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Topical Use of Jatyadi Oil in Normal and Dexamethasone Suppressed Wound Healing in Animal Studies

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ABSTRACT

The present study was undertaken to appraise the topical effects of Jatyadi Taila [JT] on normal and dexamethasone (DEX) suppressed wound healing in albino Wistar rats. Incision and excision wounds were experimentally induced in the animals. Topical application of JT on normal incision wound caused a significant ($p<0.05$) increase in wound breaking strength when compared to control and DEX treated animals. JT significantly ($p<0.05$) improved tensile strength in JT+DEX treated group as compared to DEX group. A significant ($p<0.01$) rise in hydroxyproline levels were exhibited in JT+DEX treatment as compared to normal and DEX control. JT reversed the DEX suppressed inflammation which was evident from the histo-pathological studies showing increased neutrophils and macrophage infiltration. Dense collagen deposition and high fibroblast counts were observed in the photomicrograph sections of JT+DEX treated group. The present study suggests the substantial potential of JT in wound management in both normal and delayed healing.

Keywords: Jatyadi oil; dexamethasone; HillSlope; hydroxyproline; anti-oxidants

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INTRODUCTION

Wound healing is a natural process of the body to correct the breach in tissue due to injury. Optimum healing of a wound is a prerequisite for the general wellbeing of an individual. Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [1].Wounds can be caused due to the result of surgery (incisional type), trauma, burns or a predisposing disease like diabetic or arthritic ulcers, venous or arterial diseases which are of the non-healing types. They generally cause a physical impairment in the injured area due to disruption of the orderly arrangement of the tissues. When tissue is destroyed by injury, the healing process proceeds through three phases. An inflammatory phase characterized by cell proliferation and migration; a proliferative phase dominated by collagen deposition and angiogenesis; and a maturation phase involving resolution of inflammation and scar maturation [2].

When the healing process deviates from its normal path, it leads to undesirable effects like over healing, under healing or non-healing. In chronic or non-healing wounds, sequence of normal wound healing progressing through inflammation, granulation tissue formation and remodeling often fails due to aberrant prolongation of the inflammatory phase. The chronic/ non healing wound is a cause of concern as they are more prone to infections. They neither improve after 4 weeks nor heal in 8 weeks. Millions of people who suffer from diabetes or who are undergoing treatment with corticosteroids are prone to chronic wounds at some point in their life. If the physician cannot control the infection in these chronic wounds, the patient may become further compromised by additional tissue damage, bacteremia, sepsis or deeper wound infections that may require the surgeon to consider additional surgery or possible amputation of a portion of a limb to spare adjacent viable tissues or to save the patient [3]. The physician's use of allopathic formulation mainly consists of antibiotics, creams and ointments for external application in wound healing. The emergence of resistant microorganisms has worsened this situation. The non-healing wound is a profound cause of morbidity amongst the affected. Chronic wounds are thus a debilitating factor to a person's quality of life as it inflicts significant pain and compromises with the mobility of a person thus forcing them to undergo hospitalization. This has imposed a huge financial burden. Moreover the healthcare system is weighed down with the pressure to develop an efficacious and cost effective wound management care which causes significant reduction in the time required for wound management.

As a result, the possibility of deriving alternative, cost effective therapies from traditional plant-based medicines has been explored [4]. About 163 species of plants were used as wound healing plants in Indian systems of medicine such as Ayurveda, Siddha, Unani and folk medicine [5,6]. Ayurveda is the most practiced form of traditional medicine in India and globally gaining recognition. The current study is to scientifically prove the wound healing efficacy of Jatyadi Taila (JT) in normal and dexamethasone suppressed wound healing. Classic Ayurvedic text, Sharangdhar Samhita mentions about the excellent wound healing and antimicrobial activity of JT. Traditionally, the use of this oil is as soothing, cleansing and healing oil meant for external application in wounds, ulcers and burns (Ayurmantra). Very little scientific study has been done to prove the proclaimed benefits of this oil in wound healing. A preclinical study was done to determine the wound healing efficacy of JT in excision wound model of Albino Wistar rats [7]. However JT has not been experimentally tested on the granulation and collagenation phases of wound healing. Therefore the following study has been proposed to assess the wound healing properties of JT in albino Wistar rats.

MATERIALS AND METHODS

Animals and their maintenance

Healthy inbred male albino rats [IAEC/KMC/71/2013] of Wistar strain weighing between 150 g - 250 g were used in the studies after obtaining the animal ethical approval. They were individually housed and maintained on normal food and water ad libitum. Animals were periodically weighed before and after the experiments. The rats were anaesthetized during infliction of wounds. All the surgical interventions were carried out under ketamine anesthesia (10mg/kg body weight). Animals were closely observed for any infections and the ones showing any signs of infection were separated and excluded from the study. Except the test drug, no other chemotherapeutic cover was provided to the animals.

Drug application

The test oil was applied twice daily for a period of 10 days in incision wound model and 21 days in excision wound model. The dose of the oil applied was 0.75ml/wound/day

Wound models

Incision wound model (Collagenation phase): [8]

The rats were anaesthetized using ketamine. The fur on the back of the rats were shaved and wiped with alcohol. Two lines of about 6 cm each were drawn on either side of the rat, 1cm away from the vertebral column. Using a sharp, sterilized surgical blade, wound was made along the length of the drawn lines. The wound was wiped with alcohol and allowed for hemostasis. Thereafter, the parted wound was sutured every 1 cm apart using a surgical needle with tapering ends and a size zero silk thread. The day of induction of the wound was considered as day 0. The application of the oil started from day1. The sutures were removed on the 8th day and the application of the oil was continued till the 10th day. The skin breaking strength was measured on the 10th day post wounding by constant water flow technique [9].

Measurement of Skin breaking strength

Rats were sacrificed by overdose of ketamine. The rat was placed on its stomach on a wooden platform. Two Babcock forceps were attached to either side of the wound. One of the forceps was attached to a rigid metal stand and the other forceps was connected to a hook attached to a string which was passed over a pulley. At the other end of the string, a polypropylene bottle was attached. Water from the reservoir flowed into the bottle through a rubber tubing which could be clamped to stop the flow. The flow of water was maintained at a constant rate.

Water was allowed to flow into the bottle. As the weight of the bottle increased, the tension on the string connected to the forceps increased. This caused a strain on the wound which gradually began to pull apart. The weight of the bottle at which a slight opening in the wound was observed was noted. The other wounds were also treated similarly. The average wound breaking strength was calculated for each animal and the mean of all the 6 animals in each group was considered to be the skin breaking strength of that group.

Excision Wound model [10]

Animals were anaesthetized using ketamine. The fur on the back of the rats were shaved and wiped with alcohol. Using a round seal of 500mm^2 area, an impression was made on the dorsal thoracic central region of the rat, 5 cm away from its ears. A full thickness excision of the skin was made to obtain a wound of 500mm^2 area. The wound was wiped with alcohol and hemostasis was achieved. The animals were placed individually in a cage. A stainless steel mesh was placed over the bedding to prevent husk from sticking to the wound. Application of the oil started from day 1 to day 21 post wounding. Parameters measured were wound contraction and epithelialization period.

Wound Contraction

Wound contraction was determined by tracing the raw wound on a transparent sheet every alternate day from day 1 to day 21 post wounding. Wound area was assessed by retracing the wound on a graph paper. Wound contraction was calculated as the percentage of the original wound size (500mm^2).

Wound Contraction (%) = 1-[Wound Area/Original Wound Area (500mm^2)]*100

Period of Epithelialization

Days required for falling of scab leaving no visible wound behind was taken as period of epithelialization.

Excision of wound tissue for various parameters

On day 4 and 14, one animal from each group was randomly selected and sacrificed. The wound tissue was excised for hydroxyproline [11] estimation. They were placed in tightly sealed Eppendorfs and preserved at -20ºC.

Histo-pathological studies

The last part of the tissue was placed in a tube containing 10% neutral formalin solution and sent for histo-pathological evaluation.

RESULTS

Jatyadi taila was tested on incision and excision wound models on normal and dexamethasone treated Albino Wistar rats. Jatyadi taila was administered as a topical formulation at a dose of 0.75ml/ wound daily.

Drug administration

The study was done to assess the wound healing efficacy of Jatyadi taila on normal as well as dexamethasone suppressed wound in excision and incision wound models. The animals were primarily divided into normal group which included control on which no treatment was done and JT alone, which received treatment with Jatyadi alone. For assessing delayed wound healing, animals were treated with 0.17mg/kg of dexamethasone i.m. every alternate day from the start of the study. They were further divided into two groups, positive control which received only dexamethasone and JT+DEX group which received both dexamethasone as well as JT. The parameters observed in incision wound model were skin breaking strength and change in body weight. In Excision wound model, the parameters considered were wound contraction rate, HillSlope and WC50 (time taken for 50% of wound to contract), and effect on serum as well as tissue antioxidant levels.

Incision Wound Model

The Incision wound model study was done for a period of 10 days. The sutures were removed on day 8 of the study and the animals were sacrificed and skin tensile strength was measured on day 10. The skin breaking strength of all the groups was measured using Lee's constant water flow technique.

A highly significant ($p<0.05$) improvement in the skin breaking strength was observed in JT alone group (546.4±33.12 g) as compared to control (394.7±12.77 g) and positive control group (349.5±8.90 g). JT alone group also showed a significant ($p<0.05$) increase in skin tensile strength as compared to JT+DEX group (426.6±10.47). When compared with positive control, JT+DEX group showed significant ($p<0.05$) increase in skin breaking strength. However the mean difference between control vs positive control and control vs JT+DEX was not significant indicating that they did not contribute much to the healing process. The results of the study are detailed in Table 1.

Table 1: Effect of Jatyadi Taila on skin tensile strength in normal and dexamethasone suppressed incision wound

Group	No. of Animals	Tensile Strength Mean±SEM (g)
Control	6	394.7±12.77
Positive control	6	349.5±8.90
JT alone	6	546.4±33.12[abc]
JT + DEX	**6**	**426.6±10.47[b]**

[a]*p<0.01* vs control; [b]*p<0.01* vs dexamethasone; [c]*p<0.05* vs JT+DEX

Excision wound model

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Excision wound model study was carried out for a period of 21 days. The control group was left untreated. Positive control group was treated with dexamethasone (0.17mg/kg sc.) every alternate day. JT+DEX group received dexamethasone every alternate day and Jatyadi taila daily (0.75 ml/wound daily).

Effect on % Wound closure

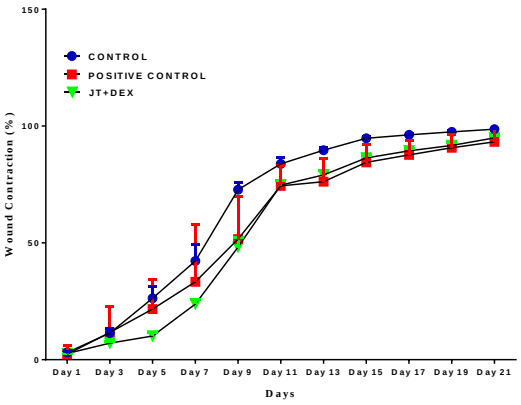

Figure 1: Comparison of percentage wound closure on alternate days in excision wound model study in control, positive control and JT+DEX treated group. The data represents Mean ± SEM of 6 rats.

The wound area of all the animals was traced on an OHP (overhead projector) sheet every alternate day from day 1 to day 21 of the study. This was traced on a graph sheet and wound area was calculated. The wound contraction was quantified as the percentage of the original wound size for each animal. The percentage wound closure on each measured day of all the groups were compared by applying 2-Way ANOVA followed by Tukey's posttest. There was decrease in wound contraction on days 3, 5, 7 and 9 on comparing JT+DEX group with control and positive control. On comparing positive control with JT+DEX group on days 1, 7, 9 and 11, there was found to be considerable difference in % wound closure. However the differences observed failed to show any statistical significance. All the groups showed near compete healing with presence of some scab tissue. The results of the study have been presented in Figure1.

Calculation of HillSlope and WC50 (No. of days required to close the wound by 50%)

In order to understand the rate of wound closure, two additional parameters viz., HillSlope and WC50 were derived with the application of nonlinear regression for asymmetric data. No statistical significant variation of Hillslope was noted across the different groups. However, the mean slope value of positive control group was found to be relatively higher at 6.241±1.399 when compared to the rest. A significant difference ($p<0.05$) was observed with WC50 of the JT+DEX treated group when compared with the control. Time taken for 50% of the wound to contract was 7.271±0.4541, 8.091±0.5263 and 9.015±0.2607 for control, positive control and JT+DEX treated groups respectively. The details are presented in Table 2.

Table 2: Comparison of the effect of Jatyadi taila on the wound parameters viz., HillSlope and WC_{50} in excision wound model

Treatment	n	HillSlope	WC_{50} (Days)
Control	6	5.850±1.070	7.271±0.4541
Positive control	6	6.241±1.399	8.091±0.5263
JT + DEX	6	5.524±0.7635	9.015±0.2607[a]

[a] *p<0.05* vs control

Period of Epithelialization

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The number of days required for complete closure of the wound or fall of scab tissue leaving no raw wound behind was counted. It was observed that control group took around 15 days for complete wound closure. In delayed wound healing, both the groups (positive control and JT+DEX) took around 19-21 days for complete epithelialization. There was significant ($p<0.05$) difference in epithelialization period on comparing control group with positive control and JT+DEX group. The results are shown in Figure 2.

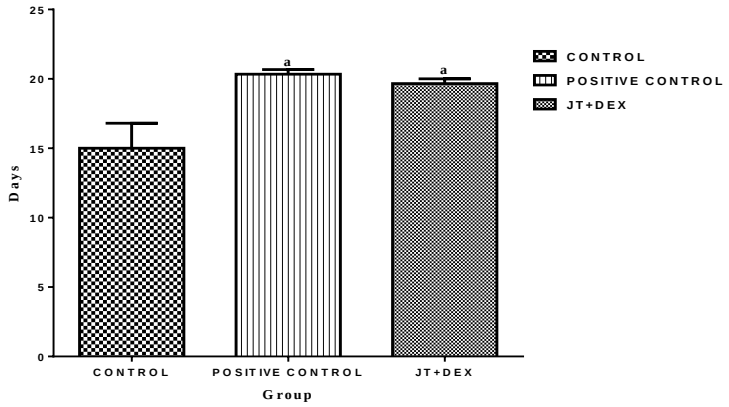

Figure 2: Comparison of the effect of Jatyadi taila on epithelialization period in excision wound model study in control, positive control and JT+DEX treated group. The data represents Mean ± SEM of 6 rats.

Estimation of Hydroxyproline (OHP)

A standard concentration plot was constructed with hydroxyproline at 5 different concentrations of 5, 10, 20, 40 and 60 µg/ml. a line of good fit was obtained with the coefficient of determination R2 calculated to be 0.9960. With the help of linear regression equation, y=mx+c, the concentrations of the unknown samples were determined.

It was observed that JT+DEX group (35±0.25) showed significant ($p<0.01$) increase in OHP levels on comparison with control and positive control group. However there was no statistical difference observed between control (30.75±0.50) and positive control (29.38±0.37) group. The details of the study are presented in Figure 3

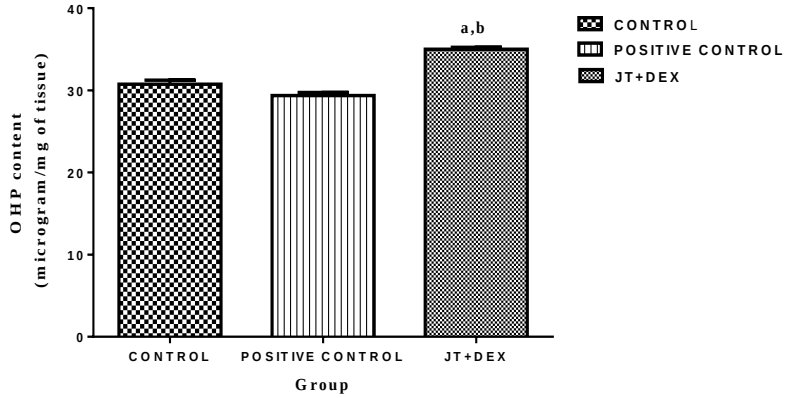

Figure 3: Comparison of hydroxyproline content in control, positive control and JT+DEX treated groups. The data represents Mean ± SEM of 8 rats

Histo-pathological studies

Histo-pathological studies were carried out on 4th and 14th day wound tissue. Following are the photomicrograph sections of the tissue studied under 45X magnification. Haematoxylin and Eosin (H&E) staining and Masson's trichrome staining (MT) were employed to observe for collagen formation, fibroblast proliferation, inflammatory cell infiltration and neovascularization. The results are presented in Figure 4.

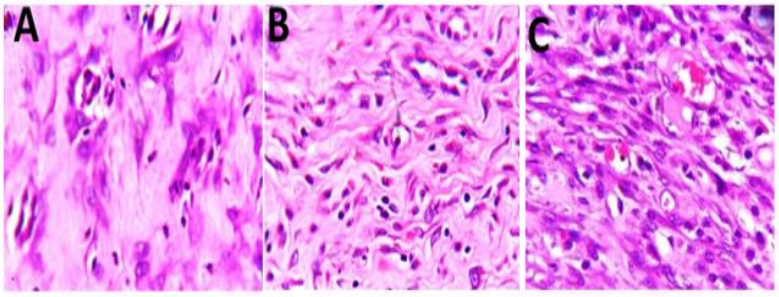

Figure 4: Photomicrograph of wound tissue from A- control; B,- positive control and C- JT +DEX treated animals

DISCUSSION

It was observed in JT alone group that Jatyadi taila showed a remarkable increase in skin breaking strength as compared to control and positive control group. Moreover it also showed a significant improvement in the tensile strength as compared to JT+DEX group. JT+DEX group also showed a significant increase in skin breaking strength as compared to positive control. It can be concluded that Jatyadi taila has a highly potent effect on improving the tensile strength of the re-sutured wound. It should be noted that Jatyadi taila has a more prominent effect on normal wounds than on the delayed wound. This can be attributed to the suppressant effect of dexamethasone on the required inflammatory process that happens early in the wound healing course. But since this oil also shows a better tensile strength in treated wounds than untreated wounds in dexamethasone suppressed wound healing, it can be observed that Jatyadi taila markedly reversed the delay in wound healing to a significant extent. This may be due to high collagen turnover and stabilization of the fibres. It can therefore be concluded that Jatyadi taila promoted the collagenation phase in both normal and delayed wound healing.

Excision wound model was studied for a period of 21 days during which every alternate day, the wound area was traced and calculated. Wound contraction is one of the most important parameters to understand the extent of healing. The contraction is mainly facilitated by the deposited collagen in the granulation tissue and the phenotypic form of fibroblasts i.e. myofibroblasts. Myofibroblasts provide the contractile tension required for wound closure. Percentage wound closure is thus an indication of improved collagenation phase and healthy fibroblast numbers than normally seen in delayed healing.

Dexamethasone is known to have a suppressant effect on inflammatory phase thus reducing the cellular response and cell signaling. As a result the growth factors responsible for increasing the collagen turn over and fibroblast proliferation are reduced. Thus dexamethasone indirectly causes suppression of the collagenation phase which in turn has inhibitory effect on wound closure and thus delays wound healing.

Jatyadi taila used in dexamethasone suppressed wound has shown to reverse this suppressant effect on wound closure. The % wound closure is similar to that of normal control animals which shows the reversal of depressant effect on wound healing. Though the wound closure is similar to control but hydroxyproline estimation and histo-pathological samples have shown an increase in collagen deposition in Jatyadi treated group as compared to control. Thus Jatyadi taila aids the collagenation phase.

Epithelialization phase is the formation of a new epithelium on the granulation tissue which overlaps with the remodeling phase. The migration of epithelial cells begins early during wound healing from the edge of the wound. However, with time the proliferation of epithelial cells also increases so that they start forming a layer and migrate towards each other to close the wound. Dexamethasone is known to inhibit the proliferation of epithelial cells by decreasing cell signaling. In this study, it was observed that the period of epithelialization was increased in dexamethasone treated group to 19 days as compared to 15 days of the

normal control group. This could be due to failure of Jatyadi taila to completely reverse the inhibitory effect of dexamethasone on epithelial cell proliferation.

Histopathology shows increased infiltration by neutrophils and macrophages on the 4th day of wounding in all the three groups. However fewer fibroblasts numbers were seen in control group whereas there was high fibroblastic proliferation in positive control and JT+DEX groups. This indicates that all the three groups were in early phase of inflammation despite the suppressant effect of dexamethasone on positive control and JT+DEX group. The 14th day histo-pathological observations have shown a reduction in neutrophils infiltration in control group. It shows the transition of wound healing in control group from early inflammatory phase to late inflammatory phase which is proved by the presence of macrophages and decrease in necrotic and ulcerative tissues as compared to day 4. However, there were high counts of neutrophils and macrophages in positive control and JT+DEX group. This indicated the overlapping of early and late inflammatory phase in these groups. However there was an increase in fibroblasts and collagen content on 14th day thus indicating an improvement in wound closure. Since the wound closure observed is similar in all three groups, however the increased collagen content in JT+DEX group indicates the improved strength of the granulation tissue in comparison to control and positive control groups. Thus it can be safely assumed that Jatyadi taila causes near complete reversal of the delay in inflammation induced by dexamethasone without causing any detrimental effect on collagenation and granulation phase. However, it is hard to determine exactly which phase it aids in inflammation.

Jatyadi taila did not show demonstrable changes in catalase and TBARS levels although there was marked reduction in GSH and total protein levels in all the three groups. This can be attributed to the increased ROS production in all the three groups with the combined catabolic effect of dexamethasone on positive control and Jatyadi treated group. However there was a significant increase in SOD levels across all the three groups. This can be as a result of increased ROS production which caused more release of SOD as a protective mechanism of the body. Jatyadi showed positive improvement in SOD levels than DEX control. Thus Jatyadi taila may have antioxidant activity. Further studies are required to verify this observation.

It can be summarized from all the observations that Jatyadi taila was found to significantly enhance normal and delayed healing of incision wounds. Dexamethasone when administered is known to decrease the skin breaking strength. Jatyadi taila was able to reverse the depressant effect of dexamethasone on skin breaking strength.

In excision wounds, Jatyadi taila reversed the depressant effect of dexamethasone on wound contraction and epithelialization and reverted back to normal wound healing path. A significant increase in collagen content of granulation tissue was observed on treatment with Jatyadi oil. Jatyadi taila was also observed to have antioxidant properties. It could thus be concluded that Jatyadi taila has a substantial potential for use in wound management in both normal and immune-compromised patients. Further studies on the effect of Jatyadi taila on diabetic wounds could provide a better insight. Moreover, Jatyadi taila could be formulated appropriately in a suitable base and accordingly assessed for its effects on wound

CONCLUSION

It could thus be concluded that Jatyadi taila has a substantial potential for use in wound management in both normal and immune-compromised patients. Further studies on the effect of Jatyadi taila on diabetic wounds could provide a better insight. Moreover, Jatyadi taila could be formulated appropriately in a suitable base and accordingly assessed for its effects on wound.

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REFERENCES

[1] Meenakshi S, Raghavan G, Virendra N, Ajaykumar SR, Shantha M. J Ethnopharm 2006; 107: 67-72.
[2] Clark RA. Am J Med Sci 1993; 306: 42 – 48.
[3] John C, Randell V, Mark A. Int J Burns Trauma 2012; 2: 126-134.
[4] Krishnan P. Curr Anaesth Crit Care 2006; 17: 21-27.
[5] Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. J Ethnopharm 2007; 114: 103-113.
[6] Biswas TK, Mukherjee B. Int J Low Extrem Wounds 2003; 2: 25–39.
[7] Shailajan S, Sasikumar M, Suhas P, Ashish A. J Ethnopharm 2011; 138: 99-104.
[8] Ehrlich HP, Hunt TK. Ann Surg 1969; 170: 203–206.
[9] Lee KH. J Pharm Sci 1968; 57: 1042-1043.
[10] Morton JJ, Malone MH. Arch Int Pharmacodyn Thér 1972; 196: 117-126.
[11] Neuman RE, Logan MA. J Biol Chem 1950; 186: 299-306.