 20: 552-559.

. Kiel, F. W. (1954) chloroquine suicide. JAMA;257: 239-242.

Kossard, S. Lee, M. S; Wilkinson, B (1997) Post menopausal frontal fibrosing alopecia: a frontal variant of lichen planopilaris. J AM-Acad-Dermatol;36 (1): 59-66.

Lamb, W. H; Court, S. (2000) Diabetes mellitus Medicine; 3 (2): 1-24.

Lobel , H.O. Coyne, P. E.; Rosenthal, P. J.(1998) Overdoses with antimalarial agents: prescribing and dispensing errors. JAMA; 280 (17): 1-3.

Madati, P.J: (1971) Chloroquine poisoning in man. East African Medicine Journal; ;48(11): 650-657.

Mason, J. R; Khurshid, K; and Frewing, L. H, (1954) Fetal chloroquine poisoning: two more cases JAMA; 188: 187.

Meyer, F; and Thorndyke, Vv. (1963) Passage of drugs across placenta. An. J. Obstet. Gynec;; 84: 1778-1798.

Murray, F. T; Orth, J. M.; Gunsalus, E. (1981) The pituitary testicular axis in the streptozotocin diabetic male rat. Evidence for gonadotroph, sertoli cell and leydig cell dysfunction. In J. Androl; 4: 265-280.

Naunyn B Der, Diabetes mellitus, Alfred Holder, Vienna, 1906.

Paz, G; Homonnai, Z. T; Drasnin N (1978) Fertility of the streptozotic-diabetic male rat. Andrologia; 10: 127-136.

Peterson, O; Beck- Neilson, H (1987) Insulin resistance: Insulin resistance and insulin- dependent diabetes mellitus. Diabetes care; 10; (suppl): 516-523.

Rollo, John Pollo, Dilly London 1798. Trinder, P.(1998) Glucose concentration An. Clin. Bioch.; 6: 24.

Wilkinson, R. J; and Davidson, R. N. (1996) Drug therapy of falciparum malaria- present practice and future prospects JAMA; 450:1.

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