

IN VIVO EVALUATION OF SUPPOCIRE PARACETAMOL RECTAL SUPPOSITORIES

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ABSTRACT

To achieve successful delivery of drug locally as well as systematically rectal route has proved its potential. The main objective of this research work was to evaluate the *in vivo* performance of paracetamol Suppocire CP suppository. Initially, suppositories were prepared, evaluated for their physicochemical parameters and *in vitro* drug release. An *in vivo* investigation of paracetamol availability in rabbits was carried out in comparison with marketed paracetamol suppository and suspension in complete cross-over design. The comparison between the three different formulations in terms of whole blood concentration revealed that the extent of drug absorption from Suppocire suppository and marketed suppository is comparable and showed promise that product of similar characteristic can be formulated using an amphiphilic suppository base. Bioavailability study showed comparable absorption after rectal and oral administration. A rectal irritation study exhibited high irritation profile for marketed suppository to rectal mucosa as compared to Suppocire suppository which showed no irritation.

Keywords: Suppository, Suppocire bases, Paracetamol, Bioavailability, Rectal irritation.

INTRODUCTION

Rectal drug delivery is well known for its certain advantages over the oral drug delivery. Palatability and taste, which are the prime concern in oral dosage forms for children need not to be considered in case of rectal drug delivery. Thus, suppositories can prove to be a useful alternative dosage form in pediatric patients. The rectal mucosa [pH 7-8] has an abundant supply of blood vessels and lymphatic vessels. The most important aspect of this route is bypassing the first hepatic metabolism. In addition, specific rectal membranes properties are responsible for the absorption of drug.¹

Paracetamol is well-established nonprescription antipyretic and analgesic drug.² It has strong antipyretic and mild analgesic action with some anti-inflammatory activity as compared to other anti-inflammatory drugs. It has low toxicity and high therapeutic index, which is an important consideration in selection of drug for pediatric patients. Paracetamol is well absorbed through rectum, though rectal absorption is slower than oral administration. The relative bioavailability is 80% to that of oral administration.³

Suppositories are solid rectal dosage form and prepared using either fatty bases or water soluble bases. Polyethylene glycol bases being hydrophilic have the inherent risk of traumatizing sensitive rectal mucosa. Natural fatty bases for e.g. Cocoa butter have the drawback of polymorphism; Semi synthetic bases are produced from vegetable oils and chemically modified during their manufacture.⁴ Drug release from suppositories and subsequent absorption through the rectum involves several stages, starting from suppository melting or softening at rectal temperature, followed by drug migration through the suppository mass and its transfer from suppository surface to the rectal environment, and finally drug solubilization in rectal fluids and drug permeation across rectal membrane.⁵ The rectal absorption occurs mainly by passive diffusion and the drug release from suppository is likely to be an important factor in determining the drug concentration in the rectal fluids and hence its absorption rate. This means, both the drug solubility and the excipients characteristics play a crucial role in the rate of drug absorption.⁶ Furthermore, the excipients properties can affect not only the rate, but also the extent of absorption, especially for drugs that undergo saturable presystemic metabolism.⁷ For such drugs, the magnitude of first pass effect could vary with the drug release rate from suppositories. When studying drug availability after rectal administration, the vascularization of the rectal tissue must be considered. The superior and middle rectal veins drain the rectal blood into the hepatoportal system, whereas the inferior rectal vein conveys the blood directly to the inferior vena cava. In contrast to the oral administration, a fraction of the drug entering the systemic circulation could bypass the liver. Therefore, it is reasonable to assume that the fraction of the drug undergoing hepatic first pass

effect can also depend on the placement and the extent of spreading of the suppository within the rectum.⁸

Compounds of either a hydrophilic and lipophilic nature may be readily absorbed through mucosal membranes. Suppositories when placed in rectum come in contact with rectal mucosa may cause irritation or mucosal damage. Pharmaceutical adjuvants like polysorbates, sodium lauryl sulphate, sodium deoxycholate and polyoxy ethylene stearate have also been reported to cause histological changes in rectal mucosa.⁹

Human rectal mucosa is composed of columnar epithelium, the lamina propria and the doubled layer muscularis mucosae. The epithelial surface consists of closed packed columnar cells with some areas interrupted by crypt regions, which contains mucous producing goblet cells. Throughout the rectal mucosa there are lymphoid nodules covered by columnar surface epithelium. The lamina propria consists of two layers dense a cellular collagen layer and loose connective tissue layer. In the lamina propria, there exist superficial blood vessels and inflammatory cells including macrophages, neutrophils and lymphocytes. The muscularis mucosa contains smooth muscle cells and larger vessels. Histological changes in rectal mucosa, their type and degree of occurrence can be correlated to irritative effects of the suppository under study.¹⁰

In the present study semi-synthetic base Suppocire CP have been investigated for its *in vivo* performance in rabbits and evaluated for the irritation property in comparison to marketed suppository in rats.

MATERIALS AND METHODS

Paracetamol was obtained as generous gift sample from IPCA Laboratories, Mumbai, India. Suppocire CP (Gattefossé, France through Colorcon Asia Pvt. Ltd. Goa, India) and labrasol (Gattefossé, France through Colorcon Asia Pvt. Ltd., Goa, India) were obtained. Sodium chloride (NaCl, AR grade), sodium hydroxide (NaOH, AR grade), 1-nitroso-2-naphthol (AR grade), sodium nitrate (AR grade), anhydrous sodium sulphate (AR grade) and nitric acid were purchased from S.D. Fine Chemicals, Mumbai, India. Organic solvents such as solvent ether (AR grade), methanol (AR grade), chloroform (Spectrometric grade) and formalin were purchased from RanChem, Mumbai, India. Freshly prepared distilled water was used throughout study.

Preparation of paracetamol suppositories

Paracetamol suppositories containing 250 mg of the drug were prepared by the fusion method using a metal mould with six cavities as discussed in previous article.^{11,12,13} The drug powder was passed through a mesh sieve of 100 μ m prior to its incorporation into

Suppocire CP base in combination with 5% w/w labrasol. Drug displacement value of the Suppocire CP base was determined in presence and absence of labrasol. The prepared suppositories were evaluated for mechanical strength, disintegration time, pharmacokinetic and rectal irritation profile.

In vivo rectal bioavailability

In vivo bioavailability of Suppocire suppository and marketed suppository was carried out in complete cross-over design. Six healthy adult rabbits of either sex each weighing 3-3.5 kg were used in the study. The rabbits were divided into two groups containing three rabbits each. Animals were fasted 20 h prior to experiment but given free access to water. Before administering the dosage form, control blood samples of one milliliter were obtained from marginal ear vein of all the rabbits. On the first day of experiment, three rabbits were given Test suppository and another three rabbits were given marketed oral paracetamol suspension (COFAMOL®, Cosme Remedies Ltd., Goa, India). After fifteen days elapse, rabbits who had earlier received test formulation were administered marketed paracetamol suspension and vice-versa. After a wash out period of fifteen days, the marketed paracetamol pediatric suppository Suppol® (Meridian Pharma, Goa, India) was administered to all six rabbits. Blood samples (1 ml) each was collected at time intervals of 0.5, 1, 2, 4, 6 and 8 h after administration of the formulations. Each of blood samples was collected in the test tube containing 0.3 ml of 3.8% w/v sodium citrate. The blood was stored at 4°C until assayed at the earliest time.

Determination of paracetamol in whole rabbit blood

The concentration of paracetamol in whole human blood was determined using spectrofluorimetric method at emission wavelength of 548 nm and excitation wavelength 467 nm. To 0.3 ml of blood, sufficient purified water was added to make the volume to 4 ml. After addition of 2.5 g of NaCl, the mixture was shaken with 10 ml of solvent ether (AR grade) for one hour on mechanical shaker. The mixture was then centrifuged (12000 rpm) and 7 ml of organic layer were transferred to glass stoppered test tube containing 1.5 ml of 0.01N NaOH. Test tube was further shaken for 10 min on vortex mixer and 1 ml of alkaline layer was transferred to another stoppered tube. To the obtained alkaline layer, 1 ml of 0.01% w/v methanolic solution of 1-nitroso-2-naphthol, 1 ml of sodium nitrate (0.1% w/v) and 1 ml of nitric acid (1N) were added consecutively. The mixture was heated at 40±2°C for 40 min. After cooling to room temperature, it was transferred to 25 ml volumetric flask and to it 10 ml purified water and 5 ml chloroform (Spectrometric grade) were added. The flask was again shaken on mechanical shaker for 30 min. The mixture was then centrifuged and lower organic layer was transferred to stoppered test tube and dried over one gram of anhydrous sodium sulphate. The fluorescence of dried organic layer was measured using Shimadzu RF5000 spectrofluorimeter. Blank was run simultaneously using control blood samples. The concentration of paracetamol in whole rabbit blood was obtained from standard calibration curve.

Data analysis and determination of pharmacokinetic parameters

Whole blood concentration- time data for paracetamol were analyzed using compartmental pharmacokinetic methods. The elimination rate constant was determined as a slope of the linear regression. Maximum blood concentration (C_{max}) and the corresponding sampling time (T_{max}) for paracetamol were recorded as observed. Area under curve versus time was calculated by trapezoidal method. Relative bioavailability "F" was also calculated. Statistical evaluations of the differences in pharmacokinetic parameters obtained with the tested formulations were carried out by applying Student's t- test ($\alpha = 0.05$) for paired data.

Rectal irritation studies

Twenty rats of either sex, weighing between 200-250 g were selected and fasted 36 h prior to experiment but given free access to water. Before rectal administration of suppository, the bowel content in the lower rectum were evacuated by pressing down on

the abdomen. The rats were divided into five group containing four rats each. The suppository under investigation (Test and Marketed) was cut into suitable size and dimension and administered into the rectum. At designated times of 4 and 8 h after administration, the rats were sacrificed and the rectum was isolated. The excised tissue were washed with normal saline fixed in 10% w/v formalin and dehydrated, embedded in paraffin and cut into sections 5 to 6 μ m thick. The sections were stained using Harris's hematoxylin and eosin method.¹⁴ The tissues were critically observed under optical microscope for any mucosal damage. Photomicrographs were taken and histological changes were noted.

RESULTS AND DISCUSSION

Preparation of paracetamol suppository

White colored suppositories having disintegration time less than 18 min were prepared using Suppocire CP base. The prepared suppositories passed drug content test according to British Pharmacopoeia. Suppocire CP suppositories released 50% paracetamol at 1.48 h and 90% at 2.67 h, respectively. The hardness of less than 0.6 kg was noted for test suppository.

In vivo rectal bioavailability

The blood level profiles obtained from single dose administration of test paracetamol suppository, marketed suppository and marketed oral suspension are given in Fig. 1. Paracetamol was absorbed rapidly from the developed suppository showing peak concentration of 8.10 μ g/ml at one hour post administration. The levels started declining after that and at 4th hour the blood showed negligible concentration of paracetamol. The blood samples withdrawn at 24 h showed no paracetamol. Analysis of variance (ANOVA) at 5% significance level indicated no significant difference in the plasma concentrations and the area under the concentration time curve (AUC_∞) within the rabbits. Drug from the marketed oral suspension was also absorbed rapidly within 1 h giving peak concentration of 7.52 μ g/ml. The drug blood concentration declined gradually with time. Analysis of variance at 5% significance level indicated no significant difference in plasma concentration and the AUC_∞ within the rabbit. Student's t-test, at 5% significance level indicated no significant difference in the blood concentration and the AUC_∞ between the test suppository and marketed paracetamol suspension.

Similarly paracetamol also got rapidly absorbed from marketed suppository showing peak concentration level of 8.33 μ g/ml at one hour. The levels started declining after one hour and at 4th h the blood showed negligible concentration of paracetamol. Analysis of variance (ANOVA) at 5% significance level showed no differences in blood concentration and AUC within group.

The average AUC_∞ for test suppository and marketed paracetamol suspension was found to be 23.25 and 21.64 μ g.h/ml, respectively and for marketed paracetamol suppository it was 23.248 μ g.h/ml. Statistical test was carried out to evaluate the difference between the drug blood concentration (μ g/ml) of test suppository and marketed paracetamol oral suspension and found to have no significant difference at 5% level. Similarly AUC_∞ of paracetamol test suppository and marketed suspension was statistically evaluated and found to be statistically not significant. Similarly the blood levels and AUC_∞ of marketed suppository and test suppository were found to be comparable with no significant difference. Blood level data of marketed oral suspension was also comparable with marketed suppository. This means that prepared Suppocire CP suppositories has almost same drug release characteristic as that of marketed suppository.

The blood concentration data of the test suppository, marketed oral suspension and marketed paracetamol suppository formulations were evaluated by statistical moment analysis to determine the mean residence time (MRT). MRT can be defined as the mean residence time for the intact drug molecule to transit through the body and involves a composite of all kinetic processes including *in vivo* release from the dosage form, absorption from the body, and all disposition process. The Pharmacokinetic data is given in Tables 1, it

was observed that MRT of test formulation and marketed suspension were almost similar being 2.58 and 2.57 h, respectively and that of marketed suppository was 2.11 h. The relative bioavailability was calculated as follows,

$$F = \frac{[AUC^\infty]_{\text{Test}}}{[AUC^\infty]_{\text{Ref}}}$$

showed value of 1.074. Thus, we can say that rectal administration of test paracetamol suppository has comparable bioavailability to

that of marketed oral suspension. Marketed paracetamol suppository gave slightly higher peak levels of 8.33 µg/ml as compared to marketed suspension and developed suppository at 1 h and relative bioavailability of 1.062, statistical test indicate that the MRT of all three formulations has no difference. They have almost equal mean residence time. No interferences by p-amino phenol a known metabolite of paracetamol, in this assay have been reported.

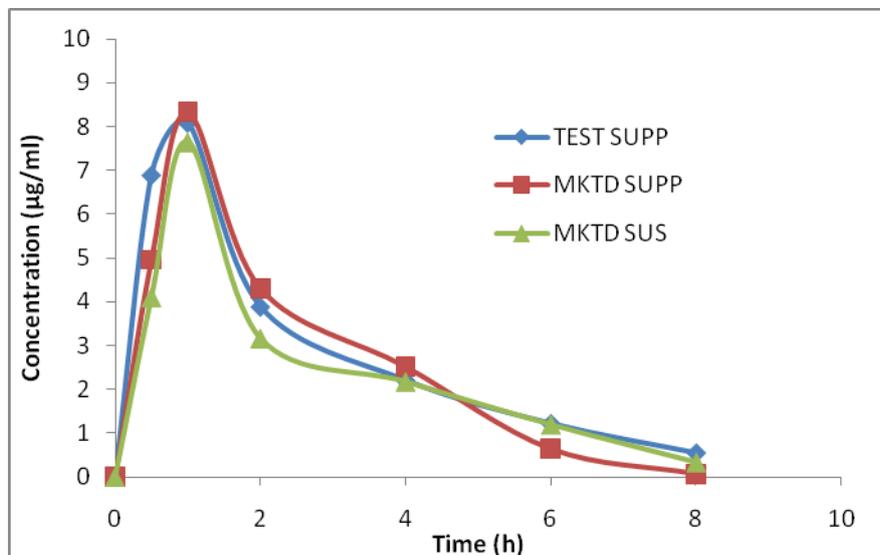


Fig. 1: Pharmacokinetic profile of paracetamol in whole rabbit blood from Test suppository (Test SUPP), Marketed suppository (MKTD SUPP) and marketed suspension (MKTD SUS)

Table 1: Comparative pharmacokinetic parameters of paracetamol test, marketed (MKTD) suppository and suspension.

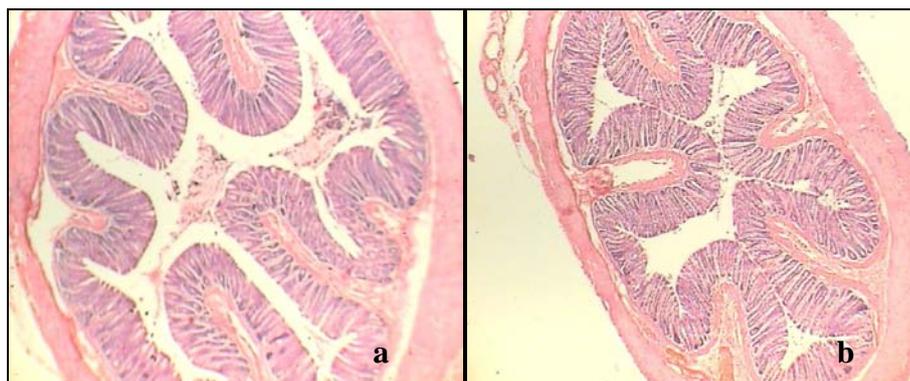
Formulation	C _{max} (µg/ml)	AUC ₀₋₁ (µg/ml)	AUC _{0-∞} (µg/ml)	T _{max} (h)	MRT (h)	K _e (h)	F
TEST Suppository	8.10±0.13	21.43±0.042	23.00±0.210	1	2.58±0.021	0.32±0.03	1.062
MKTD Suspension	7.65±0.181	20.072±0.431	21.640±0.121	1	2.57±0.032	0.40±0.12	1.074
MKTD Suppository	8.33±0.163	21.633±0.0388	23.248±0.113	1	2.11±0.025	0.48±0.03	

Rectal Irritation

Fig. 2 shows the photomicrograph normal rat rectal mucosa depicting different layers and other observations. Table 2 lists the histological observations of rat rectal mucosa at designated time intervals after administration of test suppository and marketed suppositories. As shown in Fig 2 and Table 2, administration of test suppository did not have any severe effect on rat mucosa except mild desquamation of epithelium slight increase in number of goblet cells at the end of 4 and 8 h after administrations. At 8 h, the tissue showed mild hyperplasia of goblet cells, i.e. number of goblet cells in the crypt region was slightly

more than normal. Goblet cells are mucous producing cells, which increase in number in presence of very mild irritant. No erosion or inflammatory changes were observed.

Administration of marketed suppository led to erosion of epithelial cells exposing the underlined lamina propia indicating its irritative effect. Metaplasia of epithelia also observed at 8 h post administration which is indicative of presence of irritant in contact with rectal lining for long time. It can be clearly seen that the marketed suppository caused severe irritation to rectal tissue as observed by complete erosion of mucous membrane.



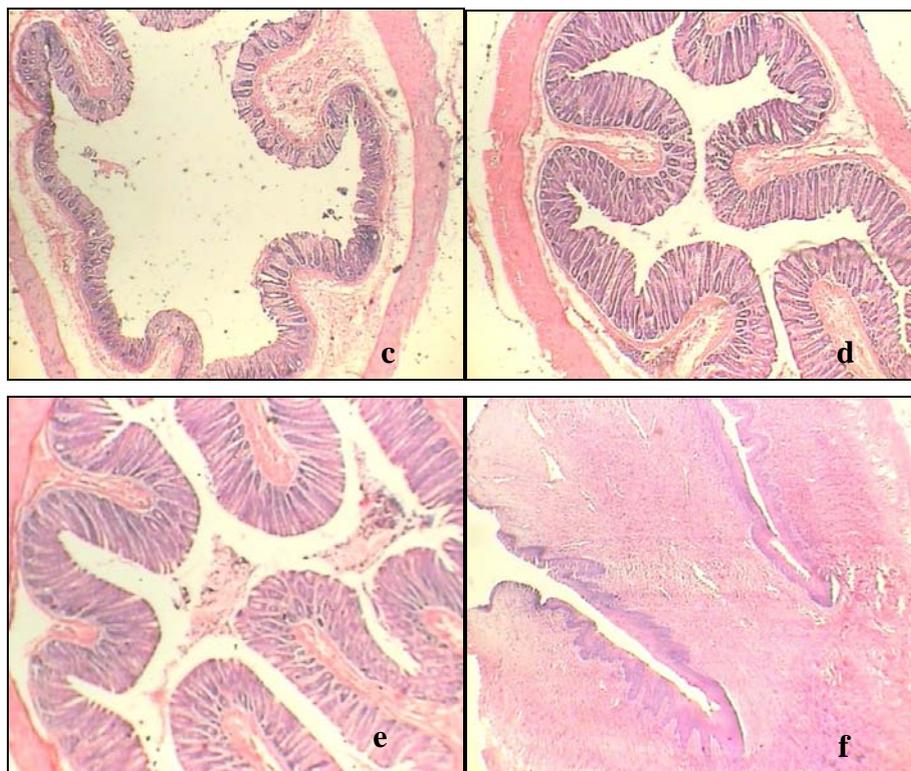


Fig. 2: Photomicrograph of rat rectum for a) Control group b) blank Suppocire CP suppository, c) after 4 h for test suppository d) 8 h for test suppository, e) 4 h for marketed suppository & f) 8 h for marketed suppository.

Table 2: Histopathological changes occurred in rectal linings after administration of paracetamol test and marketed suppositories in rat (n=4).

Group	Time (h)	Observation
Control group	-	Closely packed columnar epithelium underlined by lamina propria and muscularis mucosae. Epithelial layer containing crypt region containing goblet cells. Whereas lamina propria containing acellular collagen layer and connective tissue layer.
Blank Suppocire suppository	4 & 8	Undamaged columnar epithelium underlined by lamina propria and muscularis mucosae. Epithelial layer containing crypt region containing goblet cells. Lamina propria containing acellular collagen layer and connective tissue layer was intact.
Test Suppository	4 & 8	Mild desquamation of epithelium cells, mild infiltration of neutrophils. Lamina propria was normal.
Marketed Suppository	4 & 8	Complete erosion of superficial epithelial cell wall. Hyperplasia of goblet cells to high extent. High infiltration of neutrophils. Complete destruction of lamina propria

CONCLUSION

In conclusion, paracetamol was rapidly absorbed through rectal route and oral route. Both marketed suppository and test suppository have almost same bioavailability which was further comparable with oral suspension. A good correlation between blood levels obtained after administration of test, marketed suppository and oral suspension indicates a therapeutic efficacy. The developed Suppocire CP suppositories caused slight irritation to rectal mucosa, while marketed suppository resulted in severe inflammation of tissue. The slight irritation occurred to rectal lining for drug loaded Suppocire CP could be due to the high percentage of labrasol used in the suppository formulation, otherwise placebo Suppocire CP base itself has no irritation to mucosal linings.

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REFERENCES

- De Boer AG, Breimer DD. Hepatic first-pass effect and controlled drug delivery following rectal administration. *Adv. Drug Del. Rev.* 1997; 28: 229-237.
- Forrest JAH, Clements JA, Prescott LF. Clinical pharmacokinetics of paracetamol. *Clin. Pharm.* 1982 ;7: 93-107.
- Anderson BJ, Holford NHG. Rectal paracetamol dosing regimens: determination by computer simulation. *Pediatr. Anaesth.* 1997; 7: 451-455.
- Coben LJ, Liberman HA. In the theory and practice of Industrial Pharmacy, Eds. L. Lachman, Lieberman HA and Kanig JA, 3rd edition, Varghese publishing house, 564, 1987.
- Realdon N, Ragazzi E, Dal Zotto M, Dalla Fini G. Layered excipient suppositories: the possibility of modulating drug availability. *Int. J. Pharm.* 1997; 148: 155-163.
- Shangraw RF, Walkling WD. Effect of vehicle dielectric properties on rectal absorption of acetaminophen. *J. Pharm. Sci.* 1971; 60 (4): 600-602.
- Pagay SN, Poust RI, Colaizzi JL. Influence of vehicle dielectric properties on acetaminophen bioavailability from polyethylene glycol suppositories. *J. Pharm. Sci.* 1974; 63 (1): 44-47.
- De Mynck C, Lefebvre RA, Remon JP. The influence of formulation on the relative bioavailability of indomethacin suppositories in dogs and rabbits. *Int. J. Pharm.* 1994; 104: 1-10.

9. Chien YW, In Novel Drug Delivery Systems, 2nd Ed., Marcel Dekker Inc., New York, 198, 1992.
10. Nishihata T, Hirotsu Y, Yamazaki A, Takahashi K, Yoshitomi H. Preliminary study on sustained-release particles prepared with hydrogenated soya phospholipid and cholesterol. Int. J. Pharm. 1988; 42(1-3): 257-260.
11. Liversidge GG, Grant DGW, Padfield JM. Preformulation studies on ingredients of suppository bases. J. Pharm. Pharmacol. 1979; 31: 53.
12. Te Wierik GH, Eissens AC, Besemer AC, Lerk CF. Preparation, characterization, and pharmaceutical application of linear dextrans. III. Drug release from fatty suppository bases containing amyloextrin. Pharm Res. 1994; 11(1): 108-10.
13. Shegokar R, Singh KK. *In vitro* release of paracetamol from Suppocire Suppositories: role of additives, Malaysian J. Pharm. Sci. 2010; 8 (1): 1-15.
14. Human GL, In Animal tissue Technique, 4th ed., Freeman WH, San Francisco, 127 (1979).